Vol. 32 **No. 3** **July-September 2009**

Drugs and Pharmaceuticals
Current R & D Highlights
(Genetic Basis of Diseases)

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We express our gratitude to Dr. S.K. Rath, Scientist, Toxicology Division, C.D.R.I. Lucknow for his tenacious cooperation and persistent support for this issue of Current R&D Highlights

Guest Editorial

Genetic Basis of Diseases, the Brighter Tomorrow

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It is estimated that there are about 5000 human diseases having recorded genetic link with single gene defects with Mendelian traits. OMIM (Online Mendelian Inheritance in Man) database statistics says that there are at least 1,600 Mendelian and an additional 2,123 Mendelian-suspected phenotypes for which the molecular basis is unknown. Furthermore, there are several human diseases which are multifactorial and their genetic link is less known and seldom explored. The phenotypes of these less known diseases are different, complicated and in many cases influenced by environment and geographical locations. As per the current knowledge of genetics it is really difficult to identify a disease which does not have a genetic basis. The disease may not be as such based upon one or few mutations in the genome but might be the effect of few mutations that make the subject susceptible to the diseases. Strictly speaking, according to classical definitions of genetics, such diseases may not fall into any types like autosomal dominant or recessive or sex-linked but still have a strong genetic basis. Their transmission from generation to generation also may or may not follow classical Mendelian principles. Identifying the exact genetic basis of such diseases is the real challenge that scientists face today. Taking together all the disease phenotypes globally, about 10% of married couples are at high recurrence risk of producing offspring affected by a Mendelian disorder. This estimation also may increase if we consider consanguineous marriages which occur by choice rather than by accident, contributing to about 19% of all births. The challenges therefore, are grand for identifying the genetic basis which will of course dictate the research to contain the disease especially by applying molecular genetic tools like RNA interference. Having a battery of full proof technologies in identifying genetic alterations like, mutations, single nucleotide polymorphisms, copy number variations single-gene disorders may be characterized fully at the molecular level in the coming decade. But complex diseases like diabetes, hypertension, psychosomatic diseases, will take decades of intensive research to be fully dissected out in different human populations.

Further, establishing their correlation with specific phenotypes in several cohorts will be more challenging. Well planned whole genome association studies conducted across the globe with consideration of well defined ethnicity, exact geographical location, phenotypes, true healthiness are expected to be more informative and more specific and truly potent to dissect the genetic basis of the diseases. At least I am optimistic with the myriad of ways that are being employed in exploring the human genome and their phenotypes, the rate of adoption of new technology in different laboratories across the globe and the developments in the area of bioinformatics are bound to give surprises, challenges and new beginnings in human disease biology. The days are not far from actual translation of knowledge to the bed side of the patient.

Genetic Basis of Human Diseases

It is apparent that every medical condition with the exception of trauma has a genetic component. As is often evident from the patients family history, many common disorders such as hypertension, heart disease, asthma, diabetes mellitus and mental illness are significantly influenced by the genetic background.

Genes carry all the information that determines an organisms' characteristics. When genes are working properly, our bodies develop and function smoothly. But a single gene – even a tiny segment of a single gene – go awry, the effect can be dramatic-deformities and disease, even death.

To function correctly, each cell depends upon thousands of proteins to work properly. Sometimes, gene mutation prevents one or more of these proteins from doing so. By changing the instructions on genes for making a particular protein, a mutation can cause the protein to malfunction or missing entirely. When a mutation alters a protein that plays a critical role in the body, it can disrupt normal function and cause a medical condition. Cancer has a genetic basis, since it results from mutations in genes controlling growth and differentiation.

The detection of mutations is an important diagnostic and prognostic tool and may identify individuals with an increased risk for the development of certain chronic diseases.

Hypertrophic cardiomyopathy (HCM) is the most common cause of sudden cardiac death (SCD) in the young (including trained athletes), who are often unaware of their underlying condition. Early diagnosis of HCM is important, since at-risk individuals may be advised not to participate in competitive sports and should undergo regular cardiac screening to assess the risk of sudden cardiac death.

HCM is caused by a variety of mutations in genes encoding contractile proteins of the cardiac sarcomere, especially in cardiac myosin heavy chain beta (*MYH7*), myosin binding protein C (*MYBPC3*), and cardiac troponin T (*TNNT2*). To date, over 700 individual mutations have been identified.

Mutations in the genes coding for 3 sarcomeric proteins (*MYH7*; *MYBPC3*; *TNNT2*), most commonly are estimated to account for about 60% of all familial cases of HCM. Clinical testing for variants in most of these genes is available and can provide valuable therapeutic and prognostic information.

Genetic testing for mutant genes is the most definitive method for establishing the diagnosis of HCM, and some genotype-phenotype correlations can be useful to address DNA analysis in specific genes.

The field of pharmacogenomics seeks to identify genes that alter drug metabolism or confer susceptibility to toxic drug reactions.

Genetic diagnosis of human disorders is only the beginning to be used in clinical settings, predictive testing holds the promise of allowing earlier and more targeted interventions that can reduce the morbidity and mortality associated with various disorders.

Sheela Tandon

Epigenetic Mechanisms in Etiopathology of Complex Neuropsychiatric Disorders and the Epigenetic Therapeutics

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Epigenetics is an exciting recent development which is leading us fast to understand the etiopathology of complex non-Mendelian diseases at molecular level, and also showing promise for the development of therapeutics targeting the primary epigenetic cause of illness. An array of epigenetic drugs developed recently are effective in animal models of various neuropsychiatric disorders. One class of such epigenetic drugs, HDAC inhibitors (HDACi), is remarkable in its ability to ameliorate the symptoms in variety of complex neuropsychiatric diseases. This suggests that it will be helpful if the clinical trials are designed based on patients, grouped together by common pathophysiological mechanism of disease rather than based on the affected genes. This article reviews the recent investigations on epigenetic mechanisms underlying etiopathology of complex neuropsychiatric disorders and also the current status in the development of epigenetic therapeutics.

Cancer, life style diseases like diabetes, cardiovascular disorders, and most of the neurological and psychiatric diseases, are complex in etiopathology and cannot be explained by Mendelian genetic mechanisms [1]. Most of the neuropsychiatric disorders are characterized by chronic, persistent symptoms. Even the treatment modalities (such as antidepressants, mood stabilizers) take long time, in weeks or months, to show the response. Since epigenetic mechanisms such as DNA cytosine methylation, histone H3 methylation at certain lysine residues, exert lasting control over gene expression without altering the gene sequence or genetic code, the role of epigenetic or chromatin remodeling mechanisms is suspected in the disorders of brain and behavior [2]. Recent evidence from our lab and that of others [3-8] indeed suggest that neuropsychiatric disorders affecting cognition and reward circuitry, arise due to the epigenetic dysregulation of gene expression. It is also hypothesised that it is highly unlikely that many genes governing a particular brain circuit such as the one controlling emotions (as behavior is polygenic), can undergo mutations leading to behavioral disorder. So, we proposed that there must be repressive epigenetic mechanisms such as DNA methylation, histone methylation, histone deacetylation, non-coding RNA-mediated transcriptional gene silencing, causing dysregulation of many genes in particular brain circuitry, resulting in malfunction in the circuitry, thus causing the neural and behavioural disorder. Indeed, our research findings [9] validate our hypothesis. We show that chronic psychosocial defeat stress-induced depression and related mood disorders are

caused by dysregulation of hundreds of genes in reward circuitry due to increased transcriptional repressive methylation marks on histone H3. The maladaptations in the circuitry and the resulting mood disorder symptoms lasted even up to a month after the cessation of defeat stress. It is also known that epigenetic marks are reversible, unlike changes in gene sequences and so the disorders can be treatable. Interestingly, the normalization of stress-induced mood disorder by administering an antidepressant (imipramine) for a month, our studies show for the first time, the de-repression of many epigenetically repressed genes by reducing the methylation marks on histone H3 [9].

Epigenetics is the study of changes in gene expression that occur without a change in DNA sequence and are meiotically or mitotically heritable [10,11]. The regulation of various genomic functions, including gene expression are achieved through the methylation of DNA, the post-translational modifications (PTMs) of histone proteins, exchange of histone variants, and RNA-based silencing. The two main epigenetic mechanisms work in concert, with alterations in DNA modification (cytosine methylation) affecting chromatin conformation and vice versa [11]. The organismal behaviour, i.e., phenotype, is not decided by genotype alone, rather strongly shaped by epigenotype (i.e., environmental effects on gene functions). Recent example of differential level of gene expression profile in various tissues from monozygotic twins provide beautiful example of environmental impact on gene function (cf 12]. Fraga *et.al*. [13] show that twins are epigenetically similar during the early years of life, but later exhibited huge differences in their overall content and genomic distribution of 5-methyl cytosine DNA and histone acetylation. Meaney and collegues [14] show how epigenetic mechanisms are involved in encoding early life experience into long-term shaping of behaviour. The differential parental care (high grooming vs low grooming) received rat pups determines their adulthood response to stress. High grooming and licking of pups induces DNA demethylation and histone acetylation on the promoters of hippocampal glucocorticoid receptor (GR) genes resulting into mature development of the stress circuitry and hence proper response to stress in adulthood. On the contrary, low groomed pups grow with methylated GR promoter and fail to cope up stress very well.

Thus, decoding the epigenetic language underlying long-lasting neuro-glial and behavioral changes in response to chronic perturbations to brain will not only give an insight into etiologic and pathogenic mechanisms but may also help to develop drugs to target the primary epigenetic causes of the disease process [15]. The excitement is enormous at the prospect of bringing into reality the drugs that work at epigenetic level. A number of epigenetic drugs are at various stages of clinical trials showing great promise in the treatment of cancer. The development is also encouraging for treating various neuropsychiatric disorders, and other complex diseases. However, their effectiveness against particular disease has yet to be maximized keeping the adverse effects on other tissues and systems, as majority of these are non-specific compounds working against many of the similar members of the epigenetic target family [12,16,17,18].

Epigenetic Players in Genome Regulation

Epigenetic mechanisms are involved in regulating every molecular event where DNA is the substrate, i.e., transcription, recombination, replication and DNA repair. Packaging of DNA in chromatin ensures that the organization, and readout of the genetic information happens in a proper spatial and temporal sequence during cellular differentiation and organismal development [11]. The fundamental repeating unit of

chromatin, the nucleosome core, is an octamer protein made up of two copies each of H2A, H2B, H3, and H4 histones or variants thereof. 147 bp of DNA in approximately two superhelical turns is wrapped around the octamer of core histone proteins. When associated with other components, higher-order nucleosomal structures are formed. The N-terminal tails of various histones that form the core protrude from their globular domains. These tails undergo post translational modifications (PTMs) by the enzymes which are termed as 'writers' (such as HATs, HMTs, kinases, to add acetyl, methyl and phosphate group, respectively), 'readers' (such as the proteins having bromo, chromo, PHD, Tudor, 14-3-3 domains which can read the write ups on various amino acid residues on the histone tails; bromo for reading acetyl group, chromo, PHD and Tudor for reading or recognizing methyl group and 14-3-3 for phosphoryl group) and 'erasers' (such as HDACs, DMTs, Phosphatases for removing acetyl, methyl and phsophoryl group, respectively from the histone tail) [15].

Thus the variations introduced into nucleosome array structures by the writers, readers and erasers cause differences in chromatin architecture that results in more 'open' versus 'closed' states, loosely correlating with 'euchromatin' versus 'heterochromatin' states that most of the time signifies 'active' versus 'inactive' states of gene expression, respectively [11,15]. Covalent histone modifications such as acetylation, methylation, phosphorylation, ubiquitinylation, sumoylation, ADP ribosylation, histone variants, and ATP-dependent chromatin remodeling complexes, work together to alter the chromatin fiber [11,15]. Histone acetylation, a charge-altering modification, negates the positive charge on the 3-amino groups of lysine, causing weakening of histone: DNA contacts, opening up of the chromatin, allowing space for transcriptional machinery and so correlated with transcriptional activation [11]. In contrast, histone hypoacetylation correlates with closing in of chromatin and so gene repression and silencing [11,15]. The lysine residues on histone tails are acetylated by histone acetyltransferases (HATs), a large family of enzymes, and deacetylated by histone deacetylases (HDACs), another large family of enzymes. Both histone H3 and H4 undergo polyacetylation at nearby lysine residues in their N termini. Methylation not only affects DNA, and histones, but RNA and nonhistone proteins too, at least to varying degrees in different organisms. Histone methylation, unlike acetylation, can correlate with either transcriptional activation (H3 K4, H3K36) or repression (H3K9, H3K27, H4K20), depending on the histone and lysine residue being methylated (Lachner *et al* 2003). Histone methylation may also facilitate DNA methylation, which induce further repression or silencing of the gene [11,15]. To add to the complexity of gene regulation, multiple lysines or arginines on histone or nonhistone protein can be methylated resulting in enormous possible biological readouts. To further add complexity in gene regulation through the complexity of methylation, individual lysine residues can be mono-, di-, or trimethylated and arginine residues can be mono- or dimethylated. Various methylation reactions are mediated by distinct classes of histone methyltransferases (HMTs) and demethylases (KDMs). There is potential differences in regulatory mechanisms as unlike acetylation, methylation does not alter the positive charge of the targeted lysine or arginine [11]. Thus, methylation of histones combined with acetylation and also other modifications gives enormous array of possibilities and controlled addition and removal of specific PTMs leading to unique combinations can constitute a sort of 'epigenetic indexing code' which might correspond to distinct physiological states and genomic functions [15].

Similarly, for cytosine methylation in

CpG dinucleotides the writer is DNMT (DNA methyl transferases), which are *de novo* methyltransferases DNMT3a and -3b, that add methyl group at previously unmethylated sites, and the maintenance methyltransferase, DNMT1, that copies pre-existing methylation patterns onto newly replicated DNA strands. For reading this message of gene silencing, i.e. DNA methylation, are readers called MBD (methyl binding domain protein) such as MeCP2, MBD1. Then arrive the co-repressor protein complexes containing HDACs at the scene, followed by histone methyl transferases, HP1 (heterochromatin protein 1), which ultimately shut down the gene activity. There is evidence of active DNA demethylation, however, the demethylase is yet to be discovered [11].

Another recently discovered epigenetic player is non-coding RNAs (ncRNAs) which induce gene silencing by regulating chromatin structure [19]. The non-coding RNA, Xist (X-inactivespecific transcript), is involved in silencing the inactive X chromosome in females. siRNA (short interfering RNA) and microRNA (miRNA) molecules have been shown to target homologous genes for silencing. This aspect of epigenetic regulation is still in infancy [19].

Epigenetic Mechanisms in Neuropsychiatric Disorders

Recent evidence shows that epigenetic or chromatin remodeling events are critical to cellular differentiation, development and organismal behaviour such as learning and memory. Its role has been investigated recently in several important phenomena in the brain, including neuronal differentiation, neurodegeneration, memory formation, circadian rhythm, seizure, drug addiction and mood disorders [2]. It appears that epigenetic mediated transcriptional dysregulation is hallmark in most of the disorders affecting brain and behaviour [2,15,16,17].

Epigenetic mechanisms and their contribution to the long-lasting forms of plasticity observed in the adult mammalian brain has only recently become an area of major interest in neuroscience [2,15]. The alteration in histone modifications and chromatin remodeling in brain in response to chronic cocaine, stress and antidepressant appear quite stable, lasting over a month at least. However, there are instances where a repressed state of a gene (BDNF) can be quickly reversed by neuronal depolarization via phosphorylation of a chromatin remodeler MeCP2 [cf. 20]. Thus, epigenetic modifications in neuronal circuitry can be pretty quick as well, in response to neuronal stimulation, as well as quite slow taking weeks in response to chronic stress events or psychotropic drugs [20]. The epigenome is thus dynamically responsive to synaptic activity and serves as a link between experience, genetic predisposition, and changes in neural function. Chromatin modifications are quite dynamic and change in a cell-specific manner during development and in behaviour, i.e. in response to external stimuli as well as environmental perturbations such as traumatic experience, ischemic insults, drugs of abuse, chronic stressful situations [15]. Here are the studies done in last 5-6 years that implicate the eepigenetic dysregulation of transcription in etiopathology of a number of disorders affecting brain.

Psychotic Disorders

In schizophrenia and bipolar disorder patients with psychosis [21], DNMT1, an epigenetic modulator, was found upregulated in GABAergic medium spiny neurons of the cerebral prefrontal cortex. In yet another study an increase in DNMT1 levels, along with a decrease in reelin and glutamic acid decarboxylase 67 (GAD67), has been shown in GABAergic medium spiny neurons of the caudate and putamen nuclei from schizophrenia patients [21].

Neurodevelopmental Disorders

Rett syndrome and related disorders grouped together as Autism Spectrum Disorders (ASD) also appear to be the consequence of epigenetic dysregulation of genes in early developmental stage. Post mortem brain samples from several autistic individuals revealed monoallelic or highly skewed allelic expression of GABAA receptor subunit (GABR) genes, unlike biallelically expression in control brain samples [22]. There is suggestion that ASDs might be due to stalled brain development as a result of epigenetic dysregulation and so, de-repression of epigenetically repressed genes could be the answer therapeutically. Mutation of MeCP2 gene, causes Rett syndrome, an X-linked neurodevelopmental disorder mainly affecting girls. The gene codes for an epigenetic protein that binds to 5-methylcytosine residues and causes transcriptional repression of bound genes [23]. In a landmark study using transgenic mouse approach, conditional over expression of MeCP2 postnatally in MeCP2 deficient mice helps in partial rescue of Rett like features.

Epigenetic silencing and loss of expression of the fragile X mental retardation 1 (FMR1) gene due to expansion of a CGG repeat in its 5'-untranslated region results in Fragile X syndrome [cf. 12].

Mental retardation, where low histone acetylation is the hallmark, also appear to be due to epigenetic disturbance in early developmental stage causing change in gene transcription pattern in various brain areas [16]. It might help to develop drugs that can work at epigenetic level and perturb or restore the stalled neuronal and synaptic circuitry. This might significantly improve the quality of life for patients suffering from neurodevelopmental disorders, and their families. It is to be noted that HDACi has proved effective in improving the memory in mouse with mutation in CBP, a HAT.

Addiction

The addiction associated neural adaptations in reward circuitry has been extensively studied using epigenetic tools. Most of the contributions to this line of scientific investigation have been by Nestler and collegues. Recent work from his group [2,3,5,6] has focused on understanding the molecular mechanisms that underlie long-lasting neuroadaptations in response to chronic exposure to drugs of abuse such as cocaine.

Chronic exposure to drugs of abuse cause long-lasting changes in the brain's reward circuit as well as associated memory circuits, resulting in the addiction phenotype. Nucleus accumbens (NAc)- the ventral striatum, is a critical center in reward circuitry where drug-induced neuroplastic changes and alteration in gene expression, largely driven by dopaminergic signalling, can be seen [2,3,6,15]. The addiction associated changes at cellular, molecular and behavioural level persist even after months of abstinence and delta FosB induced chromatin remodelling events in NAc appear to be the underlying mechanisms [2,6,15]. Acute cocaine drives the expression of Fos family genes in NAc within minutes of administration and this is associated with rapid and transient increase in H4K5 acetylation and Ser10/K14 H3 phospho-acetylation at the c-fos promoter [2,15]. CBP, with its intrinsic HAT activity, plays role in tcocaine's behavioural response [15]. Cocaine-induced chromatin remodeling is behaviorally relevant as systemic or intra-NAc administration of HDAC class I and II inhibitors such as butyrate and TSA, and class III HDAC Sirt1/2 activator resveratrol, or mice deficient in HDAC5, potentiates behavioral responses to cocaine. On the contrary, viral-mediated overexpression of HDAC4 and HDAC5, but not HDAC9, in the NAc and administering class III HDAC inhibitor sirtinol has the opposite effect [2,5,6] suggesting some specificity of HDAC action. Moreover, CBP-deficient mice show reduced cocaine-induced

locomotor activity [15].

Chronic cocaine treatment upregulates many genes in NAc, like FosB, Bdnf and Cdk5, genes implicated in persistent neuroadaptations. The repeated cocaine resulted in induced H3 acetylation on the promoter region of these genes to increase their transcription [2]. The transcription factor (TF) delta FosB and CREB are implicated in the development of addicted state in chronic drug treatment and recent efforts [6] helped in uncovering genome-wide cocaine targets regulated by delta FosB and CREB and the TF-induced histone acetylation and methylation changes,utilizing the power of ChIP on chip (Chromatin Immunoprecipitation couples to microarray) technique. This landmark study shows, for the first time, how chronic cocaine affects brain reward pathway inducing dysregulation of hundreds of gene activity, to cause addiction phenotype. This landmark genome wide study gave an insight into the epigenetic regulatory mechanisms underlying neural adaptations to chronic cocaine and uncovered targets to work on for developing drugs to treat addiction [6,24].

There is evidence that epigenetic regulatory mechanisms are also implicated in chronic ethanol addiction [15].

Depression and Related Mood Disorders

Chronic stressful stimuli induce depression, post traumatic stress disorder and related mood disorders in mouse models. Like in addiction, in mood disorders too reward circuitry is severely affected. Using psychosocial stress (a model of depression) Nestler and collegues [2,4,9] show how depression like phenotype in mice arise due to changes in chromatin around the regulatory regions of BDNF in hippocampus, and at the promoters of hundreds of genes in NAc, as a result of alteration in transcriptionally repressive methyl marks on histone H3 K9 and K27. Thus, epigenetic mechanisms are also implicated in stress-induced neuroadaptations underlying mood disorders. One of the transcription factors critical in neuroadaptations in accumbens, and altered behavioural changes, is CREB (cyclic AMP response element binding protein). Mapping CREB regulatory network in NAc and comparing the global epigenetic repressive methylation marks (H3K9,27) show how CREB mediated and CREB-independent dysregulation of hundreds of genes cause the depression and related disorders [9].

Interestingly, imipramine, a commonly prescribed tricyclic antidepressant, restored the mood disorders by reversing the epigenetic marks on many of the dysregulated gene promoters [9]. In another mouse model of social isolation-induced depression, anxiety and related mood disorder, similar epigenetic mechanism, i.e., repressive H3 methylation marks resulted repression of hundreds of gene promoters were also found [9]. In a recent paper from Nestler group [7] fluoxetine, another commonly prescribed SSRI antidepressant, has been shown to restore the mood by normalizing the the dysregulated gene expression in accumbens, similar to what an HDAC1/2 inhibitor MS-275 did. So, antidepressant drugs can act at epigenetic level and HDACi has antidepressant action.

Na butyrate, an HDAC inhibitor, also restores the anxiety and related mood disorder affecting a neurotrophic factor BDNF [25]. BDNF dysregulation has also been shown in hippocampus of defeat-induced depression model by H3 K27 methylation on its promoter III and IV resulting in downregulation of BDNF transcripts III and IV. Interestingly, antidepressant imipramine caused restoration of mood by increasing the H3K9 acetylation on the BDNF promoters [4]. In neurogenic niche of the hippocampus, in subgranular zone of the dentate gyrus, phosphoacetylation of H3 affects cfos gene function following stress induced in forced swim test [26]. Thus, the BDNF gene regulation in hippocampus by epigenetic or chromatin remodeling events,

and global changes in epigenetic mechanisms at the regulatory regions of hundreds of genes, emphasize that the epigenetic mechanisms are involved in etiopathology as well as therapy of complex neuropsychiatric disorders [15].

Stress, anxiety and related mood disorders have been shown to impair neurogenesis and adult neurogenesis provides a cellular mechanism to generate lasting changes in existent neuronal circuitry. Adult neurogenesis is known to be critical in neuronal functions such as learning and memory and has been implicated in the and pathogenesis and treatment of depression [reviewed in 27]. Recent evidence suggests that neural progenitors or stem cells and newborn neurons in the neurogenic niche of adult brain, appear to be modulated by epigenetic mechanisms which may influence their turnover, survival, phenotypic maturation, integration and function in the adult brain.

The epigenetic changes caused by HDACi such as valproate, alters gliogenic destined neural progenitors to neurogenic fate in sub granular zone (SGZ) of mouse hippocampus [27]. Inhibition of HDACs caused a de-repression of a neurogenic transcription factor NeuroD which induced the epigenetic regulation of gene expression characteristic of neuron. In the same study it was also shown that a non-coding RNA can induce neurogenic fate in the SGZ progenitors [27]. In another interesting study [28], oxidative stress and inflammation appear to cause bias in neural progenitor cells' differentiation toward astrocytes by modulating activity of Sirt1, a class III HDAC, in redox-dependent manner, in another neurogenic niche in adult brain, SVZ (sub ventricular zone). This change in Sirt1 activity dependent on redox state, acts like a metabolic epigenetic switch. Likewise, few more studies highlight the critical role epigenetic regulatory mechanisms play in stem cell biology.

However, the details of various epigenetic regulatory mechanisms are still lacking in neural stem cell biology and neurogenesis. Understanding the epigenetic mechanisms which control neuronal stem cells has immense relevance to identification of drug targets that could promote the process of adult neurogenesis thus facilitating possible repair of neuronal damage and holds promise for either adjunct or stand alone treatment modalities. Recent investigations have shown that cell fates of the progenitors can be reset by the alteration of epigenetic marks or the histone code, and the converted or reprogrammed cells are functional when transplanted *in vivo* [29]. It appears that epigenetics might provide novel technology for improving regenerative medicine.

Neurodegenerative Diseases

Recent findings that HDAC inhibitors ameliorate the mouse model of various neuro-degenerative disorders suggest implications of epigenetic mechanisms in these disorders [cf. 16]. It speaks of the role of histone acetylation-deacetylation and gene regulation in various brain circuitry. In heterozygous CBP (CREB binding protein, with HAT activity) mice, the long term memory is severely affected due to less histone acetylation. Administration of HDAC inhibitors like SAHA and TSA improved the performance of these mice in learning and memory related behavioural tasks, using two different mouse models where CBP was deficient in brain. The following paragraphs review the studies showing evidence that HDAC inhibitors can slow down the neurodegeneration and improve the overall pathological symptoms in Huntington's, Parkinson's, Alzheimer's, Spinal muscular atrophy, Amyotrophic lateral sclerosis and stroke.

Huntington's Disease (HD)

HD, an inherited, late onset autosomal-dominant neurodegenerative disorder characterized by progressive motor, psychiatric and cognitive decline due to

polyglutamine (CAG) expansion in the 5' coding region of the huntingtin (htt) gene. Since Htt is a transcription factor, transcriptional dysregulation of many of the target genes appear to cause the HD pathogenesis. Mutant huntingtin results in the formation of aggregates of mutant polyQ protein which bind and functionally impair coactivators such as CBP. CBP is a HAT and so its impairment causes histone deacetylation, dysregulation of CBP/CREB-mediated gene expression, ultimately resulting in neuronal death in striatum and cortex [cf. 16,17].

HDACi have been shown to rescue lethality and degeneration of photoreceptor neurons in a *Drosophila* model of polyglutamine disease. It can also attenuate neuronal loss, improve motor function and extend survival in R6/2 mouse model for HD. Even though HD is an inherited disease due to gene mutation, epigenetic drug such as HDACi can reverse the pathophysiology by restoring the functions of many repressed or epigenetically dysregulated genes, the ones affected due to impairment of Htt and CBP [cf. 16,17].

Parkinson's Disease (PD)

Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by selective death of dopaminergic neurons, predominantly in the substantia nigra. The treatment remains elusive as no drug so far to halt the death of dopaminergic neurons. The etiology of PD, which is mostly sporadic, is still not clear. The familial PD can arise as a consequence of deficient neuronal protein, α-synuclein [cf. 16,17].

HDAC inhibitors have recently been shown to ameliorate the progressive neurodegeneration associated with PD [16,17]. The evidence for epigenetic dysregulation in PD came recently in a study using a *Drosophila* model of PD where it was shown that targeting α-synuclein to the nucleus promoted its toxicity while its sequestration in cytoplasm promoted protection. It has also been shown that nuclear α-synuclein binds histones and inactivates HATs including CBP, p300 and P/CAF, resulting in histone hypoacetylation and apoptosis. Treatment with HDAC inhibitors such as sodium butyrate or vorinostat *in vivo* or *in vitro* rescued α-synuclein- induced toxicity [16,17]. Recently, SIRT2, an NAD-dependent class III HDAC that works differently than zinc-dependent HDACs of other classes, appear to be involved in PD. The knockdown of Sirt2 by siRNA reduced the neurotoxic effects of α-synuclein [cf. 17]. Treatment with AGK2, a specific inhibitor of SIRT2, increased α-tubulin acetylation with the formation of large α-synuclein inclusions and rescued dopaminergic neurons from death both in vitro and in a Drosophila PD model [cf. 17]. Thus, both zinc dependent HDACs as well as SIRT2 could be potential drug targets for PD.

Alzheimer's Disease (AD)

AD is also a progressive neurodegenerative disorder characterized by deterioration of memory and changes in personality, leading to dementia. Nearly 2% of the population in developed countries are affected and the prediction is for threefold increase in next 50 years. A common feature in neurodegenerative disorders (HD, PD and AD) is intraneuronal aggregates such as plaques that interfere with transcription and affect the neuroplasicity and cognition. This phenomenon is more documented in HD and PD [16,17]. However, in AD too plaques can cause transcriptional dysregulation [30].The characteristic features of AD neuropathology are accumulation of extracellular β-amyloid (Ab), called plaque, resulting from aberrant processing of normal APP, and intracellular neurofibrillary tangles due to hyperphosphorylation of Tau protein [16,17].

Administration of valproic acid, an HDACi, causes decrease in Ab production in brain of PDAPP (APPV717F) AD transgenic

mice. Although direct demonstration of improvement in synaptic plasticity or cognitive impairment has been awaited, recent investigations by Tsai and collegues [cf. 17] show that HDACi restores histone acetylation status and learning and memory in a mouse model of neurodegeneration. Conditional expression of p25, a Cdk5 activator implicated in neurodegeneration, using inducible system, the investigators show the role of environmental enrichment induced increase in histone acetylation and remodeled chromatin in amelioration of learning and memory, even after significant neurodegeration had occurred in these transgenic mice. Administration of HDACi sodium butyrate significantly improved associative and spatial learning in these mice. This mimicking the effects of HDACi by environmental enrichment emphasize that experience-dependent plasticity acts through epigenetic or chromatin remodeling to ameliorate plasticity and cognitive deficits [cf. 16,17]. In another study HDAC2 overexpression in mice show decrease in dendritic spine density, synapse number, synaptic plasticity, resulting in impairement in memory formation. On the contrary, HDAC2 knock out mice show improvement in memory formation. Further, it has been shown that HDAC2 binds to the promoter and regulate many genes and affects synaptic formation and neuroplasticity in mouse hippocampus [cf.17]. The impairment in memory of HDAC2 overexpressing mice was ameliorated by treatment with vorinostat, the HDACi.Treatment with another HDACi, 4-Phenylbutyrate, to Tg2576 mouse model of AD, reversed spatial memory deficits by restoringTau hyperphosphorylation in hippocampus but without affecting Ab levels [cf. 16,17]. 4-phenylbutyrate treatment increased the expression of GluR1, PSD95 and MAP2 by restoring the significant loss of histone H4 acetylation in the cortex, emphasizing that the underlying neuroprotective mechanisms involve normalization of transcriptional dysfunction via epigenetic mechanisms. Daily administration of a relatively low dose of valproic acid (30 mg/kg, i.p.) in the APP23 transgenic mouse model of AD, reduced Ab plaque number and ameliorated memory deficits [cf. 16,17]. Treatment of triple transgenic mouse model of AD with nicotinamide, a class III HDACi protected against memory impairments and decreased Tau pathology but without affecting Ab load or production [cf. 16,17]. Thus, class III HDACs also seem to be involved in the pathology of AD.

Spinal Muscular Atrophy (SMA)

SMA, an autosomal-recessive inherited motor neuron disease and leading cause of infant mortality, is characterized by weakness and atrophy of voluntary muscles caused by degeneration of α-motor neurons in the anterior horn of the spinal cord. This hereditary disease is due to homozygous deletion of the SMN1 gene which encodes the full-length survival motor neuron protein. A related gene, SMN2, located within the same chromosomal locus, is ubiquitously expressed and encodes an unstable SMN protein devoid of C-terminal residues. Although SMA patients defective in SMN1 gene carry at least one copy of SMN2, the amount of functional SMN protein encoded by SMN2 is not enough to combat progressive degeneration of motor neurons. It has been shown that the severity of SMA is inversely correlated with SMN2 gene copy number and SMN protein expression [cf.17].

Epigenetic mechanisms appear to be implicated in this neurodenerative disease too. There is evidence that difference in severity of the disease is due to epigenetic repression of SMN2 gene by DNA cytosine methylation at CpG islands and, treatment with HDACi vorinostat and romidepsin help to bypass SMN2 gene silencing [31]. More studies with various HDACi such as sodium butyrate 4-phenylbutyrate, valproic acid, M344,

vorinostat, TSA and romidepsin, show increase in SMN2 mRNA and protein levels *in vitro*, as well as *in vivo* in transgenic mouse model of the disease. Interestingly, the SMN2 promoter associates with HDAC1 and HDAC2, but not with HDAC3–5 [cf. 17], indicating that differentHDAC isoforms play selective roles in regulating SMN gene expression. Administration of sodium butyrate to a transgenic mouse model (Smn1-/- SMN2) increased the level of SMN protein, ameliorated symptoms, and increased their lifespan [cf.17]. Oral administration of valproic acid in drinking water suppressed the degeneration of spinal motor neurons and muscular atrophy, improved motor function, motor-evoked potential and neuromuscular junction formation [cf. 17]. This was associated with increased levels of SMN mRNA and protein in spinal tisues, and also increased levels of Bcl-2 and Bcl-XL, the anti-apoptotic proteins. In a recent pilot trial 4-phenylbutyrate and valproic acid increased SMN mRNA and or protein level in the leukocytes of SMA patients and improved their functional muscle performance [cf. 17].

Amyotrophic Lateral Sclerosis (ALS)

ALS, an adult-onset neurodegenerative disease characterized by progressive loss of motor neurons in the brain, brain stem, and spinal cord cause general weakness, muscle atrophy, paralysis, and eventual mortality within five years of disease onset [cf. 17]. Most of the cases are sporadic, with only about 10% termed as familial form of ALS. Approximately 20% of the familial ALS appear to be due to gain-of-function mutations in the gene encoding Cu/Zn superoxide dismutase1 (SOD1), a key antioxidant enzyme. Mutant Cu/Zn SOD1 mice exhibit ALS-like features such as intracellular aggregates of SOD1 in brain and spinal cord, behavioural abnormalities, and premature death. Till now, no causative mutation has been found in sporadic ALS and recent evidence implicates epigenetic mechanism and transcriptional dysregulation in predominant sporadic ALS (SALS) [17]. Morahan *et al* [32] mapped DNA methylation across whole genome in brain DNA samples of 10 SALS patients and 10 neurologically-normal controls. Significant alteration in DNA methylation in SALS brain samples was found at 23 genes. Interestingly, the genes were ones involved in calcium homeostasis, neurotransmission, oxidative stress and cell death pathways were significant.

Treatment with 4-phenylbutyrate starting before or shortly after symptom onset resulted in extended survival and improved symptoms in SOD1/G93A transgenic mice [cf. 17]. Co-administration of phenylbutyrate and riluzole, the only FDA-approved drug for treating ALS, to the same transgenic mice, was more effective than either drug alone in extended survival and improving disease conditions [cf. 17]. Similarly, valproic acid and lithium produced greater and more consistent benefits than valproic acid alone in delaying the onset of disease symptoms, prolonging lifespan, and decreasing neurological deficits. Combined treatment with 4-phenylbutyrate and AEOL 10150, a catalytic antioxidant, also had an additive effect on survival period, with associated attenuation in oxidative damage in the spinal cord of the ALS mice [cf. 17]. Thus, it seems that multiple molecular mechanisms, including epigenetic ones, are implicated in the pathophysiology of ALS.

Ischemic Stroke

Heart attack and stroke is now global epidemic and according to the World Health Organization report of 2005, 15 million people suffer stroke worldwide each year. Of these, 5 million die and another 5 million are permanently disabled. Most of the drugs in Stage III clinical trials have failed [16]. It is critical to dissect the molecular mechanisms so that novel therapeutics can be developed for neuroprotection, rescue and repair of neurons under inflammation. Brief ischemic insults

have been shown to induce selective, delayed death of hippocampal CA1 neurons resulting in severe cognitive deficits. In global ischemia, aberrant accumulation of REST/NRSF, a transcriptional repressor RE1 silencing transcription factor (also termed as NRSF) occur in vulnerable hippocampal neurons, resulting in silencing of target genes essential for neuronal function [33]. Recent findings that REST recruits CoREST, G9a, a methyl transferase, and MeCP2 to promoters of target genes and induce epigenetic silencing of target genes in vulnerable hippocampal neurons implicates REST-dependent epigenetic remodeling in the pathogenesis of global ischemia [34]. Normally, the activity of REST/NRSF is very high during embryogenesis as it plays critical role in terminal neuronal differentiation. In neural progenitors and non-neural cells high activity of REST causes repression/silencing of a number of neuron-specific genes, allowing nonneuronal transcripts to be expressed. As the progenitors differentiate into neurons, REST induces a set of epigenetic modifications resulting in gene expression patterns that distinguish neuronal from non-neuronal cells. It is to be noted that dysregulation of REST and its target genes is implicated in the pathogenesis of Alzheimer's disease, Huntington's disease, epilepsy, Down syndrome and X-linked mental retardation too [cf. 16].

HDACi appear promising for therapeutic intervention in stroke associated neurodegeneration. Administration of an HDACi SAHA to mice at 0 and 6 h after induction of ischemic stroke in middle cerebral artery occlusion (MCAO) model, reduced infarct volume, prevented H3 deacetylation, and this was associated with increased expression of neuroprotective genes Bcl-2 and Hsp70 [cf. 16]. In another study, aberrant DNA methylation was observed in the brains of mice subjected to mild ischemic brain injury by the MCAO model; administration of the 5-aza-2'-deoxycytidine, a demethylating agent, and trichostatin A, an HDACi, conferred stroke protection in mice brain subjected to mild, but not severe ischemic damage [cf. 16]. In an intracerebral hemorrhagic model of stroke, administration of valproic acid caused HDAC inhibition, increased H3 acetylation and transcriptional activation of many genes; also repressed proinflammatory genes like Fas-L, IL-6, and MMP-9 [cf. 17]. However, the identity of the HDAC isoform/s involved in HDACi-mediated neuroprotection remains unclear. So, in neuroinflammation and neurotrauma associated with hypoxic conditions such as ischemic induced brain damage HDACi show not only protective role, slow down the neuronal damage and induce the generation of new neurons in neurogenic niche [cf. 16,17].

Epigenetic Therapeutics

Although the underlying epimutations (epigenetic changes that cause or predispose an organism to a disease) remain unclear in most of these complex neuropsychiatric diseases, many epigenetic therapeutic agents developed recently show usefulness in pre-clinical studies. Some of these HDACi compounds are progressing through clinical trials or have become approved treatments for particular disease conditions [cf. 12].

DNA Methyltrasferase (DNMT) Inhibitors

To de-repress the downregulated or silenced genes two classes of DNA methylation inhibitors were synthesized, nucleoside analogs and nonnucleoside analogues. Nucleoside analogues such as 5-azacytidine (5-aza-CR) and 5-aza-2-deoxycytidine (5-aza-CdR, decitabine), synthesized in the 1960s have been extensively studied and inhibit DNA methylation at micromolar dose and induce cellular differentiation. Despite being toxic at high doses, low-dose decitabine has a 49% overall response rate and a 20% complete response

rate in elderly, high-risk myelodysplastic syndrome (MDS) patients [cf. 12]. However, their instability in aqueous solutions and susceptibility to deactivation by cytidine deaminase put limitation on the use of original analogues. But, the recently discovered methylation inhibitor, Zebularine [1-β-D-ribofuranosyl-2(1H)-pyrimidinone] which inhibits cytidine deaminase and is stable in aqueous solution, can be used orally, and shown to preferentially kill cancer cells. Unfortunately, its clinical development is also limited due to higher dosage and poor bioavailability, as observed in rats, mice, and monkeys [cf. 12]. One question about all nucleoside analogues which need to be resolved is whether their effects are due to demethylation or the cytotoxic effects caused by depletion of DNMTs. Further modification will require in order to reduce the toxicity associated with this drug class.

Nucleoside analogues are made up of either a ribose or deoxyribose moiety fused to a modified cytosine ring and upon phosphorylation by kinases the nucleoside gets converted to a nucleotide and, once incorporated into DNA, it prevents methylation. Under normal circumstances, DNMTs flip cytosine rings out from newly synthesized DNA, forming an intermediate complex and incorporating a methyl group from *S*-adenosyl-L-methionine (Ado-Met) onto the C5 position; the enzyme is then released from this complex. The presence of a modification at this position causes the enzyme to become trapped in a DNA-protein adduct, preventing further methylation of progeny DNA by depleting genomic DNMT stores [cf. 12].

Another DNA methylase inhibitor class, non-nucleoside analogues, are small molecules that either bind the active site of the enzyme DNMTs or prevent expression of the enzymes without incorporating into DNA. These inhibitors are somewhat less toxic than nucleoside analogues. A small molecule, RG108, inhibits the human DNMT1 activity by binding to the catalytic pocket. It has shown concentration-dependent demethylation of genomic DNA with little cytotoxicity but has not entered clinical trials so far. Recently, in a mouse contextual memory model RG108 was shown to directly inhibit DNMT enzyme activity and it had similar effects on memory as that of zebularine., i.e. both blocked memory consolidation. Few other non-nucleoside analogues such as hydralazine, the anesthetic procaine, and the antiarrhythmic procainamide have DNA demethylation activity and being studied in Phase II [cf. 12].

MG98, the non-nucleoside analogue that works at DNMT expression level, an antisense oligonucleotide inhibitor of human DNMT1, inhibits the translation of DNMT1 mRNA by targeting its 3-untranslated region. However, its partial response in a patient with renal cell carcinoma prevented it from being undertaken in Phase II [cf. 12].

Some naturally occurring promising DNA methylation inhibitor, psammaplins, which are bromotyrosine derivatives extracted from the sponge *Pseudoceratina purpurea*, have the ability to inhibit both histone deacetylases (HDACs) and DNMTs. Psammaplin A, in particular, has potent histone deacetylase inhibitory activity (61). Another one with promise is green tea extract EGCG [(-) epigallocatechin-3-gallate], a competitive inhibitor of DNMT, showing the potential for anticancer therapy (62); neither psammaplins nor EGCG have entered clinical trials, however [cf. 12].

Histone Deacetylase (HDAC) Inhibitors

The histone deacytylase inhibitors (HDACi) comprise a large, diverse class of drugs and each member having a different functional group with HDAC-inhibitory activity. HDACi block the activity of HDACs resulting in increased acetylation of lysine residues on histone tails and thus restoring gene function. HDACi can also cause

acetylation of non-histone proteins, like transcription factors, tumor suppressors. Various HDACi show promising antitumor activity in phase I, II and III clinical trials, with variable efficacy [35]. These were well tolerated and act by inducing cell cycle arrest, apotosis, and cell death, by derepressing many silent genes. The FDA approval for SAHA (suberoylanilide hydroxamic acid) for the treatment of cutaneous T-cell lymphoma in October, 2006 caused lots of excitement that led to the development of a variety of HDACi. Now, a large number of combination therapy involving HDACi have shown promise for the treatment of a variety of malignancies, such as pancreatic cancer, prostate cancer, leukemia, a number of difficult to treat cancers, as well as of non-cancer disorders, such as neurodegenerative disorders and mood disorders including bipolar disorder and depression [12,18,36]. In addition, HDACi alone have shown much promise in the treatment of various neurological and psychiatric disorders, such as Parkinson's disease, Alzheimer's disease, Rubinstein-Taybi syndrome, Rett syndrome, Friedreich's ataxia, Huntington's disease, multiple sclerosis, inflammation associated with traumatic brain injury, anxiety, and schizophrenia. The diverse utility and wide range of mechanistic actions shown by HDACi warrant the development of more selective HDACi and better combination therapies [18,36].

At present, there are four main classes of HDACi both isoform-nonselective and selective have been developed. They are synthetically derived as well as from natural sources (reviewed in 12].These are: short-chain fatty acids (SCFAs), hydroxamic acids, benzamides, and cyclic tetrapeptides. Most of these are relatively nonselective HDAC inhibitors; however, few isoform selective inhibitors have also been discovered.

Short-chain Fatty Acids (SCFAs)

These drugs easily pass through the bood-brain barrier (BBB) and so have been in use since long to treat various neuropsychiatric disorders. The original SCFAs are valproate, or valproic acid, with mood-stabilizing and anticonvulsant or antiepileptic properties, that bind to the active site of the enzymes and inhibit class I and class IIa HDACs, but not of class IIb [cf. 12]. Sodium butyrate and 4-phenylbutyrate also pass through BBB and inhibit most class I and II HDACs. Butyrate fails to inhibit HDAC6 the class IIb HDAC, as the acetylation levels of its substrate α-tubulin[11], remain unaffected by sodium butyrate treatment. Butyrate is naturally produced in the colon and appear to be the most potent antiproliferative drug in this SCFA class [cf. 12].

Unlike newly discovered hydroxamates, SCFAs, are relatively weak inhibitors of HDACs, as their efficacy is in the millimolar range. They appear to have pleiotropic effects on other enzymes, have low specificity and low bioavailability. Valproate, in combination with other drugs shows more tumoricidal activity; while decitabine has shown promise in Phase I and II studies against advanced leukemia, with all-*trans* retinoic acid has shown positive outcome in myelodysplastic syndrome and relapsed or refractory AML, and so on [cf. 12]. Tributyrin, a sodium butyrate analogue, inhibits proliferation and induce differentiation and apoptosis of leukemia cells *in vitro* [cf. 12]. Once the molecular mechanisms become clear, novel SCFAs with strong efficacy might emerge alone or in combination, in the treatment of various leukemias and other non-cancer disorders. All of the above SCFAs have been effective in ameliorating the symptoms of neuropsychiatric diseases in mouse models.

Hydroxamates

Hydroxamates such as Trichostatin A (TSA) and suberoylanilide hydroxamic acid (vorinostat, also known as SAHA) are very promising potent HDACi effective at

micromolar or even lower range. The hydroxamate moiety appears to bind the zinc ion at the HDAC active site to inhibit the enzyme activity of most of the zinc-dependent HDACs including HDAC6 [cf. 12]. TSA, an antibiotic derived from *Streptomyces hydroscopicus*, and SAHA, a synthetic anticancer agent, have been quite promising as antitumor agents in undergoing clinical trials. SAHA (Vorinostat, Zolinza) already got an FDA approval in 2006 for the treatment of persistent cutaneous T cell lymphoma (CTCL). After SAHA, some newer hydroxamic acid containing HDACi are progressing toward clinical studies; these are LAQ824 and PDX-101. PDX-101, a sulfonate that inhibits HDACs at nanomolar concentrations, has entered Phase I clinical trials. LAQ824 in combination with 13-*cis*-retinoic acid, showing antitumor activity in human malignant melanoma has entered clinical trial [cf. 12].

TSA and SAHA, permeable to the blood–brainbarrier (BBB), have shown remarkable effects in pre-clinical neurological and psychiatric disease models. Now the clinical trials are being planned for these potent and less toxic hydroxamates HDACi in various neuropsychiatric diseases. SAHA also acts as a protein acetylase and interfere with the function of chaperones and their client proteins through disruption of HDAC6, a tubulin deacetylase [cf. 12].

Benzamides

These HDACi are amide derivatives of benzoic acid, two of which have reached the clinical trial phase, CI-994 (N-acetyldinaline) potent antitumor activity at micromolar range when given orally, and CI-994, is often used in combination with paclitaxel or carboplatin, with anti-tumor response in several Phase I studies [cf. 12].

MS-275, a benzamide having radiosensitizing properties on human prostate carcinoma and glioma cell lines. MS-275 is also in Phase II clinical trials for the treatment of various tumor types. It prefrentially works against HDAC1, compared with HDAC2, 3 and 9 [cf. 12].

Cyclic Tetrapeptides

HDAC inhibitorts in this class are cyclic tetrapeptides containing an α-epoxyketone group that allows them to alkylate, thus deactivating, the HDAC catalytic pocket. The epoxyketone moiety can be substituted, as in the case of various analogs of this class where similar functional groups, such as pentafluoroethyl and trifluoromethyl ketones, sulfydryls, or retrohydroxamate, can target the catalytic pocket. The HDAC-inhibitory activity of these compounds depends on the degree of hydration of the electrophilic ketone, which chelates the zinc ion. Depsipeptide, the most promising member of the class has completed several Phase I studies as antitumor agent [cf.12]. Aplidine, a cyclic depsipeptide isolated from the marine tunicate *Aplidium albicans*, and entering phase II for antitumor activity against hematological malignancies and solid tumors [cf. 12]. Trapoxins A and B, other hydrophobic cyclotetrapeptides derived from the fungus *Helicoma ambiens*, cause irreversible HDAC inhibition by binding covalently to the HDAC via its epoxide group. These act at nanomolar concentration, but are toxic [cf. 12]. Apicidin (OSI-2040), a potent antifungal and antiprotozoal metabolite derived from two *Fusarium* species, lacking the terminal α-epoxyketone, has antiproliferative activity against various cancer cell lines [cf. 12].

The cyclic hydroxamic acid containing peptides (CHAPs) that reversibly inhibit HDACs at low-nanomolar concentration are in fact hybrids of trichostatin and trapoxin. The compounds contain a hydroxamate side chain attached to the cyclic peptide core in place of the epoxyketone and change in the methylene chain length can lead to the development of many different CHAPs, each with its own properties [cf. 12].

Recent efforts led to design and synthesis of more selective HDAC inhibitors. MS-275, a synthetic benzamide derivative, preferentially blocks the activity of HDAC1, compared with HDAC2, 3, and 9, and has little activity against HDAC4, 6, 7, and 8 [cf. 12]. It passes the BBB easily and can be administered orally, with no severe side effects. Recent finding [7] shows that infusing MS275 in brain for few weeks reverses the depressive mood disorder by de-repressing hundreds of genes in nucleus accumbens of mice, thus acting as potential antidepressant.

Another specific HDACi Apicidin, a cyclic tetrapeptide, acts mostly against HDAC2 and 3 in the low nanomolar range, sparing HDAC1 or class II HDACs. It has slight inhibitory effect on HDAC8 but in higher nanomolar range [cf. 12]. Romidepsin (FK-228), another cyclic tetrapeptide, has potent inhibitory action against HDAC1 and 2. Tubacin is highly selective for HDAC6, which causes deacetylation of a-tubulin, a microtubule component [37]. Suramin, a symmetric polyanionic naphthylurea, and its structural analogs, have been shown to inhibit the activity of human NAD_+-dependent class III HDACs called SIRT1 and SIRT2 [cf. 17].

Nicotinamide, also known as niacinamide, with good bioavailability, is a precursor of NAD_+ and a competitive class III HDAC inhibitor that can be given orally [cf. 17]. CNS acting drugs can act at epigenetic level. Some of the psychotropics that are in clinical use, such as valproate, a mood stabilizer, fluoxetine and imipramine, antidepressants, have been shown recently to have epigenetic effects in brain, in addition to their commonly understood modes of action. The SCFA valproate can de-repress genes in brain and also in tumors by inhibiting HDACs. The drug can also demethylate DNA both *in vitro* and *in vivo* [cf.12]. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI) antidepressant, can induce genes encoding the epigenetic regulators such as MeCP2 and MBD1, the methyl binding domain proteins (MBDs), by activating the serotonergic (5-HT) system in the adult rat brain. Induction of HDAC2 mRNA accompanied the MBD protein increase, and concomitant decrease in histone H3 in various serotonin projection areas: the caudate-putamen, the frontal cortex, and the dentate gyrus of the hippocampus [38]. Development of depression-like behavior in adult male mice on perinatal exposure to methyl mercury, is reversed by chronic treatment with fluoxetine. This reversal in mood disorder by the antidepressant was due to increase in H3 acetylation at BDNf promoter IV and transcription of the gene [39].

Imipramine, a tricyclic antidepressant, can also restore the depression- like symptoms in defeated mice by de-repressing the *Bdnf* transcript III and IV in mouse hippocampus irepressed after chronic stress. This was achieved by imipramine induced hyperacetylation of histone H3 at *Bdnf* promoters III and IV, and downregulation of HDAC5 [4]. Using chromatin immunoprecipitation and microarray (ChIP-chip) in genome wide study Nestler and collegues show that imipramine could restore mood disorder by reversing the altered repressive H3 methylation marks (H3 K9, K27) on many of the dysregulated gene promoters [9]. Intradermal fluoxetine (a SSRI antidepressant) pellet, and intra accumbens infusion of MS-275 (an HDACi) could also restore the mood disorders in this same social defeat mouse model by reversing the defeat-induced gene expression pattern in the accumbens and it is noteworthy that many of the genes restored by these two diverse drugs were common [7].

Limitations in the Development of Epigenetic Therapeutics

The lack of target specificity is a major concern in the long term use of epigenetic drugs. DNA methylation inhibitors, in addition to demethylate genes that got silenced due to

hypermethylation, might also cause global demethylation. This could then lead to dangerous situation as some of the oncogenes as well as retrotransposons can get de-repressed or activated. Similar situation can arise in the long term use of HDACi which show pleiotropic effects on multiple HDAC species. Since HDACi also appear to demethylate DNA, this can also aggravate situation if some of the oncogenes get activated [12,16,17,18,36].

The HDACi have to be used in combination with other existing drug for treating cancer and other disorders. This might be because of the fact that this is being attributed to their still somewhat low level of success, relative to established drugs. However, in spite of this limitation, these drugs are being tried in refractory or drug-resistant situation where combining these HDACi with the failing drug becomes effective once again. In treatment resistant depression, HDACi together with antidepressant might prove really promising. There is evidence now [25] that butyrate paired with an antidepressant fluoxetine, is much more effective in restoring the depression phenotype in mouse, than as single drug. This action of HDACi is what is being dubbed as 'genome softener,' [40] that will increase the plasticity of the genome to reinvigorate treatment response in refractory neuropsychiatric diseases.

Future Prospects of Epigenetic Therapeutics

Despite the promising results demonstrated by many novel HDAC and DNMT inhibitors (epigenetic drugs), considerable improvement is still needed. Overall, epigenetic therapeutics appear to be quite promising in the treatment of complex diseases such as cancer and many others, including the neuropsychiatric ones.

The efforts are on to increase the specificity of epigenetic drugs by better understanding the structure and function of target molecules. The newly emerged advanced, high throughput technology like ChIP on chip, ChIP-seq, MeDIP-seq will help in mapping entire epigenome in normal and diseased tissues, which will identify etiological epimutations (epigenetic changes that can cause or predispose for the disease) that can serve as biomarkers of a disease. This will lead to screening of the combinatorial small molecule library in search for better epigenetic therapeutics with potential to significantly improve the treatment of complex diseases.

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Genetics of Diabetes

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Introduction

Diabetes mellitus belong to diverse group of metabolic disorders characterized by persistent hyperglycemia. The two most common forms of diabetes are type-1 diabetes which is also known as insulin-dependent diabetes and type-2 diabetes i.e. non-insulin-dependent. It is long known that both type-1 and type-2 diabetes are influenced by environmental as well as genetic factors. The other forms of Diabetes mellitus are maturity onset diabetes in the young children (MODY), and diabetes due to mutations in mitochondrial DNA. Sometime the patients do not correspond to the clinical characteristics of type-1 or type-2 diabetes then one should take into consideration the other forms of diabetes which often go undiagnosed in spite of significant prevalence.

Diabetes affects health in various ways. There is an abnormal metabolism of glucose which results into hyperglycemia and hyperlipidaemia. These lead to long term complications which are associated with the disease and are responsible for morbidity, disability and premature death in young adults. These are cardiovascular, peripheral vascular, ocular, neurologic and renal abnormalities. Sometimes the disease is associated with reproductive complications causing problems for both mothers and their children. The incidence of the disease is increasing due to various factors, hence, causing very significant social, psychological and financial burden all over the world.

Type 1 Diabetes

Epidemiology

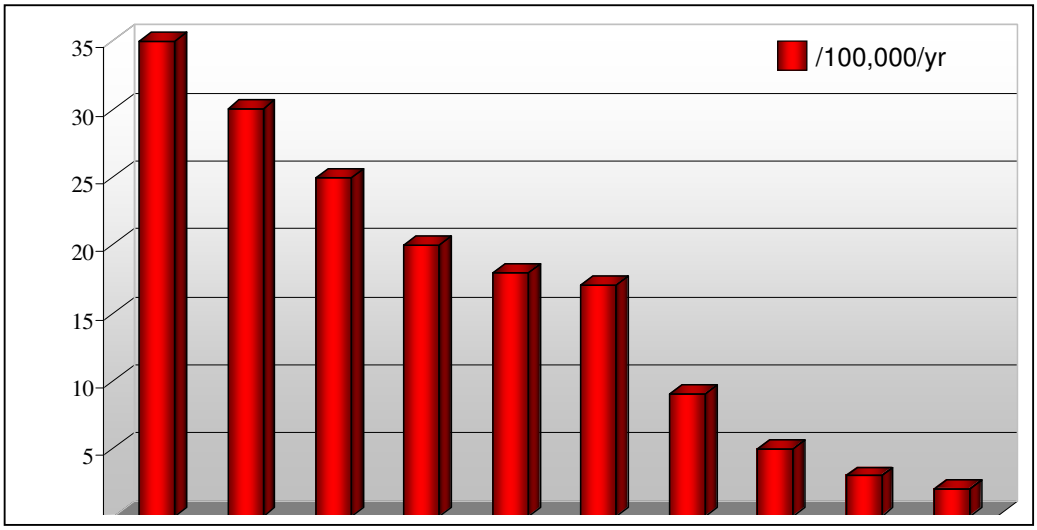
Monitoring the incidence of type 1 diabetes may provide important information about the etiology of the disease. Type 1 diabetes mellitus (T1DM), is an autoimmune disorder (Gariani *et al*., 2009). It accounts for 10% of diabetes diagnosed, affecting approximately 1.4 million people in the United States (US) and 10–20 million worldwide (Atkinson & Maclaren 1994; The DIAMOND Project Group 2006; Gale 2002). In the United States, 30,000 new cases occur annually and 40% of patients diagnosed are under the age of 20 (The DIAMOND Project Group 2006; Gale 2002; LaPorte *et al*. 1995). Recent, studies suggest that the incidence of T1DM may be on the rise and the increasing incidence in younger children is of the greatest concern.

Incidence rates of pediatric T1DM vary widely throughout the world. Onkamo *et al.*, (1999) reviewed pooled data from 37 studies (from 1960 to 1996), and observed an overall 2.8% to 3.0% per year global increase in the incidence of T1DM (Onkamo *et al*., 1999). However, a study by Kostraba *et al*., (1992) suggested a slightly negative, but not significant, trend in T1DM incidence in children and adolescents (Kostraba *et al*., 1992). The World Health Organization's DIAMOND study reported incidence rates from over 100 Centers ranging from 0.1/100,000 per year in China and Venezuela to 37.8/100,000 per year in Sardinia and

42.9/100,000 per year in Finland (The DIAMOND Project Group 2006;). A large number of studies have been published supporting the rising incidence of T1DM, especially in the younger age groups (Kavonen *et al*., 2000; Ehehalt *et al*., 2008; Harjutsalo *et al.*, 2008; Weets *et al*., 2007; Green 2001; Schoenle *et al*., 2001; Charkaluk *et al*., 2002; The SEARCH Study Group 2007; Cotellessa *et al*., 2003; Schober *et al*., 2008; Rangasami *et al*., 1997; Gardner *et al.,* 1997; Karvonen *et al*., 1999). One of the most notable and recent study in the United States, includes a population-based study of incidence rates of T1DM from 10 study locations by The SEARCH for Diabetes in Youth Study. The Search Group found an overall incidence of T1DM in children 0–19 of 24.3 per 100,000 person years with the highest rates observed among the 5–9 and 10–14 age groups with rates of 22.9 and 33.9 per 100,000 respectively (The SEARCH Study Group 2007). The incidence of diabetes in various countries is shown below in Figure 1. There is still some speculation as to whether there is an increase in the incidence in the older adolescent groups or not.

While the autoimmune nature of T1DM continues to be under investigation (Sabbah *et al*., 2000; Ziegler *et al*., 1999), the underlying mechanisms responsible for the rise of T1DM, especially in the younger age groups, remain unknown. However, the "accelerator hypothesis" proposed by Wilkin, is one of the more compelling theories (Wilkin 2001; Wilkin 2006A; Wilkin 2006B). This hypothesis suggested that increasing body weight in younger children acts as an accelerator mechanism for an increased risk of developing T1DM. In fact, an inverse relationship was found between age at diagnosis and body mass index (BMI) at diagnosis and at 12 months after diagnosis, as well as weight at diagnosis and weight change since birth. Essentially, the age at diagnosis becomes earlier as children become heavier; suggesting that being overweight accelerates insulin resistance, leading to the development of T1DM in genetically-predisposed individuals.

Figure 1: T1D Incidence Rates Worldwide



FIN = Finland, SAR = Sardinia, SWE = Sweden, NOR = Norway, US-WI = US-Wisconsin, US-PA = US-Pennsylvania, ITA = Italy, ISR = Israel, JAP = Japan, CHI = China

Chronic childhood disorder more prevalent than T1D is asthma. More children

with beta cell autoantibodies, a hallmark of T1D, are being diagnosed with the T1D around the world each year. Although the peak age at onset is at puberty, T1D can also develop in adults. Epidemiologic studies have revealed no significant gender differences in incidence among individuals diagnosed before age 15 years (Kyvik *et al*., 2004). However, after age 25, the male to female incidence ratio is approximately 1.5. There is also a notable seasonal variation in the incidence of T1D in many countries, with lower rates in the warm summer months, and higher rates during the cold winter (Dorman *et al*., 2003).

Environmental Risk Factors

The epidemiological patterns described above suggest that environmental factors also contribute to the etiology of the T1D. In particular, the recent temporal increase in T1D incidence points to a changing global environment rather than mere variation in the gene pool, which requires the passage of multiple generations. Twin studies also provide evidence for the importance of environmental risk factors for T1D. T1D concordance rates for monozygotic twins are higher than those for dizygotic twins (approximately 30% vs. 10%, respectively) (Hirschhorn, 2003). However, most monozygotic twin pairs remain discordant. Thus, T1D cannot be completely genetically determined.

Environmental risk factors are thought to act as either 'initiators' or 'accelerators' of beta cell autoimmunity, or 'precipitators' of overt symptoms in individuals who already have evidence of beta cell destruction. They may function by mechanisms that are directly harmful to the pancreas, or by indirect methods that produce an abnormal immune response to proteins normally present in the cells. The T1D environmental risk factors that have received most attention are viruses and infant nutrition.

Enteroviruses, especially Coxsackie virus B (CVB), have been the focus of numerous ecologic and case-control studies (Dahlquist *et al*., 1998). CVB infections are frequent during childhood and are known to have systemic effects on the pancreas. Recent prospective studies are helping to elucidate the role of viruses to the etiology of T1D. For example, enteroviral infections occurring as early as *in utero* appear to increase a child's subsequent risk of developing the disease (Dahlquist *et al*., 1995, Hyoty *et al*., 1995). Other viruses, including mumps (Hyoty et al., 1993), cytomegalovirus (Pak et al., 1988), rotavirus (Honeyman et al., 2000) and rubella, (McIntosh and Menser, 1992) have also been associated with the disease.

Another hypothesis that has been the subject of considerable interest relates to early exposure to cow's milk protein and the subsequent development of T1D. The first epidemiologic observation of such a relationship was by Borch-Johnsen et al., who found that T1D children were breast-fed for shorter periods of time than their non-diabetic siblings or children from the general population (Borsh-Johnsen et al., 1984). The authors postulated that the lack of immunologic protection from insufficient breast-feeding may be responsible for the increased risk for T1D later during childhood. It was also postulated that shorter duration of breast feeding may indirectly reflect early exposure to dietary proteins that stimulate an abnormal immune response in newborns. Most recently it has been hypothesized that the protective effect of breast-feeding may be partially due to its role in gut maturation (Kolb and Pozzilli, 1999; Harrison and Honeyman, 1999; Vaarala, 1999). Breast milk contains growth factors, cytokines, and other substances necessary for the maturation of the intestinal mucosa. Breast-feeding also protects against enteric infections during infancy, and promotes proper colonization of the gut. Interestingly, enteroviral infections can also interfere with gut immunoregulation, which may explain the epidemiologic associations between viral

infections and T1D.

The role of hygiene in the etiology of T1D is also currently being explored (McKinney et al., 1997; Marshall et al., 2004). It has been hypothesized that delayed exposure to microorganisms due to improvements in standard of living hinders the development of the immune system, such that it is more likely to respond inappropriately when introduced to such agents at older (compared to younger) ages. This explanation is consistent with recent reports indicating that factors such as day care attendance (McKinney et al. 1997), sharing a bedroom with a sibling, and contact with pets are protective against T1D (Marshall et al., 2004). Further studies are needed to determine if improved hygiene can explain the temporal increase in the incidence of T1D worldwide.

Type-2 Diabetes

Type-2 diabetes is a complex metabolic disorder characterized by hyperglycemia and associated with a relative deficiency of insulin secretion, along with a reduced response of target tissues to insulin (insulin resistance). Its metabolic and clinical features are heterogeneous; people with type-2 diabetes range from those of normal weight or underweight with a predominant deficiency of insulin secretion (in whom slowly evolving type-1 diabetes should be considered) to the more common obese person with substantial insulin resistance. (Zimmet et al., 2001).

The genetic determinants of type 2 diabetes are still poorly defined, except in the few people with an early-onset, dominantly inherited form of the disorder (maturity-onset diabetes of the young), in whom specific genetic mutations have been identified (e.g., of the glucokinase gene) (Williams & Pickup 1999).

The metabolic syndrome

Started as a concept rather than a diagnosis (Reaven & Banting 1988), it was realised that insulin resistance is associated with a variety of cardiovascular risk factors, including central adiposity, glucose intolerance, dyslipidaemia and hypertension. The World Health Organization subsequently provided a definition that can be used for individual diagnosis (WHO 1999). As insulin resistance is difficult to determine in routine practice, diagnosis of the metabolic syndrome in people without diabetes requires demonstration of impaired glucose tolerance (IGT), together with two of the following: hypertension, obesity, dyslipidaemia (hypertriglyceridaemia or low level of high-density lipoprotein cholesterol), or microalbuminuria. The increase in cardiovascular risk is associated with IGT is not as great as that associated with diabetes itself, but nevertheless remains substantial (Zimmet et al., 2001).

Epidemiology

It is calculated that worldwide there are now 150 million people with diabetes, and that this number will rise to 300 million by 2025 (Zimmet et al., 2001). In Australia, the AusDiab study reported in 2000 that 7.4% of the population aged 25 or over had diabetes (type-2 in 90%), and that about 50% were undiagnosed (Dunstan et al., 2002). Prevalence increases progressively with age, so that more than 20% of the population aged over 60 have type 2 diabetes (Dunstan et al., 2002). The prevalence of type 2 diabetes has more than doubled in Australia since 1981, and the total number of cases has increased threefold (Dunstan et al., 2002). As the prevalence of type-1 diabetes is low in Asian, Indian, Middle Eastern and African populations, type-2 diabetes would constitute well over 90% of diabetes cases worldwide.

Precise figures for the prevalence of the metabolic syndrome are not generally available, but, in Australia, the AusDiab study showed a 16% prevalence of impaired glucose metabolism (IGT or impaired fasting glucose [IFG]) (Dunstan et al., 2002). This suggests

that, in a developed country, for every person with type 2 diabetes probably at least two or more have the metabolic syndrome. Moreover, the AusDiab data, which are probably representative of most developed countries, demonstrate that various cardiovascular risk factors, including hypertension and dyslipidaemia, become progressively worse with progression from normal glucose tolerance to IGT/IFG to diabetes. In addition, polycystic ovary syndrome, which is increasing in frequency with increasing adiposity in the community, is strongly associated with insulin resistance and glucose intolerance (Norman et al., 2001). There are major ethnic differences in susceptibility to type 2 diabetes, which are probably largely genetically determined; people of Micronesian, Polynesian, Aboriginal and Torres Strait Islander, Indian or Chinese background are at substantially increased risk (Zimmet et al., 2001).

While there is good evidence for a strong genetic contribution to both obesity and diabetes, the increase in these conditions in both developed and developing countries appears to be due to a changing balance between energy intake and energy expenditure through physical activity (Zimmet et al., 2001). Much is written about the unhealthy Australian diet, but, although we may have substituted hamburgers and chips for our parents' lamb chops and sausages, the total calorie intake and macronutrient composition have changed little over the past 50 years. However, our physical activity levels have probably diminished by half (Prentice et al., 1995).

The tendency for the increased prevalence of type 2 diabetes to be concentrated in lower socioeconomic groups in developed countries and higher socioeconomic groups in developing countries (Mohan et al., 2001). probably reflects the adoption of a "healthier" lifestyle by better educated people in developed countries, while it is generally the affluent in developing countries who enjoy a high calorie intake and low level of physical activity.

Role of Genetics in the Development of Diabetes

Type-1 Diabetes

It has been reported that first degree relatives have a higher risk of developing T1D than unrelated individuals from the general population. Which is approximately 6% vs. <1%, respectively (Dorman and Bunker, 2000). Genome wide analysis has revealed that there may be more than 20 regions of the genome which may be involved in the genetic susceptibility to T1D. The highest risk has been repoted for the HLA gene cluster at chromosome 6. This region contains several hundred genes known to be involved in the immune response. Those most strongly associated with the disease are the HLA class II genes (i.e., HLA-DR, DQ, DP).

IDDM1

The HLA class II genes, also referred to as *IDDM1*, contribute approximately 40-50% of the heritable risk for T1D (Hirschhorn et al., 2003). When evaluated as haplotypes, DQA1*0501-DQB1*0201 and DQA1*0301-DQB1*0302 are most strongly associated T1D in Caucasian populations. They are in linkage disequilibrium with DRB1*03 and DRB1*04, respectively. Specific DRB1*04 alleles also modify the risk associated with the DQA1*0301-DQB1*0302 haplotype. Other reported high risk haplotypes for T1D include DRB1*07-DQA1*0301-DQB1*0201 among African Americans, DRB1*09-DQA1*0301-DQB1*0303 among Japanese, and DRB1*04-DQA1*0401-DQB1*0302 among Chinese. DRB1*15-DQA1*0602-DQB1*0102 is protective and associated with a reduced risk of T1D in most populations.

Individuals with two high risk DRB1-DQA1-DQB1 haplotypes have a significantly higher T1D risk than individuals with no high

risk haplotype. The T1D risk among those with only one susceptibility haplotype is also increased, but effect is more modest. Relative risk estimates range from 10 – 45 and 3-7, respectively, for these groups, depending on the race (Dorman and Bunker, 2000). In terms of absolute risk, Caucasian individuals with two susceptibility haplotypes have an approximately 6% chance of developing T1D by age 35 years. The loss of dominant protection is usually associated with *HLA-DQB1*0602* haplotypes is consistent with a locus centromeric to *HLA-DQB1* being a major determinant of MHC-associated susceptibility, and perhaps the true T1D susceptibility locus (Zaheed et al., 2008).

Aspartic acid at position 57 of the DQB chain is encoded by DQB protective allele, while alanine is at the same position at the predisposing alleles (Morel et al., 1988) (20). If the protective alleles at DRB1 are taken into account, then the presence /absence of DQB chain Asp 57 explain the association of DQB1 alleles in a complete and satisfactory way. The most predisposing allele in a study was DQB1*0201 which may facilitate homo-dimerization and T-cell activation (Morel et al., 1988). HLA-DQ8 (DQA1*0301/ DQB1 *0302) is known to be a major genetic predisposing factor in the Caucasian type 1 diabetic population (Todd et al., 1987). However, several studies also reported that type 1 diabetes susceptibility is likely to be modulated by other HLA molecules such as HLA-DR (Sheehy et al., 1989; Monos et al., 1988). Results from DQ8-matched case-control studies indicated that different DQ8-DRB1*04 haplotypes were associated with variable risks of type 1 diabetes development in a hierarchical rank of DQ8-DRB1*0405 > DQ8-DRB1*0402 > DQ8-DRB1*0401 > DQ8-DRB1*0404 > DQ8-DRB1*0403 (or 0406). This rank suggested that distinct DRB1*04 alleles provided variable degrees of protection (Undlien et al., 1997; Cucca et al., 1995). Experimental model (transgenic mice) demonstrated coexpression of DR4 (0401) with DQ8 reduced the incidence of spontaneous diabetes when the mice also transgenically expressed costimulatory molecule B7.1 on ß-cells (Wen et al., 2001). However, mechanisms by which DR4 manifest the effect are not clear. It was also demonstrated that transgenic expression of H2-E^d (murine homologue of HLA-DR) in NOD mice also decreased the incidence of diabetes (Hanson et al., 1996).

Brorsson, et al. (2009) developed novel methods for identifying new genes that contribute to the risk of developing type 1 diabetes within the Major Histocompatibility Complex (MHC) region on chromosome 6, independently of the known linkage disequilibrium (LD) between human leukocyte antigen (HLA)-DRB1, -DQA1, -DQB1 genes. They combined single nucleotide polymorphism (SNP) genotyping data with protein-protein interaction (ppi) networks to identify disease-associated network modules enriched for proteins encoded from the MHC region. Approximately 2500 SNPs located in the 4 Mb MHC regions were analysed in 1000 affected offspring trios generated by the Type 1 Diabetes Genetics Consortium (T1DGC). The most associated SNP in each gene was chosen and genes were mapped to ppi networks for identification of interaction partners. A total of 151 genes could be mapped to nodes within the protein interaction network and their interaction partners were identified. Five protein interaction modules reached statistical significance using this approach. The identified proteins showed well known pathogenesis of T1D, but the modules also contained additional candidates that have been implicated in beta-cell development and diabetic complications. Hence the extensive LD within the MHC region makes it important to develop new methods for analyzing genotyping data for the identification of additional risk genes for T1D. Combining genetic data with knowledge about functional pathways provided new insight into

mechanisms underlying T1D. (Brorsson *et al*., 2009).

In addition to *IDDM1*, two other genes are now known to influence T1D risk (Anjos and Polychronakos, 2004). These include *INS* and *CTLA-4*. The variable number of tandem repeats at the 5' end of the insulin gene (IDDM2) regulates insulin expression in the thymus. Studies on IDDM5 have led to the discovery of a novel polymorphism 163 A → G (M55V) in SUMO4 gene, which was found to be associated with T1D patients with Asian origin. Functionally SUMO4 conjugates to IkBa and negatively regulates NFkB transcriptional pathway. The M55V substitution reduces the sumoylation activity of the V55 variant, which resulted in higher NFkB dependent transcriptional activity. The polymorphisms of the cytotoxic T lymphocyte antigen 4 gene (CTLA4, IDDM12) encoding a regulatory molecule in the immune system associate with T1D and autoimmune thyroid diseases (ATD). The 3' untranslated region of this gene determines the level of soluble CTLA-4. Genetic mapping of variants conferring a small disease risk can identify pathways in complex disorders, as evidenced by quantitative alterations of candidate genes contributing to autoimmune tissue destruction. Moreover, the identification of two transcription factors that, when mutated, are responsible for severe autoimmune disease is leading to a better understanding of T cell tolerance. Both AIRE and Foxp3, identified initially via their association with genetically manipulated mice, are involved in tolerance induction in humans. Although mutations in these genes may cause rare but serious diseases, it is likely that other transcription factors will contribute to the genetic load that predisposes certain individuals to disease.

Type 2 Diabetes

T2D is, is reported to be polygenic in nature hence in part may have the genetic component. First degree relatives of T2D are about 3 times more likely to develop the disease than without any family history of the disease (Flores et al., 2003; Hansen 2003; Gloyn 2003). Concordance rates for monozygotic twins are approximately 60-90%, which is significantly higher than those for dizygotic twins. Identification of candidate genes (Barroso et al., 2003; Stumvoll, 2004) approach is one of interests of scientist working in the field of diabetes. Type 2 diabetes is associated with impaired insulin secretion. Both 1st- and 2nd-phase insulin secretions are reduced, but the effect is particularly pronounced for the 1st phase. The processes culminating in impaired insulin secretion are not fully understood, but both genetic and environmental factors are thought to play a role (Anna et al., 2009).

In the pre genome era the approach to study the genetics was to select the candidate genes because they are thought to be involved in pancreatic β cell function, insulin action / glucose metabolism, or other metabolic conditions that increase T2D risk (e.g., energy intake / expenditure, lipid metabolism). To date, more than 50 candidate genes for T2D have been studied in various populations worldwide. However, results for essentially all candidate genes have been conflicting. Possible explanations for the divergent findings include small sample sizes, differences in T2D susceptibility across ethnic groups, variation in environmental exposures, and gene-environmental interactions. most important genes are *PPARγ*, *ABCC8*, *KCNJ11*, and *CALPN10* One form of the *PPARγ* gene (Pro) decreases insulin sensitivity and increases T2D risk by several fold. Approximately 98% of Europeans carry at least one copy of the Pro allele. Thus, it likely contributes to a considerable proportion (~25%) of T2D that occurs, particularly among Caucasians. U*ABCC8* (ATP binding cassette, subfamily C, member) U. This gene encodes the high-affinity sulfonylurea receptor (SUR1) subunit that is coupled to the Kir6.2 subunit

(encoded by U*KCNJ11*U, also known as the potassium channel, inwardly rectifying subfamily J, member 11). Both genes are part of the ATP-sensitive potassium channel, which plays a key role in regulating the release of hormones, such as insulin and glucagon, in the beta cell. Mutations in either gene can affect the potassium channel's activity and insulin secretion, ultimately leading to the development of T2D. Interestingly, *ABCC8* and *KCNJ11* are only 4.5 kb apart, and not far from the *INS* gene. Variant forms of *KCNJ11* (Lys) and *ABCC8* (Ala) genes have been associated with T2D, as well as other diabetes-related traits. Because of the close proximity of these genes, current studies are evaluating whether they work in concert with each other, or they have an independent effect on T2D susceptibility. Since *PPARγ, ABCC8* and *KCNJ11* are the targets of drugs used routinely in the treatment of T2D, there are pharmacogenetic implications for maintaining good glycemic control. Response to hypoglycemic therapy may actually be related to one's genotype. Thus, genetic testing may not only help to determine who is at high risk for developing T2D, but may also be useful in guiding treatment regimens for T2D. U*CAPN10* (calpain 10) *CAPN10* encodes an intracellular calcium-dependent cysteine protease that is ubiquitously expressed (Cox et al., 2004). A haplotype that was initially linked to T2D included an intronic A to G mutation at position 43, which appears to be involved in *CAPN10* transcription. Two amino acid polymorphisms (Thr504Ala and Phe200Thr) have also been associated with T2D risk. However, it has been suggested that the coding and noncoding polymorphisms do not independently influence T2D risk, but instead contribute to an earlier age at diagnosis. Physiological studies suggest that variations in calpain 10 activity effects insulin secretion, and therefore, susceptibility to T2D. Studies from different ethnic groups indicate that the contribution of this locus to increased T2D risk may be much larger in Mexican-American than Caucasian populations.

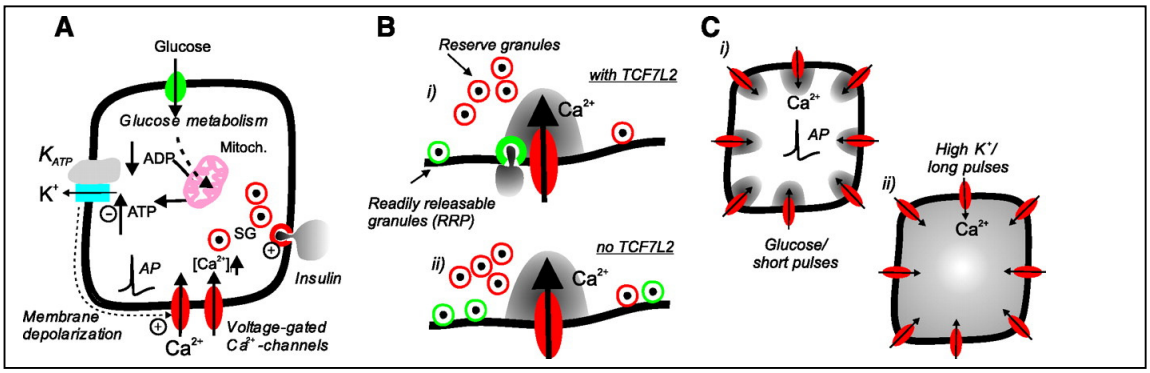
TCF7L2, the susceptibility gene with the largest effect on disease susceptibility discovered to date, was identified pre–genome-wide association by Grant et al. in 2006, with rapid replication of its consequence on diabetes susceptibility in multiple populations (Grant *et al*., 2006; Groves *et a*l., 2006; Cauchi *et al*., 2006; Damcott *et al*., 2006; Weedon 2007). *TCF7L2* was a positional candidate gene that mapped to a region of genetic linkage to type 2 diabetes in the Icelandic population on chromosome 10. However, the identified *TCF7L2* risk allele, which was present in ~28% of control subjects and ~36% of type 2 diabetic individuals, could not explain this linkage, so the finding was actually serendipitous (Grant *et al*., 2006). The precise genetic defect that causes the association of *TCF7L2* with type 2 diabetes remains unclear. There is a large number of highly correlated variants, none of which are obvious functional candidates, that show association with diabetes (Grant *et al*., 2006). The most likely candidate is the single nucleotide polymorphism rs7903146, which shows the strongest association with diabetes and resides in a noncoding region with no obvious mutational mechanism. It is clear, however, that the effect of the *TCF7L2* risk allele is through a defect in insulin secretion (Weedon 2007).

There have been few studies investigating the role of TCF7L2 on insulin secretion in isolated islets. Recently, a study by Shu et al. (2008) reported that silencing of *TCF7L2* by siRNA resulted in strong suppression of insulin secretion in human and mouse islets. Conversely, overexpression of *TCF7L2* stimulated insulin secretion. Exactly how TCF7L2 protein levels modulate insulin secretion was not established in the study by Shu et al. (2008).

The normal regulation of insulin secretion from pancreatic β-cells is well understood

(Figure 2) (Rorsman et al., 2003). da Silva Xavier et al. (2009) studied the effects of overexpression and knockdown of *TCF7L2* on β-cell function using an impressive battery of cell physiological techniques. They confirm that silencing of *TCF7L2* exerts a strong inhibitory effect on glucose-induced insulin secretion. By contrast, insulin secretion triggered by high extracellular K^+ was not affected. Unlike what was seen in the study of Shu et al., overexpression of *TCF7L2* did not affect insulin secretion. The inhibition of glucose-stimulated insulin secretion produced by *TCF7L2* silencing was not associated with any lowering of $[Ca^{2+}]_i$; if anything, the responses to glucose were larger in cells lacking TCF7L2. This suggests that glucose sensing in the β-cell was unaffected.

Figure 2: Mechanisms by which *TCF7L2* silencing reduces glucose-stimulated insulin secretion.



A: Stimulus-secretion coupling of the β-cell. Glucose, via mitochondrial (mitoch.) production of ATP and an increased ATP-to-ADP ratio, causes closure of ATP-sensitive K^+ (K_{ATP}) channels and elicits action potentials (APs) that are associated with the opening of voltage-gated Ca^{2+} channels. The increase in $[Ca^{2+}]_i$ stimulates exocytosis of insulin-containing secretory granules (SGs). *B*: The insulin granules belong to different functional pools, which differ with regard to release competence. The vast majority of granules did not attain release competence and belong to a reserve pool (red granules). A small fraction of the granules are immediately available for release: the readily releasable pool (RRP) (green granules). Many readily releasable pool granules are situated in close proximity of the voltage-gated Ca^{2+} channels (*i*). In the absence of TCF7L2, the Ca^{2+} channels may detach from the secretory granules and $[Ca^{2+}]_i$ increases in the wrong part of the β-cell (*ii*). *C*: Localized increases in $[Ca^{2+}]_i$ (gray zones) close to the Ca^{2+} channels during brief action potential–like stimulation (*i*) and the global elevation produced during protracted (e.g., high K^+) stimulation (*ii*).

Although it remains to be determined exactly how a reduction of TCF7L2 inhibits insulin secretion, the report of da Silva Xavier et al. (2009) is significant because it is one of the first detailed studies of the mechanisms by which the diabetes gene affects insulin secretion. The fact that the most strongly associated diabetes susceptibility variants in TCF7L2 are all in the noncoding region suggests that they affect disease susceptibility by influencing the expression levels (**Jin**, **Liu 2008**). The finding that silencing TCF7L2 is associated with strong inhibition of insulin secretion whereas overexpression, if anything, stimulates insulin secretion makes it tempting to speculate that diabetes results from reduced TCF7L2 expression. It is therefore unexpected that the only study thus far that has compared

TCF7L2 expression in nondiabetic and diabetic human islets found that type 2 diabetes is associated with a fivefold increase in TCF7L2 mRNA levels (**Lyssenko et al., 2007**). Future studies will be required to assess how more subtle variations in TCF7L2 level impact on β-cell function. In addition, studies investigating the temporal and specific TCF7L2 isoform expression profiles in human islets are required to fully elucidate the role of this key tran scription factor in islet development and function.

Maturity-Onset Diabetes of the Young

Maturity Onset Diabetes of the Young (MODY) is a group of diabetes disorders that affects about 2% of people with diabetes. MODY is often not recognized and people may be treated as Type 1 or Type 2 diabetes by their doctors. MODY has four main characteristics: (i) Diabetes presents at a young age, usually less than 25 years of age. (ii) MODY runs in families through several generations. A parent with MODY has a 50% chance of passing on MODY to their child. This is called autosomal dominant inheritance. (iii) People with MODY do not always need insulin treatment and can often be treated with diabetes pills or meal planning alone. (iv) People with MODY do not produce enough insulin; this is different to Type 2 diabetes where people frequently produce lots of insulin but don't respond to their insulin.

MODY displays an autosomal dominant pattern of inheritance, generally spanning three generations (Stride and Hattersley, 2002). There are at least six forms of MODY, each of which is caused by a mutation in a different gene that is directly involved with beta cell function (Winter, 2003). Table 1 lists the MODY genes that have been identified to date. Because ~15% of MODY patients do not carry mutations in any one of these genes, it is anticipated that other genes that cause MODY will be discovered in the near future (Demenais et al., 2003; Frayling et al., 2003; Kim et al., 2004).

Table 1 MODY Genes

Type	Gene	Locus	#Mutations	%MODY
MODY1	*HNF4A*	20q12-q13.1	12	~5%
MODY2	*GCK*	7p15-p13	~200	~15%
MODY3	*HNF1A*	12q24.2	>100	~65%
MODY4	*IPF1*	13q12.1	Few	
MODY5	*HNF1B*	17cen-q21.3	Few	<3%
MODY6	*NEUROD1*	2q32	Few	

GCK (glucokinase) U. The *GCK* gene is currently the only MODY gene that does not regulate the expression of other genes. Rather, the *GCK* gene plays a key role in glucose metabolism and insulin secretion. Thus, the clinical course of MODY2 patients differs from the prognosis associated with other types of MODY. MODY2 patients have a mild fasting hyperglycemia that is present from birth, and generally stable throughout life. There may be a mild deterioration of normoglycemia with age, but patients with MODY2 mutations are usually asymptomatic. Most are detected during routine medical screening. During pregnancy women with MODY2 mutations are often diagnosed. However, the outcome of the pregnancy can be influenced by whether the mother and / or

fetus carry the mutation. When both mother and fetus are MODY2 positive, there is generally no effect on birth weight. However, MODY2 negative fetuses which are carried by MODY2 positive mothers are typically large for gestational age due to maternal hyperglycemia. In contrast, if the fetus, but not the mother, carries the MODY2 mutation, their birth weight will be reduced by approximately 500g due to reduced fetal insulin secretion, which inhibits growth. U*HNF4A* (hepatocyte nuclear factor 4-α).

Mutations in promoter and coding regions of the *HNF4A* gene cause MODY1. *HNF4A* is expressed in many tissues, including the liver and pancreas. It regulates hepatic gene expression, and influences the expression of other MODY genes such as *HNF1A,* which causes MODY3. In the beta cell of the pancreas, it directly activates insulin gene expression. Mutations in the *HNF4A* gene also have been associated with T2D (Silander et al., 2004). U*HNF1A* (hepatocyte nucleara factor 1-α). MODY3, the most frequent cause of the disease, results from mutations in the *HNF1A* gene. *HNF1A* is expressed in the liver and pancreas. It can also influence *HNF4A* expression, indicating a connection between MODY1 and MODY3. This suggests that the MODY transcription factors form a regulatory network that maintains glucose homeostasis. In addition to causing MODY3, *HNF1A* mutations have been associated with T1D (Moller et al., 1998; Lehto et al., 1999) and T2D (Pearson et al., 2004). U*IPF1* (insulin promoter factor-1). MODY4, which is a rare form of the disease, is due to mutations in the *IPF1* gene. Homozygosity for such mutations has been associated with newborn pancreatic agenesis and neonatal diabetes. Therefore, infants who carry MODY4 mutations tend to be small for gestational age. Individuals with MODY4 may also develop T2D (Cockburn et al., 2004). *IPF1* regulates expression of glucokinase, insulin and other genes involved in glucose metabolism. U*HNF1B* (hepatocyte nucleara factor 1-β)U. MODY5, another rare form of MODY, has also been linked with MODY1 because *HNF1β* regulates *HNF4α.* However, unlike MODY1, MODY5 is also associated with renal cysts, proteinuria and renal failure. U*NEUROD1* (neurogenic differentiation factor 1)U. Mutations in *NEUROD1* are responsible for MODY6. MODY6 is also rare. Together, MODY4, MODY5 and MODY6 comprise less then 3% of all MODY cases. *NERUOD1* is expressed in the beta cells of the pancreas, the intestine and the brain. In the pancreas, it contributes to the regulation of the expression of insulin. To summarize, all MODY genes are expressed in the islet cells of the pancreas, and play a role in the metabolism of glucose, the regulation of insulin or other genes involved in glucose transport, and/or the development of the fetal pancreas. Because MODY phenotypes vary depending which gene is involved (Table 2), genetic testing may also assist in the treatment of the disease.

Table 2: MODY Phenotypes

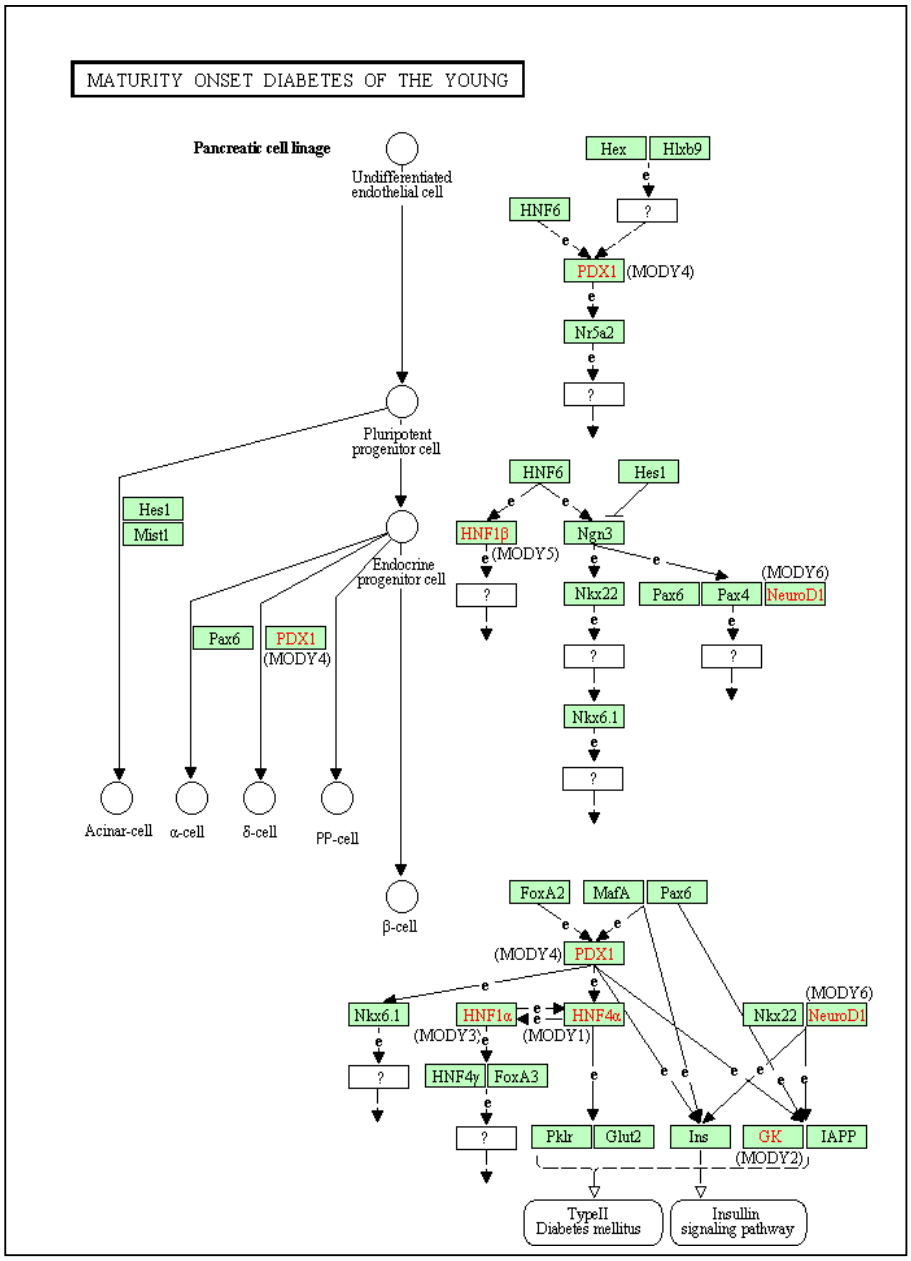
Type	Disease Onset	Complications	Treatment
MODY1	Severe	Frequent	Diet, oral agents, insulin
MODY2	Mild	Rare	Diet
MODY3	Severe	Frequent	Diet, oral agents, insulin
MODY4	Moderate	Little data	Oral agents, insulin
MODY5	Severe	Renal cysts	Oral agents, insulin
MODY6	Severe	Little data	Diet, oral agents, insulin

Feature

The pathway of Maturity-Onset Diabetes of the Young is summarized in Figure 3. From this figure it is quite clear that different forms of MODY are result of differences in the pathway genes which reveals the importance of genetics in studying Maturity-Onset Diabetes of the Young

The present review focused on the genetics of diabetes highlighting the importance of various genes which may be involved in the causation of various types of diabetes. The genome wide search and linkage analysis will throw more light in this direction in future.

Figure 3: Maturity onset diabetes of the young



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Editors

Feature

Beta Thalassemia: Diagnosis and Prevention

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Thalassemia is an autosomal recessive '*Hereditary Anemia*' resulting from defects in hemoglobin production and the most extensively studied genetic disease at the biochemical and genetic level. Till date β thalassemia, remains a model for understanding the molecular pathophysiology of the disease. Beta thalassemia is caused by complete absence or reduced synthesis of β globin chain leading to an imbalance of α and β chain ratios.

The disease affects multiple organs and thus associated with considerable morbidity and mortality. The clinical diversity of β thalassemia has resulted largely due to the amount of the precipitation of excess α chains which, damages the red cell precursors and its progeny. Spleen destroys these deformed red blood cells and causes marked anemia in thalassemic patients. Individuals who are carriers, clinically asymptomatic while those who are homozygous, suffers from hypochromic, microcytic and hemolytic anemia. Such patients require regular blood transfusion and iron chelation therapy to sustain their life.

Thalassemia is recognized as global public health problem. WHO has estimated that in next 20 years an approximately 900,000 births of thalassemia major children will take place worldwide. Out of these, Asian Indian and Middle Eastern regions will be accounting 95% of thalassemia births, if no preventive measures are to be taken.

The remarkable phenotypic diversity of the β thalassemia is prototypical of how a wide spectrum of disease severity can be generated in single gene disorders. The ability to predict phenotype from genotype has important implications for the screening of beta-thalassemia carriers, for genetic counseling and prenatal diagnosis and for planning the appropriate treatment regimen.

Beta Thalassemia is widespread throughout the Mediterranean region, Africa, Middle East, Indian subcontinent & Southeast Asia. In India prevalence, of β Thalassemia ranges from 1-17% in different parts of the country. The distribution of the disease is heterogeneous and estimated as high as 9.2-17% in Gujratis and Tamils, 7% in western Maharashtra, 6.5% in Punjabis, 3.7% in Bengalis [Eastern India] and around 4% in Uttar Pradesh.

Inheritance of Thalassemia Gene

The thalassemia allele is passed from parents to children by an autosomal recessive pattern of inheritance. A child inherits one β globin gene from each parent. When both parents carry normal β globin genes, the child will inherit two normal β globin genes. When one of the parents carries an affected β globin gene and the other parent carries a normal

healthy β globin gene, each child born to these parents has a one in two or a 50% chance of inheriting the affected thalassemia gene from the carrier parent, as shown in figure 1.

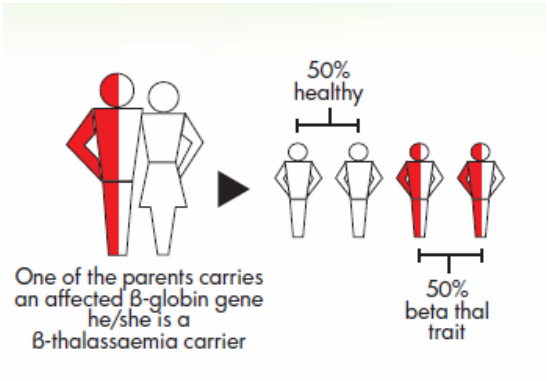

Fig 1: Inheritance of thalassemia gene when one parent is carrier and other is normal

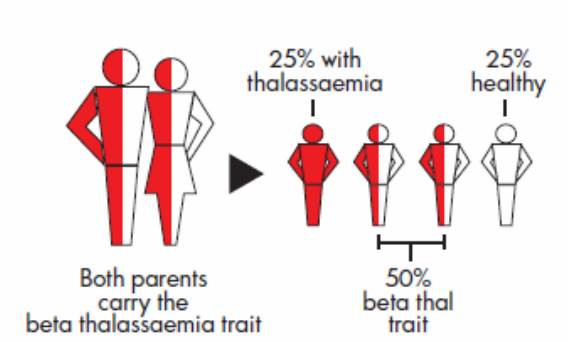

Fig 2: Inheritance pattern of thalassemia gene when both the parents are carriers

Although being a carrier of the thalassemia trait has no adverse health effects, if both the parents are carrier then there is a one in four or 25% chance of having the child with thalassemia major (Figure-2).

Types of Thalassemia

β Thalassemia Major: This is the phenotype where patients inherit both the defective β globin alleles in homozygous or in double heterozygous state. Symptoms appear within six months to one year of age. These patients suffer from severe anemia.

They need regular blood transfusion at the interval of 3-4 weeks and Iron cheating therapy for survival of their life. The common clinical features of β-thalassemia includes: pallor, lethargy, poor appetite, failure to thrive, irritability and difficulty settling, developmental delay and haemolytic anemia. Splenomegaly, hepatomegaly, growth failure with bone changes, fractures and leg ulcers can be seen in childhood. Regular transfusion therapy to maintain hemoglobin levels of at least 9 to 10g/dL allows for improved growth and development and also reduces hepatosplenomegaly due to extramedullary hematopoiesis as well as bone deformities. Hypertransfusion regimens lead to iron over load tissues and its resultant cardiac, liver and endocrine complications: darkly pigmented skin, bronze diabetes, hypopituitarism, hypothyroidism, hypoparathyroidism, hypogonadism, hepatic complications including cirrhosis and most importantly cardiac iron overload. Out of 1 million transfusion dependent thalassemic patients only 39% get adequate iron chelation and approximately 3000 die each year due to inadequate iron chelation. Besides the enormous burden in health resources, chronic transfusions with daily chelation compromise the quality of life, often resulting in non-compliance. Some patients especially develop transfusion-transmitted viral infections, with associated serious morbidities.

β Thalassemia trait: It is an asymptomatic disorder associated with prominent abnormalities of erythrocyte morphology but with little or no anemia, and with elevated component of HbA_2.

Chromosomal location of β globin gene

The β globin gene cluster spans 70kb on the short arm of chromosome 11(11p15.5). The β globin gene is the only expressed gene in the normal adult, expression of the δ and γ gene is minimal.

The β globin gene cluster is under the control of the β-locus control region (β-LCR), a dominant regulatory element, essential for

the expression of all the downstream genes in the complex. This region consists of five DNase 1 hypersensitive (HS) sites (designated 1-5). [Figure 3]

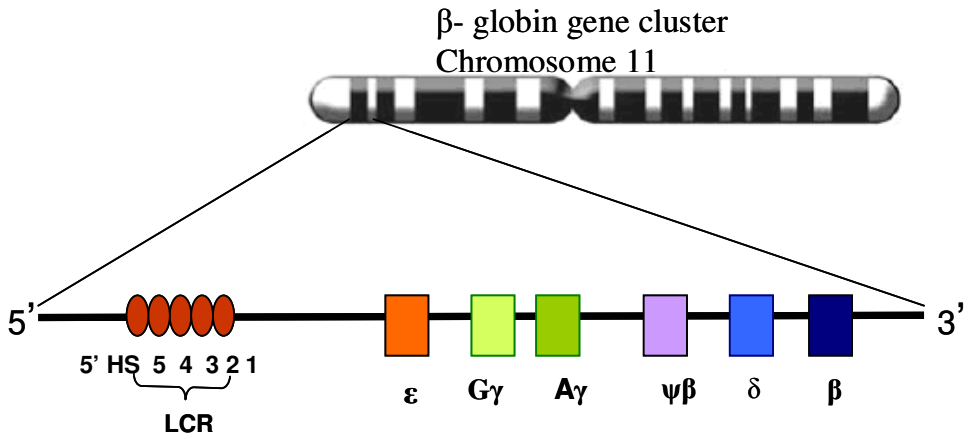

Fig 3: Chromosomal location and structural organization of β globin gene cluster

The general structure of the β globin gene is typical of the other globin loci. The genomic sequence of β gene alone spans 1.6kb and codes for 146 amino acids; the transcribed region is confined in three exons separated by two intervening sequences (IVS).[Figure 4]

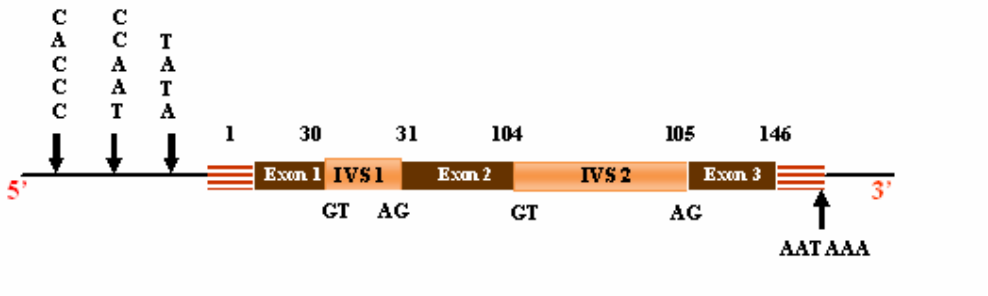

Fig 4: Structure of β globin gene showing three exons, introns, consereved sequences and poly A tail

Molecular Mechanisms

Over 300 mutations affecting β globin gene have been identified in patients with thalassemia.The common mutations are all point mutation because of single base substitution and insertion or deletion of few bases. These mutations are found within the beta globin gene itself or its immediate 5'and 3' flanking sequences. These mutation results in deficit of beta chain production that ranges from minimal (β^+) to a complete absence (β^0).

The phenotypic variability depends on the degree of globin chain imbalances between α and β globin chain. The greater the imbalance more is the severity. Any inherited or acquired factor that is able to reduce the degree of globin imbalance in thalassemia major may produce milder clinical form of thalassemia. On the other hand in β thalassemia heterozygotes; factors which increase the globin chain imbalance turns asymptomatic carrier to severe thalassemia phenotype.

Diagnosis of Beta Thalassemia

Hematological methods

Red Cell Indices: The hematological parameters are measured by automated Red Cell Counter Equipment. This is used to define the size, volume, and Hb content of the RBCs in terms of mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). This also measures various parameters viz. WBC count, RBC count and platelets count. Thalassemia is diagnosed where the size and volume of red blood cells and the concentration of hemoglobin inside them are significantly reduced (MCV < 75fL, MCH < 27pg).

Low MCV also indicates the presence of Iron deficiency. If further laboratory tests (such as Total iron binding capacity (TIBC) and ferritin) exclude iron deficiency, as the cause of a low MCV, additional tests are carried out to confirm the presence of the thalassemia trait and to determine its type.

Hemoglobin electrophoresis: It is used for the separation of different Hb types present in a blood sample. It performed on Cello-gel strip at alkaline pH. Hemoglobin acts as a negatively charged protein at this pH and migrates towards the anode. Different types of Hb molecules differ in the molecular weight, size and sequence of amino acids .These properties give the Hb molecule a particular charge and mobility in an electric field; on basis of which they are separated.

Hemoglobin Chromatography: It enables quantitative measurement of the different components of the hemoglobin molecule - i.e. Hb A, Hb A_2, and Hb F. These levels are determined by automated Hb Variant Equipment. It also assists in detecting the common abnormal hemoglobin variants (Hb S, C, DPunjab, E, OArab and Lepore) along with the normal fractions of the hemoglobin.

The most characteristic hematological finding of the β thalassemia trait is an elevated levels of Hb A_2 (>3.5), a classical diagnostic feature whereas in the case of thalassemia major the level of Hb A_2 may or may not be increased but majority of the cases have high levels of Hb F.

Molecular methods

To confirm and to provide prenatal diagnosis, DNA Mutation analysis for the beta globin gene is necessary. The molecular study helps in establishment of genotype and phenotype correlation of the disease.

Prevention

The permanent treatment available for thalassemia is unsatisfactory and also causes economic and emotional burden on patients and their families as well as on society. Therefore, prevention of the affected child either by detection of the carrier status of the couples, premarital screening or prenatal diagnosis seems to be more useful option for couple at risk.

Thalassemia is a preventable disease, a fact well demonstrated by countries such as, Greece, Sardinia and Cyprus which were amongst the first to establish successful national programmes, significantly reducing the birth of affected children and in some cases the birth rate of thalassemia major child has reached zero.

Carrier Screening

As thalassemia is the recessive genetic disease so the prospective approach for the carrier screening is the most suitable option for the control of the disease. One can know their carrier status by simple hematological test. Such programmes have shown tremendous success in many at risk populations like Sardinia, Cyprus, Greece, Turky, UK, Tusania, Srilanka, China, Malaysia and Thailand. In India, it is desirable to have carrier and high risk couples screening programmes based on public awareness to prevent the disease.

Pre-marital Screening

In thalassemia the pregnancy termination is unacceptable to some persons due to cultural or religious variations therefore premarital screening is more suitable option. At risk couples are identified prior to marriage, on the assumption that they will then decide to separate and each find another, non carrier partners.

Prenatal Diagnosis

Prenatal Diagnosis at risk couple, play a most effective role in the management of thalassemia. Prenatal diagnosis is accomplished by DNA analysis of the fetus. The methods which are commonly used for fetal sampling are Chorionic villus sampling (CVS), amniocentesis and cordocentesis.

Genetic Counseling

Genetic counseling is the most complex but socially acceptable preventive method for all genetic diseases. In most cases, simple laboratory tests can identify whether a person carries the thalassemia allele or not. However, before any laboratory tests are carried out, it is important that individuals receive genetic counseling where possible. It provide them information, advice and guidance on why they should be tested, and what the results of the test will mean for them. Otherwise provision of this information should rely on a good health education programme.

Screening and Prenatal Diagnostic services are available at various centers in India. SGPGI is among one of them and one of the most advance centre which actively participating in management and prevention programme of thalassemia.

Further Readings

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Genetic Basis of Cancer

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Our bodies are made up of billions of cells that grow, divide, and then die in a predictable manner. Cancer occurs when something goes wrong with this system, causing uncontrolled cell division and growth. Cancer cells often lump together and form a mass of extra tissue, also known as a tumor, which continues to grow. As it grows, a cancerous tumor outgrows its birthplace and moves on to other places. It may damage and invade nearby tissues. Cancer is one of the most significant killer disease around the world although in fact, it's more accurate to say it's a collection of about 100 similar diseases. Cancer can be cured, but the cure rate depends on variety how soon it was diagnosed, and how healthy the person's body is. Breast cancer, skin cancer, colorectal cancer, cervical cancer, prostate cancer and uterine cancer, are quite survivable if they're discovered in time.

A person's genetic makeup is a complete set of instructions on how the body is "supposed" to be built. The body's genetic material consists of genes, made up of coils of deoxyribonucleic acid (DNA). The potential for understanding human genetics increased greatly when the Human Genome Project successfully identified and mapped all the genes on human chromosomes. Genetic techniques can be used to study individual genes to learn more about specific disorders.

Genetic tests are used to diagnose certain disorders (for example, hemochromatosis and chromosomal disorders such as Down's syndrome and Turner's syndrome). Genetics is also increasing the ability to predict what disorders a person is likely to develop. For example, women with certain abnormalities in the *BRCA* genes are more prone to develop breast and ovarian cancers. These predictions may allow disease prevention and screening to be tailored much more to each person. Advances in techniques that assess people's genetic characteristics and increased understanding of human genetics have improved diagnosis of genetic disorders before birth. Genetic screening can be used to counsel parents about the risks of passing on a genetic disorder to their offspring.

Hereditary cancer is a cancer that develops as a result of a gene mutation passed down from a parent to the child. Inheriting a gene mutation does not necessarily mean that person will develop cancer, but increases their risk factor. Research and studies have found that certain gene mutations increase the chances of a person to develop certain kinds of cancers, depending on family history.

The Most Common Hereditary Cancers are:

Breast Cancer: A lump or thickening of the breast; discharge from the nipple; change in the skin of the breast; a feeling of heat; or enlarged lymph nodes under the arm.

Tests for Breast Cancer

The SPOT-Light test counts the number of HER2 genes in a small sample of removed tumor (A healthy breast cell has two copies of the HER2 gene, which sends a signal to cells, telling them when to grow, divide and make repairs). The removed piece is stained with a

chemical that causes any HER2 genes in the sample to change color.

Ovarian Cancer: Abdominal swelling; in rare cases, abnormal vaginal bleeding; digestive discomfort.

Tests for Ovarian and Cervical Cancer

Positron emission tomography with a glucose analog (PET-FDG) may better detect the presence of cervical cancer that had spread to surrounding lymph nodes than traditional CT or MRI scans.

Prostate Cancer: Urination difficulties due to blockage of the urethra; bladder retains urine, creating frequent feelings of urgency to urinate, especially at night; bladder not emptying completely; burning or painful urination; bloody urine; tenderness over the bladder; and dull ache in the pelvis or back.

Tests for Prostate Cancer

Prostate Specific Antigen (PSA) - Prostate Specific Antigen may help detect prostate cancer early.

Colorectal Cancer: Rectal bleeding (red blood in stools or black stools); abdominal cramps; constipation alternating with diarrhea; weight loss; loss of appetite; weakness; pallid complexion.

Tests for Colorectal Cancer

DR-70 is a simple blood test that screens for 13 different cancers at the same time. It is highly specific and catches cancer long before you would suspect anything was amiss. Cancers that can be detected by the test are of the lung, colon, breast, stomach, liver, rectum, ovary, cervix, esophagus, thyroid, and pancreas, and trophoblast and malignant lymphoma.

In hereditary breast and ovarian cancers, the two diseases are often linked together. This is because they develop from the same mutated genes in cases of hereditary cancer. These genes are called BRCA1 and BRCA2.

BRCA genes control cell multiplication and are responsible for repairing damage to DNA. If the gene has been damaged, or mutated, then it can no longer perform its job properly. In the human body, we have two sets of 23 chromosomes. Each set of chromosomes contains DNA from each parent. Within each chromosome, there are thousands of genes. These genes are also in pairs. The BRCA gene is a pair, one containing DNA from the mother, and the other containing DNA from the father. If the mother or father's BRCA gene is mutated, it may be passed on to a child at conception. There is a simple blood test that can detect a mutated BRCA gene. People with breast and ovarian cancer can be tested, and if mutation is found, this will provide vital information for family members. People without cancer can also be tested as well.

Prostate cancer is the most common type of cancer that affects men. It is estimated that 5-10% of cases are developed because of genetic linkages. Studies show that 6 genes are involved in the development of prostate cancer. Since studies on identifying the genes that cause prostate cancer are still in the early stages, no tests have been developed to identify them. However, genetic counseling is an option to identify the risk factor in a person. Colorectal cancer is the 3rd most common cancer among women and men in the United States. One out of every seventeen people will develop the disease in their lifetime. 5-10% of those cases are attributed to genetics. 5% of all cancer diagnoses are related to hereditary non polyposis colorectal cancer, or HNCC.

HNCC is a syndrome in which the risk of developing colorectal cancer is heightened. It is defined by the mutation of specific genes. Studies have revealed that most cases of HNCC occur because of mutation to the MLH1 and MSH2 genes. Further studies are being conducted to identify other genes that may be responsible.

Families with HNCC generally have three

factors in common:

Three or more closely related family members diagnosed with colorectal cancer a family member diagnosed under the age of 50 colorectal cancer has affected two or more generations.

Genetic testing is available through laboratory tests. DNA is examined thoroughly for mutated MLH1 and MSH2 genes. These genes are sought because studies have shown that they cause almost all cases of HNCC.

Genetic Abnormalities: Small changes (mutations) may occur in a specific gene. These changes do not affect the structure of the chromosomes. Some mutations in a gene cause few or no problems. Other mutations cause serious disorders such as sickle cell anemia, cystic fibrosis, and muscular dystrophy. Increasingly, medical scientists are finding specific genetic causes of children's diseases.

Testing for Genetic Abnormalities: A person's chromosomes and genes can be evaluated by analyzing a sample of blood. In addition, doctors can use amniocentesis or chorionic villus sampling to detect certain chromosomal or genetic abnormalities in a fetus. If the fetus has an abnormality, further tests may be performed to detect specific birth defects.

Prevention: Although chromosomal abnormalities cannot be corrected, some birth defects can sometimes be prevented (e. g. taking folate [folic acid] to prevent neural tube defects or screening parents for carrier status of certain genetic abnormalities). Genetics may be able to help predict what disorders a person is likely to develop or how the person will respond to certain treatments.

Increased understanding of human genetics has the potential to predict how people, depending on their precise genetic makeup, will respond to certain treatments. For example, specific genes can predict how much warfarin, a blood thinner, a person is likely to require. This prediction is important because taking too much warfarin can cause serious bleeding and taking too little makes the drug ineffective, which is also risky. Gene analysis can also predict whether a person will have intolerable or only minor side effects when taking irinotecan, an anticancer drug. People likely to have intolerable side effects can be treated with a different drug. Gene technology is rapidly improving. The polymerase chain reaction (PCR) is a laboratory technique that can produce large numbers of copies of a gene, which makes studies on the gene much easier. A specific segment of deoxyribonucleic acid (DNA), such as a specific gene, can be copied (amplified) in a laboratory. Starting with one DNA molecule, at the end of 30 doublings (only a few hours later) about a billion copies are produced.

Several application methods of gene therapy are being tested presently by researchers. Gene therapy involves replacement of missing or improperly functioning genes. A gene probe can be used to locate a specific part of a gene (a segment of the gene's DNA) or a whole gene in a particular chromosome. Probes can be used to find normal or mutated segments of DNA. A DNA segment that has been cloned or copied becomes a labeled probe when a radioactive atom or fluorescent dye is added to it. The probe will seek out its mirror-image segment of DNA and bind to it. The labeled probe can then be detected by sophisticated microscopic and photographic techniques. With gene probes, a number of disorders can be diagnosed before and after birth. In the future, gene probes will probably be used to test people for many major genetic disorders simultaneously.

NEWSANDVIEWS

Genetic and Clinical Factors Relating to Warfarin Dosing.

Warfarin has a narrow therapeutic window and the hemorrhagic or thrombotic implications of over- or under-dosing can be devastating. Moreover, there is substantial individual variation in response to warfarin. The pivotal role of CYP2C9 and VKORC1 is emphasized because polymorphisms of these two genes account for approximately 40% of the variation in dose requirements. Recent studies have reported that polymorphisms in CYP4F2 might account for between 2 and 7% of the variation. Large studies published recently, including the Warfarin Genetics (WARG) study and the International Warfarin Pharmacogenetic Consortium, have added to our understanding of factors relating to warfarin dosing. Several prospective studies have evaluated genotype-guided dosing, but these have found negative results or were poorly designed. Whether genotype-guided dosing is clinically beneficial remains unclear, but studies are currently underway that will help to determine this.

(Trends Pharmacol Sci. 2009 Jul;30(7):375-86. Epub 2009 Jun 17.)

Genetic Basis to Childhood Brain Cancer Identified

Scientists have identified certain genetic mutations, the most common of childhood brain cancers, to play a key role in medulloblastoma. The team led by Dr. Michael Taylor, a pediatric brain surgeon at Toronto's Hospital for Sick Children, has found eight similar genes linked to the childhood brain cancer.

"When these eight genes are functioning normally, their role is to make a protein which tells the developing brain when it's time to stop growing. But when the genes are mutated, the brain may continue to grow out of control, leading to cancer," "Drugs are already being developed that target these types of proteins. ". During the study, the researchers looked at more than 200 tumor samples. data over a period of 3 1/2 years.

(Child Health News March 10, 2009)

Polymorphisms Determine β-Adrenoceptor Conformation: Implications for Cardiovascular Disease and Therapy.

β(1)- and β (2)-adrenoceptors are crucial regulators of cardiovascular function. Agonists and antagonists at these receptor subtypes are cornerstones in the treatment of cardiovascular disease. In humans, both of the genes encoding the β(1)- and β(2)-adrenoceptors carry frequent polymorphisms resulting in different variants of the receptor proteins. Whether the polymorphic nature of the receptors causes the clinically observed differences with respect to the response of the patients to therapeutic drugs is currently a matter of intense discussion. Specifically, novel optical methods directly assess the functional importance of beta-adrenoceptor polymorphisms on ligand-induced changes of receptor conformation. The ability to determine polymorphism-dependent differences in drug efficacy directly on the receptor level might develop into an important approach to establish individualized drug therapies based on the genetic determinants of the patients.

(Trends Pharmacol Sci. 2009 Apr;30(4):188-93. Epub 2009 Mar 9.)

New Evidence for Genetic Basis of Lung Cancer Risk in Smokers

Scientists have uncovered new evidence supporting the hypothesis that the genetic background plays a role in determining how likely it is that a smoker will develop lung cancer and what type of cancer it will be.

Genome-wide association studies by Cancer Research UK-funded scientists at the Institute of Cancer Research implicated DNA variants within regions on chromosomes 5, 6, and 15.

The latest ICR work suggests there are two independent sites on chromosome 15 involved in determining lung cancer risk. They calculated that current or former smokers carrying one copy of each of the chromosome 15 variant were at a 28% higher risk of developing lung cancer than smokers without the variants. The relative risk jumped to 80% for either former or current smokers who were homozygous for both chromosome 15 variants. The increased risk of lung cancer was not evident in carriers of the polymorphisms who had never smoked.

Additionally, the sequences on chromosomes 5 and 6 were found to influence the type of lung cancer smokers developed. Individuals who carried the chromosome 5 variation were more likely to develop adenocarcinoma, a type of non-small-cell lung cancer (NSCLC) that represents the most common form of the disease, the ICR researchers point out. The chromosome 6 variant also appeared to influence whether the carrier developed the adenocarcinoma or the squamous cell carcinoma form of NSCLC.

(GEN News Highlights)

Genetic Cause of Prostate Cancer

Scientists have made a major advance in understanding the genetic causes of prostate cancer, opening a new front in the battle against the most common malignant disease in men. Seven new genetic mutations have been identified that are present in over half of all new cases of prostate cancer, diagnosed in 35,000 men a year. The discovery helps explain why the disease runs in families. Each individual mutation increases the risk by up to 60 per cent and when all seven are present together the risk is raised three-fold.

Prostate cancer is one of the most rapidly increasing cancers here but the existing blood test for the disease is unreliable. The breakthrough means a new genetic test for prostate cancer could be developed to identify men at high risk who could be targeted for regular screening and early treatment.

(GEN News Highlights)

India's Genetic Divide may Hit Pharma Cos' R&D Plans

Global pharma firms making drugs for the Indian market may have to review their discovery and development programme, following a path-breaking discovery by top Indian and American scientists that India is genetically not a single large population.

“Drug companies engaged in clinical trails could be worried as our research shows that many groups in modern India descend from a small number of founding individuals. A common drug may not be the answer, considering the genetic variation in the Indian population. For instance, medicines tested on the Western population may not be effective on the Indian population,” said Lalji Singh, former director of the Centre for Cellular and Molecular and Biology (CCMB) who has co-authored the research findings on Reconstructing the Indian Population History, said on Thursday.

More than three-fourths of India’s over one billion people are burdened with genetic disorders. The study shows that Indians have been genetically different from other groups and this could be a major cause of recessive diseases. The incidence of genetic diseases among Indians is, therefore, different from the rest of the world.

“Drug makers may have to consider the genetic variation in the Indian population as the treatment for any disease will depend on the genetic profile of the individual,” said Kumaraswamy Thangaraj a senior research scientist at the CCMB.

In Andhra Pradesh, for instance, the

Vaishya community does not have an enzyme which metabolises anaesthesia. Breast cancer is predominant among the Parsi community, while a rare neurological disorder is rampant in the population in Chittor and Tirupati.

"Drug trials should take into account diseases that are specific to the population," said Lalji. A senior official of a top Indian drug-maker who did not wish to be identified said that pharma companies, the world over, are alive to the issue as the success of clinical trails and the efficacy of a drug hinges on the gene pool.

(The Economics Times 30th Sept., 2009)

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Clinical Utility of Genetic Tests for Inherited Hypertrophic and Dilated Cardiomyopathies

The success of the Human Genome Project and the recent discoveries in the area of genetics promise to significantly change the clinical practice of cardiology, providing new tools for more accurate diagnosis and prognosis of disease as well as for a better prediction about health risks for the family.

Rapid advances in the technology and reduction in the cost of DNA sequencing have led to increasingly rapid translation of genomic information into clinical applications. As a consequence the number of genetic tests is growing, and becoming currently available for clinical testing. However, genetic tests are generally time-consuming and expensive, and they should be used with sufficient consideration of their necessity and value in managing the patient's condition. Therefore, it is imperative that cardiologists know the basis for genetic cardiovascular disorders and the medical implications of these defects in order to improve their expertise as well as to ensure an appropriate practical use of genetic tests in the clinical setting. The purpose of this paper is to provide a concise overview of the genetic etiology as well as the clinical utilities and limitations of genetic testing for the heritable form of hypertrophic and dilated cardiomyopathies.

The Genetics of Cardiomyopathies

Diseases identified as "genetic" can be typically classified into two categories: Mendelian diseases and multifactorial diseases. Mendelian diseases or monogenic diseases are rare, and only one mutation in a given gene is responsible for inheritance of the disease in a given family. Genetic diseases, in the second group, occur more commonly in the population and are often recognized as "running in families". The genetic model underlying a multifactorial disease is often complex since it may be related to the interaction or additive effect of multiple genes as well as to the presence or absence of environmental factors. Congenital heart disease, coronary heart disease, venous thrombosis, and diabetes mellitus fall into this category.

Heritable hypertrophic and dilated cardiomyopathies are monogenic diseases, caused by mutations in key genes that lead to the absence or abnormality of myocardial proteins. Disease-causing gene mutations have been identified in approximately two-thirds of cases of hypertrophic cardiomyopathy (HCM) and about 50% of idiopathic dilated cardiomyopathy (DCM).

Various types of mutations can occur in DNA, including non-sense (stop codons), missense mutations (causing aminoacid substitution) and splice-site. Mostly, newly detected mutations for heritable cardiovascular disorders are missense. For these mutations, it is difficult to establish their pathogenicity, unless specific functional test are available. Presently, pathogenicity is presumed when the substitution affects a very conserved sequence through evolution, or it was reportedly associated to disease in independent patients.

The accurate reconstruction of family

history is crucial element for diagnosis of genetic cardiomyopathy. The family history should encompass at least 3 generations with a careful and complete history about the family members, including demographic and medical information. The family history may provide additional relevant information, such as age of onset and penetrance, and help to identify the patterns of inheritance.

In addition, information of cardiovascular tests and procedures (echocardiography, electrocardiography, Holter monitoring, implantable cardioverter-defibrillator, heart transplant) should always be recorded as part of the family history.

In autosomal dominant inheritance, family history typically reveals that the disorder is usually present in every generation, and there is a 50% chance of inheriting the mutation. In autosomal recessive inheritance, the condition appears to "skip" generations. Parents of an affected have a 25% chance of having an affected child and a 50% chance of having a carrier child in each pregnancy. In X-linked dominant inheritance, all daughters of an affected man are affected, sons and daughters of carrier women have a 50% risk of being affected. In X-linked recessive inheritance, there is a 50% chance that each son of a carrier woman will also be affected. No male-male transmission is observed.

However, inherited cardiomyopathies show a wide range of clinical presentation within the same family, with incomplete and age-dependent penetrance. Genotype-phenotype relations are complex and not yet completely understood. Considerable phenotypic heterogeneity exists even among individuals who have identical mutations at the disease-causing locus.

Conversely, mutations in different genes can result in a similar phenotype. The incompleteness of genotype-phenotype correlations has limited the use of genetic testing in clinical practice. In selected cases, DNA target analysis has, however, potential clinical value, and genetic testing should be done in patients and families.

Hypertrophic Cardiomyopathy (HCM)

Hypertrophic cardiomyopathy (HCM) is a relatively common and autosomal dominant genetic heart disease (1:500 of the general population), typically diagnosed by unexplained left ventricular hypertrophy with 2-dimensional echocardiography (or alternatively with cardiac magnetic resonance imaging). Left ventricular hypertrophy, often disproportionately affecting the ventricular septum, can range from mild (~13–15 mm) to severe (30–60 mm). The clinical spectrum of HCM is diverse, ranging from asymptomatic individuals to those with disabling symptoms of heart failure, exercise intolerance, arrhythmias and chest pain..

Moreover, HCM is the most common cause of sudden cardiac death (SCD) in the young (including trained athletes), who are often unaware of their underlying condition. Early diagnosis of HCM is important, since at-risk individuals may be advised not to participate in competitive sports and should undergo regular cardiac screening to assess the risk of sudden cardiac death.

HCM is caused by a variety of mutations in genes encoding contractile proteins of the cardiac sarcomere, especially in cardiac myosin heavy chain beta (*MYH7*), myosin binding protein C (*MYBPC3*), and cardiac troponin T (*TNNT2*). To date, over 700 individual mutations have been identified (for a list of gene mutations.

Mutations in the genes coding for 3 sarcomeric proteins (*MYH7*; *MYBPC3*; *TNNT2*), most commonly are estimated to account for about 60% of all familial cases of HCM. Clinical testing for variants in most of these genes is available and can provide valuable therapeutic and prognostic information.

Overall, clinical diversity of HCM can reflect the broad spectrum of underlying molecular cause.

Some specific mutations have been recognized to be associated with strong clinical effect. In particular, some missense mutations in the gene of the *MYH7* (R403Q, R453C, G716R and R719W) were associated with early onset and poor clinical prognosis, including sudden death. Moreover, mutations in the *TNNT2* gene (R92Q and ΔE160) have been associated with high incidence of cardiac sudden death in young men, even with mild hypertrophy. In contrast, patients with mutations in the *MYBPC3* can have a late onset and a relatively good prognosis.

Nevertheless, defining precise genotype-phenotype correlations has been limited by the low frequency of each mutation. Studies of HCM families have also shown the presence of clinical variability among individual with identical mutations. Moreover, the clinical presentation within a given kindred may vary between family members, with mild clinical symptoms manifested in one relative and early-onset heart disease in another. Such interfamilial variable expressivity may be explained by genetic and environmental modifying factors. The identification of other genetic alterations that might play a role in modulating the presentation of disease is, thus, crucial in order to improve gene-based diagnosis.

Recently, nonsarcomeric protein mutations in 2 genes (gamma-2 regulatory subunit of AMP-activated protein kinase, *PRKAG2*, and lysosome-associated membrane protein 2, *LAMP2*) involved in glycogen accumulation in cardiac myocytes have also been reported to be responsible for a less common type of HCM, known as metabolic HCM. Clinical features of metabolic HCM are also the high prevalence of electrical abnormalities (ventricular pre-excitation and atrial fibrillation) and faster progression from hypertrophy to dilation and severe heart failure with respect to HCM. Mutations in the *PRKAG2* or in *LAMP2* genes are believed to account for about 1% of all HCM and for up to 50% of HCM with ventricular preexcitation.

Genetic testing for mutant genes is the most definitive method for establishing the diagnosis of HCM, and some genotype-phenotype correlations can be useful to address DNA analyses in specific genes. For example, HCM with late onset, good prognosis and mild hypertrophy can help to target DNA analysis for *MYBPC3* mutations. Conversely, the presence a more malignant phenotype with a high risk of SCD, may guide genetic screening for *MYH7* mutations in the presence of severe hypertrophy or *TNNT2* mutations if the degree of hypertrophy is mild.

However, caution is required because genotype-phenotype correlations are based on a small population. In addition, the coinheritance of compound mutations (more than 1 mutation in a single gene, or mutations in 2 different genes) is more common than might be expected and can explain why different families that appear to have the same mutation can behave differently.

Dilated Cardiomyopathy (DCM)

Dilated cardiomyopathy (DCM) is a common (estimated 1:2500 persons) and largely irreversible form of heart muscle disease; it is the third most common cause of heart failure in the young and a major cause of heart transplantation. DCM, diagnosed by 2-dimensional echocardiography, is characterized by left ventricular dilation and impaired left ventricular systolic function, often with involvement of the right ventricle, which then lead to ventricular and supraventricular arrhythmias, conduction system abnormalities, thromboembolism, and sudden or heart failure-related death.

DCM may derive from a particularly broad range of primary causes, including infectious agents, toxins, chronic excessive

consumption of alcohol, chemotherapeutic agents, autoimmune, neuromuscular disorders, mitochondrial, metabolic and endocrine disorders.

Approximately one half of patients with the disease are found to have "idiopathic" DCM, indicating that the cause for the condition cannot be determined. About one-third to one-half of "idiopathic" DCM cases have a positive family history in ≥2 closely related relatives. These patients are considered to have familial dilated cardiomyopathy. Inheritance patterns of familial DCM include autosomal dominant, autosomal recessive and X-linked. The autosomal dominant forms are the most common inheritance accounting for about 85–90% of cases.

DCM exhibits high genetic heterogeneity as mutations in > 20 genes have been associated with the disease, such as desmin, tafazzin,*δ*-sarcoglycan, dystrophin, and metavinculin, and nuclear envelope proteins such as emerin and lamin A/C. Mutations in the sarcomere genes, which are responsible for causing HCM, are also associated with DCM. To date, sarcomere gene mutations (*MYBPC3; MYH7;TNNT2;* cardiac troponin I, *TNNI3; α-tropomyosin,TPM1;* cardiac actin, *ACTC*) account for approximately 10–16% of familial DCM.

At the present time, genetic screening in all known disease genes is not possible. However, lamin A/C (*LMNA*) is the most frequent disease associated gene for familial DCM with conduction system disease.

The *LMNA* gene encodes the two differentially spliced proteins lamin A and lamin C, the major components of the nuclear lamina, which localizes at the nucleoplasmic surface of the inner nuclear membrane as a meshwork structure Lamin interacts directly with the chromatin and also with the integral proteins of the inner nuclear membrane, thereby playing a role in maintaining the structural integrity and spatial organization of other inner nuclear membrane. During the last years, clinically distinct disease phenotypes have been attributed to *LMNA* mutations-termed "laminopathies"-ranging from accelerated aging disorders to striated muscle diseases like muscular dystrophy and cardiomyopathy..

Indeed, the *LMNA* gene is involved in up to 30–50% of patients with cardiac conduction disorders and DCM. Although mutations causing DCM can occur almost anywhere in *LMNA*, the domain coil 1B seems to be most frequently affected. The most prevalent *LMNA* mutation hot spot in familial DCM in Europe is codon 190. a missense mutation (R190W) initially described by Arbustini and colleagues in an Italian family with severe DCM and SCD. Subsequently, this variant was identified in DCM patients from other European countries, and even from Korea.

A meta-analysis of clinical characteristics of *LMNA* mutation carriers revealed that *LMNA* mutations carry a high risk of sudden death, and that this risk does not differ between subjects with predominantly cardiac or neuromuscular disease.

Pasotti *et al.* have recently shown that dilated cardiomyopathies caused by *LMNA* gene defects are highly penetrant, adult onset, malignant diseases characterized by a high rate of heart failure and life-threatening arrhythmias, predicted by New York Heart Association functional class, competitive sport activity, and type of mutation. Therefore, it is highly recommended to screen *LMNA* for genetic variants when such clinical features are present in a DCM patient. The *LMNA* genetic testing may allow useful diagnosis of mutations clearly correlated with a worse prognosis as well as identify early "presymptomatic" relatives at greatest risk for developing DCM.

Potential Benefits, Disadvantages and Appropriateness of Genetic Testing

Genetic testing is often the best way to

confirm a diagnosis in a patient with HCM and DCM, as well as to provide risk estimates for asymptomatic patients. However, genetic tests remain generally expensive technologies that are labour-intensive and time-consuming. Rapid advances in the technology and reduction in the cost of DNA sequencing are expected to decrease the costs and, thus, increase the use of genetic testing, perhaps even within the next years.

Routine and extensive genetic screening is impractical because of the genetic heterogeneity of cardiomyopathies. Genetic testing is not appropriate for every patient, but it should be used in selected cases, such as patients with an established family history of severely affected relatives and at high risk of worse prognosis. For example, clinical DNA testing for gene mutations known to be associated with a more malignant phenotype (e.g. *TNNT2* in HCM and *LMNA* in DCM) can confirm the diagnosis and help the cardiologist to stratify the risk of patient. Genetic testing unambiguously allows early identification and diagnosis of individuals at greatest risk for developing cardiomyopathy, allowing to focus clinical resources on high-risk family members.

However, the utility of DNA diagnosis for risk stratification is expected to be limited by the genetic and allelic heterogeneity of cardiomyopathies. A single gene mutation does not by itself fully explain the development of the clinical phenotype. For example, evidence is accumulating that the combined effect of more than one disease-associated mutation or genetic polymorphisms, which contribute to cardiovascular performance, may affect penetrance and severity of the disease in many families. Anyway, it should be emphasized that genetic screening is superior to clinical with respect to specificity of identification of family members at high risk.

In addition, it is extremely important that family members receive careful counselling both before and after testing on the potential risks. Relative may carry the mutation but be asymptomatic and the mutation may merely be a predisposing factor to disease in the presence of other factors, and so its presence alone does not allow accurate prediction of phenotype or prognosis. However, if a mutation is identified in asymptomatic individual, regular clinical cardiovascular screening (echocardiogram, ECG) is recommended to detect the first signs of disease that may be diminished by early treatment.

If family members are found not to carry that mutation, they can be definitively diagnosed as unaffected, and the need for serial follow up becomes unnecessary. In this case, they can be reassured that neither they nor their offspring will be at higher risk compared to the general population to develop these disorders. A specialised cardiogenetic team consisting of clinical geneticists and cardiologists should work together in order to provide the most relevant information to the patients and the relatives.

Blood samples are drawn for DNA analysis and, further consultation with the geneticists can help clarify the interpretation of the results of the DNA analysis. If the disease-causing gene cannot be predicted or investigated, DNA is stored for future research and screening, if permitted by the patient. If a pathogenic mutation is detected in the proband, the team provides genetic counselling for family members with subsequent DNA testing when family members decide to undergo genetic screening.

(Based on an article by Colombo MG, et al., 2008. Cardiovasc. Ultrasound (6): 62)

Genetic Dissection of Type 2 Diabetes

A disease can be inherited or acquired, or both. Some develop as a consequence of a single base pair exchange/mutation, so called monogenic diseases. The individual susceptibility to develop others depends on several genetic variants or combination of variants, so called polygenic diseases. While both common type 2 diabetes (T2D) and obesity may be considered polygenic diseases, there are several examples of clearly monogenic disorders with roughly the same phenotypic expression such as Maturity Onset Diabetes in the Young (MODY) that follow a clear Mendelian mode of inheritance. A complex disease often appears to be acquired; the development of obesity and T2D is triggered by environmental factors in genetically susceptible individuals. However, not all obese individuals develop diabetes; genetic susceptibility is a prerequisite. Given this interplay between genetic and environmental factors a complex polygenic disease may also be referred to as multifactorial.

1. Evidence that type 2 diabetes is inherited

There is ample evidence that T2D has a strong genetic component. The concordance of T2D in monozygotic twins is approximately 70% compared with 20–30% in dizygotic twins. Given the age-dependent penetrance of the disease, it is clear that the longer the follow-up, the higher the concordance rate of T2D clusters in families. The life-time risk of developing the disease is about 40% in offspring of one parent with T2D, greater if the mother is affected, the risk approaching 70% if both parents have diabetes. Translated into a λs-value, the recurrence risk for a sibling of an affected person divided by the risk for the general population, this means that a first-degree relative of a patient with type 2 diabetes has a 3-fold increased risk of developing the disease. Large ethnic differences in the prevalence of T2D have also been ascribed to a genetic component. The change in the environment towards a more affluent western lifestyle plays a key role in the epidemic increase in the prevalence of T2D worldwide. This change has occurred during the last 50 years. Clearly, our genes have not changed during this period but this does not exclude an important role for genes in the rapid increase in T2D, since genes or gene variants explain how we respond to the environment.

2. Thrifty genotypes or phenotypes?

A plausible explanation for the interaction between genes and environment comes from *the thrifty gene hypothesis*. Neel proposed that individuals living in an environment with unstable food supply (as for hunters and nomads) would maximize their probability of survival if they could maximize storage of energy. Genetic selection would thus favor energy-conserving genotypes in such environments. Storage of energy as fat, especially as intra-abdominal fat, is a more efficient way of storing energy than as glycogen in muscle and liver. This is what is found in the insulin-resistant phenotype and explains why offspring of subjects with T2D show early accumulation of abdominal fat.

An alternative explanation has been proposed by which these changes can be the consequence of intrauterine programming, the so called *thrifty phenotype hypothesis*. According to this hypothesis, intrauterine malnutrition would lead to a low birth weight and increased risk of the metabolic syndrome (clustering of cardiovascular risk factors like

abdominal obesity, dyslipidemia, hypertension and glucose intolerance) later in life. These findings have been replicated in several studies but the risk of a small birth weight is increased in families with the metabolic syndrome suggesting that a small birth weight could be a phenotype for a thrifty gene.

3. Prediction of future type 2 diabetes

The importance of different risk factors for T2D differ between ethnic populations but the increased risk conferred by a family history of diabetes seems more constant, although its relative effect decreases with increasing frequency of T2D in the population. The predictive value of a family history of T2D is also relatively poor in young subjects whose parents have not yet developed the disease. A low level of physical activity, abdominal obesity and presence of the metabolic syndrome also commonly confer an increased risk of T2D. In addition, an elevated glucose concentration *per se* is a strong predictor of future T2D. In a prospective study of 2115 non-diabetic individuals followed for six years within the Botnia study it was shown that individuals with a family history of T2D, with a BMI $\geq$ 30 kg/m^2, and fasting plasma glucose concentration $\geq$5.5 mmol/l had a 16-fold increased risk of developing T2D.

4. Mapping genetic variability

Traditionally, two different methods have been used to discover genes that are related to a disease: analysis of genomic regions shared by relatives more often than expected (so called linkage analysis using polymorphic markers such as micro satellites or tandem repeats) and candidate gene studies, particularly by attempts to correlate biological variation (phenotype) with variation in DNA sequences (genotype) in the form of a single nucleotide polymorphism (SNP). The most straightforward approach would be to sequence the whole genome in affected and unaffected individuals but this is for practical reasons not (yet) possible. Several approaches have been described to estimate whether an observed association can account for linkage (Table 1).

Table 1: Type 2 diabetes susceptibility genes

Year (Major Publication)	Gene (suggested)	Disease Mechanism
2000	*PPARG*	Insulin sensitivity
2003	*CAPN10*	Glucose transport
2003	*KCNJ11*	Beta-cell dysfunction
2006	*TCF7L2*	Beta-cell dysfunction
2007	*CDKAL1*	Beta-cell dysfunction
2007	*CDKN2A/2B*	Beta-cell dysfunction
2007	*HHEX/IDE*	Beta-cell dysfunction
2007	*SLC30A8*	Beta-cell dysfunction
2007	*IGF2BP2*	Beta-cell dysfunction
2007	*WFS1*	Unknown
2007	*TCF2*	Unkonown
2007	*FTO*	Obesity
2008	*MC4R*	Obesity
2008	*NOTCH2*	Unknown
2008	*ADAMTS9*	Unknown
2008	*THADA*	Unknown
2008	*TSPAN8/LGR5*	Unknown
2008	*CDC123/CAMK1D*	Unknown
2008	*JAZF1*	Unknown
2008	*KCNQ1*	Beta-cell dysfunction

Without functional support it is not always possible to know whether linkage and association represent the genetic cause of the disease. For many complex disorders this may require a cumbersome sequence of *in vitro* and

in vivo studies. In fact, the success of identifying T2D susceptibility genes by linkage has been restricted to the story of one single gene; *CAPN10*. *PPARG*, peroxisome proliferator-activated receptor gamma; *CAPN10*, calpain 10; *KCNJ11*, potassium inwardly rectifying channel, subfamily J, member 11; *TCF7L2*, transcription factor 7-like 2; *CDKAL1*, CDK5 regulatory subunit associated protein 1-like 1; *CDKN2A/B*, cyclin-dependent kinase inhibitor 2A/B; *HHEX*, haematopoietically expressed homeobox; *IDE*, insulin-degrading enzyme; *SLC30A8*, solute carrier family 30 member 8; *IGF2BP2*, insulin-like growth factor 2 mRNA binding protein 2; *FTO*, fat mass and obesity associated; *MC4R*, melanocortin 4 receptor; *NOTCH2*, Notch homolog 2 Drosophila; *ADAMTS9*, ADAM metallopeptidase with thrombospondin type 1 motif, 9; *THADA*, thyroid adenoma associated; *TSPAN8*, tetraspanin 8; *LGR5*, leucine-rich repeat-containing G protein-coupled receptor 5; *CDC123*, cell division cycle 123 homolog; *CAMK1D*, calcium/calmodulin-dependent protein kinase ID; *JAZF1*, juxtaposed with another zinc finger gene 1; *KCNQ1*, potassium voltage-gated channel, KQT-like subfamily, member 1.

5. Association studies and candidate genes for type 2 diabetes

The starting point for the candidate gene approach is the potential implications that either altered expression and/or function of a particular gene product (conferred by intronic or exonic genetic variants) may have on a biological function or disease. Extending the analysis of genes implicated in monogenic forms of diabetes has proved successful also for T2D as exemplified by *PPARG*, *KCNJ11*, *TCF2/HNF1B* and *WFS1*.

6. Obesity genes and type 2 diabetes

Obesity is clearly one of the driving risk factors behind developing T2D. However, not all subjects with diabetes are obese and not all obese develop diabetes. With this scenario in mind it is important to consider whether a possible association between a gene and T2D is in fact due to a gene associated with obesity or vice versa. *FTO*, the strongest identified obesity gene so far increases the risk of T2D. It is therefore not surprising that *FTO* was not detected as associated with T2D in the WGAS which matched for BMI. Although insulin resistance is an early detectable defect in subjects at increased risk of developing T2D, obesity, and particularly abdominal obesity, usually precedes insulin resistance in these individuals. About 40% of the variation in body fat can be attributed to genetic factors. The genetic influence is even more impressing for abdominal obesity. For example, genes are considered to explain 60% of the variance in abdominal fat in postmenopausal women. Abdominal fat tissue could provide a signal for the chain of events leading to skeletal muscle insulin resistance. Two such candidate signals are the adipocyte hormones leptin, and another tumor necrosis factor-α (TNF-α). A mutation in the leptin gene results in complete absence of the protein in the fat ob/ob mouse. Treatment of the ob/ob mouse with leptin resulted in marked weight loss. This is another example of a monogenic disorder with a similar although exaggerated phenotype as found in common obesity. Two morbidly obese children from consanguineous parents had very low circulating leptin levels due to a frame shift mutation involving a deletion of a single guanine nucleotide in the leptin gene. Whereas some progress in treatment of these children with leptin has been reported, treatment of obese subjects without mutations the leptin gene has been less successful. Obese humans actually have elevated rather than decreased levels of leptin suggestive of hormonal resistance analogous to insulin resistance and leptin levels show a strong positive correlation with the total fat mass. Moreover, contribution of common variants in the leptin receptor gene to obesity and obesity-related phenotypes including leptin levels has

remained more or less elusive. Other monogenic forms of obesity implicate the gene encoding the melanocortin 4 receptor (*MC4R*) which serves as the hypothalamic receptor for the anorexigenic peptide a-MSH (melanocyte stimulating hormone). α-MSH is derived from the product of pro-opiomelanocortin (*POMC*), which has also been associated with monogenic forms of (early-onset) obesity, either directly or indirectly . *MC4R* gene variants are expected to account for as much as 5% of severe obesity cases. Recently, variants close to *MC4R* were found to associate with obesity in a large study involving over 90,000 samples thus establishing *MC4R* as the second replicated obesity gene after*FTO*.

The list of candidate genes for both monogenic and particularly for common obesity that has been compiled through numerous one-by-one candidate gene association studies is very long. Unfortunately, many of these studies are underpowered and the field probably plagued by publication bias. With the advent of GWAS this has now changed although progress has been slower for obesity than for T2D. Thus far two genes (*FTO* and *MC4R*) have been identified in two different scans but several more scans including a substantial number of subjects is under way or already in press. Insulin-induced gene 2 (*INSIG2*) was identified in a low-density scan in the Framingham cohort where heritability estimates for BMI range between 37 and 54%. Out of 116,204 SNPs tested in 694 participants only one SNP reached overall significance. The obesity-associated b *INSIG2* genotype was present in 10% of individuals, homozygotes being about one BMI unit heavier than heterozygotes or non-carriers, regardless of sex and age. The protein encoded by *INSIG2* is also a functionally attractive candidate gene for obesity since it inhibits the synthesis of fatty acids and cholesterol. Loss of function may thus need excessive storage of surplus lipid in the form of adipose tissue. The association between *INSIG2* and obesity has been replicated in some but certainly not all populations tested including meta-analysis of obesity WGAS. A stronger candidate was then found in *FTO*. As mentioned above, the gene predisposes to diabetes through an effect on BMI with a 3 kg between-homozygote difference reflecting fat mass. The obesity-associated *FTO* risk allele is present in 16% of adults resulting in an increased risk for obesity with about 30% for one and 70% for two copies. The function of the gene product of *FTO* (fat mass and obesity associated) is largely unknown. A recent bioinformatics analysis suggests that it may serve as a 2-oxoglutarate-dependent nucleic acid demethylase. Functional data could confirm such a function and also suggested nuclear localization. The link between demethylation and fat mass remains to be elucidated. FTO genotypes seem to affect metabolic variables in line with the effect on BMI. However, there has also been found an association between FTO variants and measures of insulin resistance in obese children and adolescents that appear to be independent of BMI.

7. Genetic influences on age-related decline in mitochondrial dysfunction

Since genes are transcribed to RNA, RNA translated into proteins, and defects in proteins cause disease the ultimate goal would be to carry out a random search of expressed proteins in target tissues. This may not yet be completely feasible but the study of large-scale transcript profiles is. This approach has been successful in defining prognosis of cancers but for complex diseases affecting many target tissues it may not be that simple. Moreover, defining what is differentially expressed among more than 20,000 gene transcripts on a chip is a statistical challenge. Despite these problems analysis of gene-expression in skeletal muscle of patients with T2D and prediabetic individuals has provided new insights into the pathogenesis of the disease applying pathway analysis rather than analysis of expression of single genes. This is based

upon the assumption that if one member of the pathway shows altered expression, this will be translated into the whole pathway. Transcriptome analysis has shown that genes regulating oxidative phosphorylation in mitochondria exhibit a 20% coordinated down-regulation in skeletal muscle from prediabetic and diabetic individuals compared with non-diabetic controls. A similar down-regulation of the gene encoding a master regulator of oxidative phosphorylation, the PPARγ co-activator PGC-1α was also observed. Thus OXPHOS genes, has emerged as central in the pathogenesis of T2D suggesting that impaired mitochondrial function and impaired oxidation of fat may predispose to T2D diabetes through a "thrifty gene" mechanism. By studying young and elderly twins we could demonstrate that elderly carriers of a Gly482Ser polymorphism in the PGC-1α gene had decreased expression of the PGC-1α in skeletal muscle, suggesting that genetic variants determine the age-related decline in expression of key genes regulating oxidative phosphorylation. This study gives an example of how genetic factors, in combination with non-genetic factors, can influence gene-expression, which subsequently affects glucose and fat metabolism. The interaction between genetic and non-genetic factors may be even more complex and involve epigenetic factors such as DNA methylation and histone modifications. So far, the knowledge of the influence of epigenetic factors on the pathogenesis of T2D remains limited.

8. Gene–environment interactions

The rapid increase in T2D during the past 50 years must be ascribed to changes in the environment rather than to genes, as the genetic background has not changed during this period. But the genetic background determines how we respond to the environment. The PPARγ receptor is a good example of an interaction between genes and the environment. PPARγ activators have become a major new type of anti-diabetic drugs (thiazolidinediones) while dietary long-chain polyunsaturated fatty acids are supposedly natural ligands for PPARγ. The importance of the genetic variation in *PPARG* as a significant modulator of physiological responses to dietary fat in humans has been demonstrated in several studies. The different genotype carriers show different association between intake of total fat, fat subtypes, and obesity. There are also data to suggest that the protective effect of the Ala allele is influenced by the degree of saturation of ingested fat. This may not be too surprising since free fatty acids have been proposed as natural ligands for PPARγ.

9. Pharmacogenetics

An important goal of genetics is to use the information to improve treatment, i.e. to identify individuals who are more likely than others to respond to a specific therapy. This has been shown in neonatal diabetic patients with the *KCJN11* mutation. When the patients were switched from insulin to sulfonylurea their symptoms markedly improved. This was especially dramatic for the severe neurological symptoms often associated with the disease. Patients with MODY3 (HNF-1α mutations) are supersensitive to treatment with sulfonylureas whereas they respond poorly to treatment with metformin . It has also recently been shown that individuals with the *TCF7L2* risk genotype respond poorly to treatment with sulfonylureas, eventually as a consequence of their more severe impairment in beta-cell function.

10. Genetic prediction of type 2 diabetes

A few studies have tried to use genetic variants to predict future T2D. Lyssenko et al. showed that the Pro12Pro genotype in *PPARG* predicted future T2D in individuals with BMI ≥ 30 kg/m^2 and fasting plasma glucose >5.5 mmol/l with an OR of 1.7 . The TT risk genotype of SNP 44 in *CAPN10* increased this risk in an additive manner to an OR of 2.7. More recently we

showed that risk genotype carriers of *TCF7L2* had a 1.5-fold increased risk of developing future T2D in two independent studies. Combining the risk variants in *TCF7L2* with those in *PPARG* and *KCJN11* increased the OR to 3 (Lyssenko V, unpublished observations). In keeping with this observation, Weedon et al. showed that each risk allele of these three genes increased the OR by 1.28 yielding an additive OR of 5.78 in a cross-sectional study. It can be expected that cross-sectional studies will give higher risk estimates than prospective studies, as they tend to include more severe cases of T2D.

11. Future directions

Dissecting the genetics of T2D is still a complicated task but by no means the nightmare task that it used to be. The last 10 years, particularly the last two, have brought about tangible breakthroughs and we can now list at least eighteen genes that have consistently been shown to increase the risk of T2D. We are likely to see a doubling of this number within the next one to two years. Meanwhile, these genes explain only a small proportion (≈0.3) of the individual risk of T2D (λs of 3). Although we now seem to cover approximately 75% of the genetic map of T2D, the variants detected most likely represent "low hanging fruit" or common variants. It is possible that there are more rare variants with stronger effects not detected with our current methodology. These variants are most likely seen in patients with early-onset forms of diabetes or in individuals with a marked β-cell dysfunction. It is unlikely that genotyping using high-density DNA arrays can detect these rare variants. Their detection will rather require sequencing. Sequencing of the whole genome was once a dream, but with new technology this dream may become true in a very near future. The role of copy number variations in the pathogenesis of disease has been highlighted in the past years but the tools to detect these CNVs have limited further exploration. This problem may be solved with the introduction of new DNA chips with a much better coverage of CNVs. We are only beginning to realize that epigenetic alteration (DNA methylation, histone acetylation and deacetylation) can introduce epigenetic changes during life-time. Such changes may influence age-related changes in gene-expression and thereby contribute to age-related diseases. Until now, DNA methylation has been studied by laborious bisulfite sequencing of single genes. In the future, the possibility of whole genome DNA methylation studies may shed new light on the extent and importance of these epigenetic effects. Dissection of the genetic complexity of T2D and obesity may thus be possible after all. Besides these issues that are mostly related to or depend on methodology the real task will be to probe the interaction between genes, environment, and treatment and how to bring these results back to subjects who have developed T2D or are at risk of developing the disease.

(Based on the article written by Martin Ridderstråle et al and published in Molecular and Cellular Endocrinology, 297(2009), pp. 10-17)

Asthma Genetics and Genomics 2009

Asthma is a complex disease likely influenced by multiple genetic and environmental factors. Familial aggregation of the disease has been clearly demonstrated, suggesting that genetic factors are important influences on disease susceptibility. A variety of candidate genes have been assessed for asthma susceptibility, based on both known pathophysiology and positional cloning. Multiple potential susceptibility genes have been identified for both asthma and COPD using these approach.

To date genetic association studies on asthma have used candidate gene approach, on the basis of their location function or both. Genome studies have linked asthma-related traits to several chromosomal regions. Table 1 presents a list of 43 genes and their chromosomal location that have been associated with asthma phenotype in atleast one study of samples of greater than a total of 300 subjects. Additional criteria for inclusion included replication in at least one other population and replication with the same SNP. As can be seen from the table, these are relatively small number of genes with many replications. The most frequently replicated are: TNF alpha, IL4, FCERB, Adam33 and GSTP1. Several genes have been identified by likage and fine mapping (ADAM33, DPP10, GPR 154 and PHF11) and one gene has been identified by GWAS (ORMD3).

Table 1

Gene	Ref seq.
ADAM33	chr20
ADRB2	chr5
CCL11	chr17
CCL24	chr 7
CCL5	chr 17
CD14	chr5
CH13L1	chr1
CTLA4	chr2
CX3CR1	chr3
CYSLTR2	chr13
DPP10	chr2
FCER1B	chr11
GPR154	chr7
GSTP1	chr11
HAVCR1	chr5
IFN gamma	chr12
IL10	chr1
IL12b	chr5
IL13	chr5
IL4	chr5
IL4R	chr16
INPP4A	chr2
IRAK-3	chr12
ITGB3	chr17
LTA	chr6
MYLK	chr3
NAT2	chr8
NOD1	chr7
NOS3	chr7
NPPA	chr1
ORMDL3	chr17
PAFAH	chr6
PHF11	chr13
PTGDR	chr14
TBXA2R	chr19
NGFB1	chr19
TLR4	chr9
TLR9	cjr3
TLR10	chr4
TNF	chr6
UGB (CC10)	chr11
VDR	chr12

(Based on the article published in "Curent Opinion in Genetics and Development" vol 19(3), p. 279, June 2009 written by Scott T W; Benjamin AR and Angela R)

ABSTRACTS

A role for JAK2 mutations in myeloproliferative diseases.

Morgan Kelly J*et al.*

Annu. Rev. Med. 2008, **59,** 213-22

Myeloproliferative disorders (MPDs) are characterized by a clonal expansion of myeloid cells. Over the past two years, the identification of the JAK2V617F mutation in most cases of polycythemia vera (PV) as well as □50% of patients with essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF) has greatly advanced our understanding of MPDs. TheJAK2V617F mutation alters theJAK2 tyrosine kinase to confer constitutive activation and affect downstream signaling pathways. Data from mouse models demonstrate that the mutation is sufficient for development of PV, but additional work is needed to better understand how this allele functions in ET and IMF. Regardless of the various pathologies, the JAK2V617F discovery highlights the importance ofJAK-STAT signaling in myeloid differentiation and focuses effort on developing a clinically relevant JAK2 inhibitor.

Copy number variation and susceptibility to human disorders (Review).

Shastry, Barkur S

Molecular Medicine Reports (2009), **2(2),** 143-147

A large no. of analysis of a new form of genetic variation, known as copy no. variation (CNV), have been published recently as a new tool for understanding the genetic basis of complex traits such as diabetes, asthma, Crohn's disease, autism and bipolar disorder.

Through the use of different types of genome-wide scanning procedures, CNVs were shown to be assocd. with several complex and common disorders, including nervous system disorders. One of the common features of the regions assocd. with the complex and common disorders identified thus far is the presence of CNVs and segmental duplications. Segmental duplications lead to genome instability. Because of their location and nature (several contain genes), many CNVs have functional consequences, such as gene dosage alteration, the disruption of genes and the modulation of the activities of other genes. Therefore, these genetic variations have an influence on phenotypes, the susceptibility of an individual to disease, drug response and human genome evolution. These types of variants (gain and loss of DNA) are not restricted to humans, having also been identified in other organisms. Our current knowledge regarding CNVs and their heritability is still rudimentary, due to their location in regions of complex genomic structure and to the tech. limitations of assocn. studies. Future advances in the technol. will aid in the construction of a new CNV map, used to find the genes underlying common diseases and to understand familial genetic conditions, severe developmental defects in humans and other organisms, and genome evolution.

Epigenetic contributions to cancer metastasis.

Rodenhiser, David I

Clinical & Experimental Metastasis (2009), **26(1),** 5-18

The molecular basis of cancer encompasses both genetic and epigenetic alterations. These epigenetic changes primarily involve global DNA methylation changes in the form of widespread loss of methylation along with concurrent hyper-methylation events in gene regulatory regions that can repress tissue-specific gene expression. Increasingly, the importance of these epigenetic changes to the metastatic process is being realized. Cells may acquire an epi-genotype that permits their dissemination from the primary tumor mass or the ability to survive and proliferate at a secondary tissue site. These epigenetic changes may be cancer-type specific, or in some cases may involve a common target gene providing a selective advantage to multiple metastatic cell types. In this review, the growing volume of literature related to the epigenetic contributions to cancer metastasis. I discuss the functional importance of these epigenetic phenomena and how new epigenetic biomarkers may permit the identification of diagnostic signatures of

metastasis and the development of new cancer therapies.

Ethical and practical challenges surrounding genetic and genomic research in developing countries.

Nyika, Aceme, *et al.*

Acta Tropica (In Press)

The nature of some potential benefits and risks associated with genetic research is different from the types of potential benefits and risks associated with other types of health research such as clinical trials and biomedical research involving humans. Whereas most potential risks associated with biomedical research or clinical trials are mainly biological in nature, potential risks associated with genetic research are mainly of socioeconomic nature. Although the peculiarity of some of the aspects of genetic research and the complexity of the science involved are acknowledged, the extent to which these characteristics hinder firstly disclosure of information to participants and their communities and secondly comprehension of the disclosed information is a practical challenge that tends to be exaggerated in some cases. In this article, a brief overview of the various types of genetic research will be given in order to set the scene for some ethical and practical issues surrounding the research in developing countries that will be discussed subsequently. Case studies that illustrate some of the ethical and practical issues flagged will be given, followed by suggestions on possible ways of tackling some of the challenges in developing country settings. Nevertheless, genetic and genomic research could go a long way in providing knowledge that could be useful in the development of drugs and vaccines for many diseases affecting the developing countries.

Genetic determinants of aggressive breast cancer.

Ventura C A *et al.*

Ann. Rev. Med. 2008; **59,** 199-212.

The development and spread of breast and other human cancers are caused by the overexpression, mutation, and/or deletion of specific genes that drive these events. Finding genetic and molecular differences between cancerous and healthy cells can reveal the genetic determinants of cancer. This knowledge results in a better understanding of the carcinogenic process and improved predictive power, with implications for identifying new drug targets, designing novel therapies, and improving preclinical and clinical studies. The concepts of biomarker, genetic marker, and genetic determinant in cancer, with particular focus on the most aggressive and lethal form of breast cancer, termed inflammatory breast cancer (IBC) have been reviewed. Using IBC as an example, we describe in detail the approaches to identify the genes that are responsible for-and not merely associated with-this disease.

Genetic communication between fetus and mother: short- and long-term consequences.

Holzgreve, Wolfgang *et al.*

American Journal of Obstetrics and Gynecology, **196(4),** 372(Apr., 2007)

Cigarette smoking in those who are alcohol dependent is associated with higher morbidity and mortality. The A1 allele of the D2 dopamine receptor (DRD2) gene has been independently associated with alcohol and nicotine dependence. Whether this polymorphism is associated with nicotine dependence in those who are also alcohol dependent has not been investigated. Subjects were 84 (61 males; 23 females) Caucasian DSM IV diagnosed nicotine- and alcohol-dependent subjects sampled from consecutive admissions to a hospital alcohol detoxification ward. Data were obtained through standardised measures of nicotine and alcohol consumption and dependence severity. A1+ allelic (A1/A1 or A1/A2 genotype) compared to A1- allelic (A2/A2 genotype only) patients were characterised by higher levels of alcohol and cigarette consumption. A1+ allelic patients

reported greater alcohol dependence severity, but not nicotine dependence severity. When the combined nicotine and alcohol dose was examined, A1+ allelic patients consumed significantly more of these drugs than their A1- allelic counterparts.

Genetic epidemiology studies in hereditary non-polyposis colorectal cancer.

Scott Rodney J *et al.*

Methods in molecular biology (Clifton, N.J.) (2009), **472** 89-102

Genetic epidemiology studies in hereditary non-polyposis colorectal cancer (HNPCC) have the potential to radically improve assessment of disease risk such that more individualised information can be provided to patients susceptible to developing disease. Studies of HNPCC initially focused on disease associations and the definition of the disease and its association with different cancers within the context of an inherited predisposition. With the identification of the genetic basis of HNPCC, new insights into the disease have been forthcoming and many advances in our understanding have been made. There have been many reports examining potential modifier genes in HNPCC, yet the results remain controversial as many findings have not been replicated and therefore no clear consensus as to the role of specific modifier genes has been reached. This review focuses on some of the factors associated with disease risk in HNPCC and where some of the difficulties lie in assessing the value of genetic epidemiology studies in this disorder.

Genetic predisposition and environmental risk factors to pancreatic cancer.

Landi, Stefano

Mutation Research, Reviews in Mutation Research (2009), **681(2-3),** 299-307.

A review. Some cases of pancreatic cancer (PC) are described to cluster within families. With the exception of PALLD gene mutations, which explain only a very modest fraction of familial cases, the genetic basis of familial PC is still obscure. Here the literature was reviewed in order to list the known genes, environmental factors, and health conditions assocd. with PC or involved in the carcinogenesis of the pancreas. Most of the genes listed are responsible for various well-defined cancer syndromes, such as CDKN2A (familial atypical mole-multiple melanoma, FAMMM), the mismatch repair genes (Lynch Syndrome), TP53 (Li-Fraumeni syndrome), APC (familial adenomatous polyposis), and BRCA2 (breast-ovarian familial cancer), where PC is part of the cancer spectrum of the disease. In addn., in this review I ranked known/possible risk factors extending the anal. to the hereditary pancreatitis (HP), diabetes, or to specific environmental exposures such as smoking. It appears that these factors contribute strongly to only a small proportion of PC cases. Recent work has revealed new genes somatically mutated in PC, including alterations within the pathways of Wnt/Notch and DNA mismatch repair. These new insights will help to reveal new candidate genes for the susceptibility to this disease and to better ascertain the actual contribution of the familial forms.

Histone deacetylase inhibitors target diabetes via chromatin remodeling or as chemical chaperones?

Lawless M W *et al.*

Current diabetes reviews (2009), **5(3),** 201-9.

Globally, obesity and diabetes (particularly type 2 diabetes) represents a major challenge to world health. Despite decades of intense research efforts, the genetic basis involved in diabetes pathogenesis & conditions associated with obesity are still poorly understood. Recent advances have led to exciting new developments implicating epigenetics as an important mechanism underpinning diabetes and obesity related disease. One epigenetic mechanism known as the "histone code" describes the idea that

specific patterns of post-translational modifications to histones act like a molecular "code" recognised and used by non-histone proteins to regulate specific chromatin functions. One modification which has received significant attention is that of histone acetylation. The enzymes which regulate this modification are described as lysine acetyltransferases or KATs and histone deacetylases or HDACs. Due to their conserved catalytic domain HDACs have been actively targeted as a therapeutic target. Some of the known inhibitors of HDACs (HDACi) have also been shown to act as "chemical chaperones" to alleviate diabetic symptoms. In this review, authors discuss the available evidence concerning the roles of HDACs in regulating chaperone function and how this may have implications in the management of diabetes.

How will genetic approaches assist in the management of respiratory diseases?

Hall, Ian P *et al.*

Current Opinion in Pharmacology, **9(3),** 256 (June 2009)

There are a number of ways in which understanding the genetic basis of either disease pathogenesis or treatment response might be of value in the management of respiratory diseases. Although some single gene defect respiratory diseases such as cystic fibrosis exist, the majority of common respiratory diseases are caused by the interaction of multiple genes and environmental factors. Newer approaches including the use of genome wide association studies will help define the underlying genetic factors. Understanding the genetic basis for disease will throw new light on the pathophysiology of disease, may help redefine disease subtypes, and may also contribute to therapeutic approaches. The aim of this review is to assess the current state of our knowledge of these areas of research.

Inherited mitochondrial diseases of DNA replication.

Copeland WC. *et al*.

*Annu Rev Med.***,** 2008**; 59:**131-46

Mitochondrial genetic diseases can result from defects in mitochondrial DNA (mtDNA) in the form of deletions, point mutations, or depletion, which ultimately cause loss of oxidative phosphorylation. These mutations may be spontaneous, maternally inherited, or a result of inherited nuclear defects in genes that maintain mtDNA. This review focuses on our current understanding of nuclear gene mutations that produce mtDNA alterations and cause mitochondrial depletion syndrome (MDS), progressive external ophthalmoplegia (PEO), ataxia-neuropathy, or mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). To date, all of these etiologic nuclear genes fall into one of two categories: genes whose products function directly at the mtDNA replication fork, such as POLG, POLG2, and TWINKLE, or genes whose products supply the mitochondria with deoxynucleotide triphosphate pools needed for DNA replication, such as TK2, DGUOK, TP, SUCLA2, ANT1, and possibly the newly identified MPV17.

Identification of copy number abnormalities and inactivating mutations in two negative regulators of nuclear factor-α B signaling pathways in Waldenstroem's macroglobulinemia.

Braggio, Esteban *et al.*

Cancer Research (2009), **69(8),** 3579-3588

Waldenstrom's macroglobulinemia (WM) is a distinct clinicobiol. entity defined as a B-cell neoplasm characterized by a lymphoplasmacytic infiltrate in bone marrow (BM) and IgM paraprotein prodn. Cytogenetic analyses were historically limited by difficulty in obtaining tumor metaphases, and the genetic basis of the disease remains poorly defined. Here, we performed a comprehensive anal. in 42 WM patients by using a high-resoln., array-based comparative genomic hybridization approach to unravel the genetic mechanisms

assocd. with WM pathogenesis. Overall, 83% of cases have chromosomal abnormalities, with a median of three abnormalities per patient. Gain of 6p was the second most common abnormality (17%), and its presence was always concomitant with 6q loss. A minimal deleted region, including MIRN15A and MIRN16-1, was delineated on 13q14 in 10% of patients. Of interest, we reported biallelic deletions and/or inactivating mutations with uniparental disomy in tumor necrosis factor (TNF) receptor-assocd. factor 3 and TNF□-induced protein 3, two neg. regulators of the nuclear factor-□B (NF-□B) signaling pathway. Furthermore, we confirmed the assocn. between TRAF3 inactivation and increased transcriptional activity of NF-□B target genes. Mutational activation of the NF-□B pathway, which is normally activated by ligand receptor interactions within the BM microenvironment, highlights its biol. importance, and suggests a therapeutic role for inhibitors of NF-□B pathway activation in the treatment of WM.

Impact of genetic risk information and type of disease on perceived risk, anticipated affect, and expected consequences of genetic tests

Cameron, Linda D. *et al.*

Health Psychology, **28(3),** 307 (May 2009)

Genetic tests vary in their prediction of disease occurrence, with some mutations conferring relatively low risk and others indicating near certainty. The authors assessed how increments in absolute risk of disease influence risk perceptions, interest, and expected consequences of genetic tests for diseases of varying severity. Adults (N = 752), recruited from New Zealand, Australia, and the United Kingdom for an online analogue study, were randomly assigned to receive information about a test of genetic risk for diabetes, heart disease, colon cancer, or lung cancer. The lifetime risk varied across conditions by 10% increments, from 20% to 100%. Participants completed measures of perceived likelihood of disease for individuals with mutations, risk-related affect, interest, and testing consequences. Analyses revealed two increment clusters yielding differences in likelihood perceptions: A "moderate-risk" cluster (20%-70%), and a "high-risk" cluster (80%-100%). Risk increment influenced anticipated worry, feelings of risk, testing-induced distress, and family obligations, with nonlinear patterns including disproportionately high responses for the 50% increment. Risk increment did not alter testing interest or perceived benefits. These patterns of effects held across the four diseases. Magnitude of risk from genetic testing has a nonlinear influence on risk-related appraisals and affect but is unrelated to test interest.

In this article, a brief overview of the various types of genetic research will be given in order to set the scene for some ethical and practical issues surrounding the research in developing countries that will be discussed subsequently. Case studies that illustrate some of the ethical and practical issues flagged will be given, followed by suggestions on possible ways of tackling some of the challenges in developing country settings. Nevertheless, genetic and genomic research could go a long way in providing knowledge that could be useful in the development of drugs and vaccines for many diseases affecting the developing countries.

Inflammation and colorectal cancer.

Kraus, Sarah; Arber, Nadir.

Current Opinion in Pharmacology (2009), **9(4),** 405-410.

Patients with long-standing inflammatory bowel disease (IBD) have an increased risk of developing colorectal cancer (CRC). However, the underlying mechanisms are not entirely clear. A genetic basis for the increased risk of CRC in IBD patients is only a partial explanation. It is possible that high levels of inflammatory mediators that are produced in this setting may contribute to the development

and progression of CRC. Growing evidence supports a role for various cytokines, released by epithelial and immune cells, in the pathogenesis of IBD-assocd. neoplasia. Two key genes in the inflammatory process, cyclooxygenase-2 (COX-2) and nuclear factor kappaB (NF-□B), provide a mechanistic link between inflammation and cancer while other factors such as, TNF-□ and IL-6-induced signaling have been recently shown to promote tumor growth in exptl. models of colitis-associated cancer. This article reviews the pathogenesis of IBD-related CRC and summarizes the molecular mechanisms underlying the development of intestinal neoplasia in the setting of chronic inflammation.

International patterns of cancer incidence in adolescents.

Stiller, Charles A *et al.*

Cancer Treatment Reviews, **33(7),** 631 (Nov., 2007)

International patterns of childhood cancer incidence are well documented but equivalent information relating to adolescence is scarce. This article synthesises international data on cancer in adolescents from population based cancer registries. Total incidence ranged from 95 to 255 per million person years in the series studied. The highest rates were in Australia and among Jews in Israel and the lowest in India and Japan. Lymphomas were the most frequent cancers in western industrialised countries of the northern hemisphere and in the Middle East, and occurred in substantial numbers in all other regions. Hodgkin lymphoma outnumbered non-Hodgkin in western industrialised countries but was relatively rare in most developing countries and in Japan. Leukaemias were the most frequent diagnostic group in India, East Asia and Latin America. Melanoma was the commonest cancer of adolescents in Australia and New Zealand and moderately frequent in many other predominantly white populations but rarely seen elsewhere. Kaposi sarcoma was the most frequent cancer in both sub-Saharan African series studied. The highest rates for nasopharyngeal carcinoma were in Algeria and Hong Kong and for liver carcinoma in Hong Kong and sub-Saharan Africa. Testicular germ cell tumours were relatively frequent in predominantly white populations. Central nervous system tumours and thyroid carcinoma were most often registered in countries with higher standard of living. Osteosarcoma was moderately frequent almost everywhere. Characteristic embryonal tumours of childhood and the most common carcinomas of adulthood were rarely seen. Only osteosarcoma, ovarian germ cell tumours and, in some populations, nasopharyngeal carcinoma have their highest incidence at age 15-19 years. Total cancer incidence was higher in adolescent males than females, but there was often a female excess in melanoma and thyroid carcinoma, and Hodgkin lymphoma was at least as frequent among females as males in several countries with relatively high incidence. More complete delineation of worldwide patterns of cancer in adolescence would be facilitated by availability of more data classified in a standard way to take account of morphology.

Low frequency variants in the exons only encoding isoform A of HNF1A do not contribute to susceptibility to type 2 diabetes.

Jafar-Mohammadi Bahram *et al.*

PloS one (2009), **4(8),** e6615

There is considerable interest in the hypothesis that low frequency, intermediate penetrance variants contribute to the proportion of Type 2 Diabetes (T2D) susceptibility not attributable to the common variants uncovered through genome-wide association approaches. Genes previously implicated in monogenic and multifactorial forms of diabetes are obvious candidates in this respect. In this study, we focussed on exons 8-10 of the HNF1A gene since rare, penetrant mutations in these exons (which are

only transcribed in selected HNF1A isoforms) are associated with a later age of diagnosis of Maturity onset diabetes of the young (MODY) than mutations in exons 1-7. The age of diagnosis in the subgroup of HNF1A-MODY individuals with exon 8-10 mutations overlaps with that of early multifactorial T2D, and we set out to test the hypothesis that these exons might also harbour low-frequency coding variants of intermediate penetrance that contribute to risk of multifactorial T2D. We performed targeted capillary resequencing of HNF1A exons 8-10 in 591 European T2D subjects enriched for genetic aetiology on the basis of an early age of diagnosis (< or =45 years) and/or family history of T2D (> or =1 affected sibling). PCR products were sequenced and compared to the published HNF1A sequence. We identified several variants (rs735396 [IVS9-24T>C], rs1169304 [IVS8+29T>C], c.1768+44C>T [IVS9+44C>T] and rs61953349 [c.1545G>A, p.T515T] but no novel non-synonymous coding variants were detected. We conclude that low frequency, nonsynonymous coding variants in the terminal exons of HNF1A are unlikely to contribute to T2D-susceptibility in European samples. Nevertheless, the rationale for seeking low-frequency causal variants in genes known to contain rare, penetrant mutations remains strong and should motivate efforts to screen other genes in a similar fashion.

Molecular pathogenesis of cutaneous melanocytic neoplasms.

Ibrahim, Nageatte

Annual Review of Pathology: Mechanisms of Disease (2009), **4,** 551-579.

A review. Melanoma is the deadliest form of skin cancer without an effective treatment. An understanding of the genetic basis of melanoma has recently shed light on some of the mechanisms of melanoma genesis. This review explores the major genes involved in familial and sporadic cutaneous melanoma with an emphasis on CDKN2A, CDK4, MC1R, and MAPK pathway targets (e.g., RAS and BRAF), apoptosis regulators (e.g., BCL-2, AKT, and APAF-1), and the tumor-suppressor genes TP53 and PTEN. New directions for therapeutics based on our current knowledge of the genes implicated in melanoma are also discussed.

Natural selection and the molecular basis of electrophoretic variation at the coagulation F13B locus.

Ryan, Anthony W

European Journal of Human Genetics (2009), **17(2),** 219-227

Electrophoretic analysis of protein variation at the coagulation F13B locus has previously revealed three alleles, with alleles 1, 2, and 3 each being at high frequency in European, African, and Asian populations, resp. To det. if this unusual pattern of interpopulation differentiation reflects local natural selection or neutral genetic drift, we re-sequenced 4.6 kb of the gene, encompassing all exons, splice junctions, and 1.4 kb of the promoter, in African, European, and Asian samples. These analyses revealed three major lineages, which correspond to the common protein alleles and differ from each other at a non-synonymous substitution in exon 3 and a novel splice acceptor in intron K. There is previous evidence that these lineages are not functionally equivalent; authors therefore carried out case-control analyses and confirmed that variability at F13B modulates susceptibility and/or survivorship in coronary artery disease (P<0.05) and type II diabetes within the coronary artery disease cohort (P<0.01). Tajima's D and Fu and Li's tests did not indicate significant departures from neutral expectations. However, publicly available data from SeattleSNPs and HapMap do indicate highly unusual levels of population differentiation (P=0.003) and an excess of allele-specific, extended haplotype homozygosity within the African population (P=0.0125). Possible causes of this putative signal of selection include hematophagous

organisms, infection by pathogens that cause disseminated intravascular coagulation, and metabolic or dietary factors.

Neurobiology and treatment of Parkinson's disease

Schapira, Anthony H.V. *et al.*

Trends in Pharmacological Sciences, **30(1),** 41 (Jan., 2009)

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease and is an important cause of chronic disability. Numerous important advances have been made in our understanding of the aetiopathogenesis, pathology and clinical phenomenology of this disease, and these have underpinned advances in symptomatic treatment and the prospect that these might be extended into interventions that will slow progression. It is notable that the continuing characterisation of the downstream biochemical consequences of the genetic causes of PD serves only to reinforce this notion. Progress in the management of PD has continued, particularly in timing of drug initiation and the sequence and combinations in which drugs are used to improve long-term outcome and reduce drug-induced complications. Particular progress has been made in the field of neuroprotection, where novel therapies and clinical trial designs are being tested. This review will focus particularly upon this area.

Nicotine dependence and genetic variation in the nicotinic receptors.

Bierut, Laura Jean *et al.*

Drug and Alcohol Dependence, **104(Suppl. 1),** S64 (Oct., 1, 2009)

Technological advances have led to the discovery of genetic variants that contribute to many illnesses including nicotine dependence. A multi-stage model of the development of nicotine dependence underlies these genetic studies, and it includes a progression through several stages of smoking behavior from never smoking to nicotine dependence. The final step in this model of dependence is the progression from established smoking behavior to the development of nicotine dependence. Contrasting individuals who smoke only a few cigarettes per day, or "chippers", to heavy smoking, nicotine dependent subjects, focuses a genetic study on the transition from smoking to nicotine dependence. This approach has identified distinct genetic variants that contribute to nicotine dependence on chromosome 15 in the region of the [alpha]5-[alpha]3-[beta]4 family of nicotinic receptor genes. This region of association includes an amino acid change in the [alpha]5 nicotinic receptor protein, which is most likely a biological variant altering the risk of developing dependence. There is also evidence that other variants alter [alpha]5 nicotinic receptor gene expression and potentially the risk of smoking. The discovery of these genetic variants and their contribution to the development of nicotine dependence highlight some of the many challenges in genetic studies. The first is that the prevalence of risk alleles can vary across populations so that a genetic risk factor can have a larger or small effect in a population depending on its frequency. The second challenge is that the risk that each genetic variant contributes in the development of a disorder is small and so it is many genes along with environmental risk factors that contribute to the development of a disorder. Interestingly, recent genetic studies of lung cancer and chronic obstructive pulmonary disease demonstrate that this same region has an important genetic influence on these disorders. Finally, there are differences in the risk of developing nicotine dependence based on gender and socioeconomic status. As our understanding of the genetic contributions of nicotine dependence increases, we may improve and personalize our treatments for smoking cessation and enhance our knowledge of other smoking related diseases in those who are at high risk for the many adverse consequences of smoking.

On the origin of cancer: Evolution and

a mutation paradox.

Erren, Thomas C

Medical Hypotheses (2009), **73(1),** 124-125.

This paper discusses the different rationalization and provides an answer on the basis of evolutionary considerations regarding the question, 'Is cancer a genetic program with an unknown function'. It is stated that cancer is not a 'program', neither specific nor unspecific, and it does not have a 'function'. Cancer is generally an 'evolutionary legacy', esp. it is an outcome of changes in genes which are conditions sine qua non for evolution.

Osteoporosis in inflammatory bowel disease

Ali, Tauseef *et al.*

The American Journal of Medicine, **122(7),** 599(July 2009)

Osteoporosis commonly afflicts patients with inflammatory bowel disease, and many factors link the 2 states together. A literature review was conducted about the pathophysiology of osteoporosis in relation to inflammatory bowel disease. Screening guidelines for osteoporosis in general as well as those directed at patients with inflammatory bowel disease are reviewed, as are currently available treatment options. The purpose of this article is to increase physician awareness about osteopenia and osteoporosis occurring in patients with inflammatory bowel disease and to provide basic, clinically relevant information about the pathophysiology and guidelines to help them treat these patients in a cost-effective manner.

Pharmacoepigenetics: its role in interindividual differences in drug response.

Gomez, A. *et al.*

Clinical Pharmacology & Therapeutics (New York, NY, United States) (2009), **85(4),** 426-430

Pharmacoepigenetics potentially offers another level of explanation for interindividual variations in drug response that cannot be accounted for on the basis of genetic polymorphism. Many genes encoding enzymes, drug transporters, transcription factors, drug targets, and nuclear receptors are under epigenetic control. In addn., microRNAs (miRNAs) that govern the expression of these genes have recently been identified. This finding has implications, for example, in the context of drug resistance during cancer treatment. This review focuses on the role of DNA methylation and miRNAs in drug pharmacokinetics.

Pro-inflammatory genetic background and zinc status in old atherosclerotic subjects.

Giacconi, Robertina *et al.*

Ageing Research Reviews, **7(4),** 306 (Dec., 2008)

Inflammation and genetics are prominent mechanisms in the pathogenesis of atherosclerosis (AT) and its complications. In this review authors discuss the possible impact on AT development of several genetic determinants involved in inflammation, oxidative stress and cytoprotection (IL-6, TNF-[alpha], IL-10, CD14, TLR4, MT, HSP70). Genetic polymorphisms of these genes may affect a differential inflammatory response predisposing to AT. However, allelic polymorphisms of genes which increase the risk of AT frequently occur in the general population but, only adequate gene-environment-polymorphism interactions promote the onset of the disease. Zinc deficiency has been suggested as an environmental risk factor for AT. With advancing age, the incidence of zinc deficiency increases for several reasons. Among them, dietary intake, malabsorption and genetic background of inflammatory markers may be involved. A crucial contribution may also be played by increased oxidative stress which may lead to the appearance of dysfunctional proteins, including metallothioneins (MT) that are in turn involved in zinc homeostasis. The detection of candidate genes related to

inflammation and promoting AT and their reciprocal influence/interaction with zinc status might allow earlier appropriate dietary interventions in genetically susceptible subjects.

Prostate cancer genomics: towards a new understanding.

Witte, John S

Nature Reviews Genetics (2009), **10(2),** 77-82

Recent genome-wide assocn. and expression array studies have provided new insights into prostate cancer genetics. The germline and somatic variants identified in these studies have been proposed to predict prostate cancer risk and aggressiveness. These results are discussed in the context of their implications for the screening and treatment of prostate cancer. Recent genetics and genomics studies of prostate cancer have helped to clarify the genetic basis of this common but complex disease. Genome-wide studies have detected numerous variants associated with disease as well as common gene fusions and expression 'signatures' in prostate tumors. On the basis of these results, some advocate gene-based individualized screening for prostate cancer, although such testing might only be worthwhile to distinguish disease aggressiveness. Lessons learned from these studies provide strategies for further deciphering the genetic causes of prostate cancer and other diseases.

RNA and Disease

Cooper, Thomas A.

Cell, **136(4),** 777(Feb., 20, 2009)

Cellular functions depend on numerous protein-coding and noncoding RNAs and the RNA-binding proteins associated with them, which form ribonucleoprotein complexes (RNPs). Mutations that disrupt either the RNA or protein components of RNPs or the factors required for their assembly can be deleterious. Alternative splicing provides cells with an exquisite capacity to fine-tune their transcriptome and proteome in response to cues. Splicing depends on a complex code, numerous RNA-binding proteins, and an enormously intricate network of interactions among them, increasing the opportunity for exposure to mutations and misregulation that cause disease. The discovery of disease-causing mutations in RNAs is yielding a wealth of new therapeutic targets, and the growing understanding of RNA biology and chemistry is providing new RNA-based tools for developing therapeutics.

Sequence-based advances in the definition of cancer-associated gene mutations.

Simpson Andrew J

Current opinion in oncology (2009), **21(1),** 47-52

Recent rapid progress in DNA sequencing has permitted projects to be undertaken that are aimed at building unbiased genome-wide portraits of the underlying mutations in human tumors. This review sets out the highlights of the recent progress in this area and the rapidly evolving picture of the underlying genetic basis of human epithelial cancers Individual tumors are estimated to contain around 80 point mutations in protein coding genes of which 15 are likely to be tumorigenic. It is likely that there are hundreds of different genes that when mutated contribute to human tumorigenesis most in only a small fraction of tumors. Mutations caused by large chromosomal rearrangements also appear to be common in tumors. In prostate and lung cancers, recurrent chromosomal translocations resulting in tumorigenic fusion proteins have been identified The multitude of new mutated genes being identified in human tumors represent many new directions for experimental research into the molecular pathways that lead to tumor formation. These studies, in turn, are likely to lead to many novel approaches to targeted therapy useful in subsets of tumors with particular types of gene mutation.

Silver-Russell and Beckwith-

Wiedemann syndromes: opposite (Epi)mutations in 11p15 result in opposite clinical pictures.

Eggermann, Thomas

Hormone Research (2009), **71(Suppl. 2),** 30-35

Progress in the identification of the (epi) genetic basis of imprinting disorders has provided greater insight into the central role of imprinted genes in regular human growth. In additional to the well-known Prader-Willi, Angelman, and Beckwith-Wiedemann syndromes, imprinting disturbances have recently been identified in transient neonatal diabetes mellitus, uniparental disomy (14) syndromes and Silver-Russell syndrome (SRS). Among these diseases, the growth retardation disorder SRS is unique because it is the first human disorder associated with epigenetic mutations that affect two different chromosomes. In addition to maternal uniparental disomy of chromosome 7, hypomethylation of the imprinting control region 1 in 11p15 and maternal duplication of 11p15 have recently been described as major (epi) genetic disturbances in SRS. Interestingly, opposite (epi)-mutations are involved in the overgrowth disease Beckwith-Wiedemann syndrome (BWS). Thus SRS and BWS can be regarded as two genetically and clinical opposite clinical pictures. Although not yet completely understood, SRS and BWS can be used as models to decipher the functional link between the observed (epi) genetic mutations and the clinical features in individuals with disturbed growth. Future studies will clarify the complex basis of human growth and hopefully contribute to better-directed therapies.

Telomeres and disease.

Lansdorp PM**.**

EMBO J. 2009 Sep 2**; 28(17):**2532-40. Epub 2009 Jul 23

The telomeres of most eukaryotes are characterized by guanine-rich repeats synthesized by the reverse transcriptase telomerase. Complete loss of telomerase is tolerated for several generations in most species, but modestly reduced telomerase levels in human beings are implicated in bone marrow failure, pulmonary fibrosis and a spectrum of other diseases including cancer. Differences in telomerase deficiency phenotypes between species most likely reflect a tumour suppressor function of telomeres in long-lived mammals that does not exist as such in short-lived organisms. Another puzzle provided by current observations is that family members with the same genetic defect, haplo-insufficiency for one of the telomerase genes, can present with widely different diseases. Here, the crucial role of telomeres and telomerase in human (stem cell) biology is discussed from a Darwinian perspective. It is proposed that the variable phenotype and penetrance of heritable human telomerase deficiencies result from additional environmental, genetic and stochastic factors or combinations thereof

The clinical implications of the genetics of renal cell carcinoma.

Rosner, Inger *et al.*

Urologic Oncology: Seminars and Original Investigations (2009), **27(2),** 131-136

Over the last several decades, the advances in molecular genetics have elucidated kidney cancer gene pathways. Kidney cancer is a heterogeneous disorder. Each specific type of kidney cancer has its own histology features, gene, and clinical course. Insight into the genetic basis of kidney cancer has been learned largely from the study of the familial or hereditary forms of kidney cancer. Extirpative surgery is currently the treatment of choice for kidney cancer that is confined to the kidney. Treatment for advanced or metastatic kidney cancer is a formidable challenge with the traditional therapies currently available. However, investigation of the Mendelian single-gene syndromes, like von Hippel Lindau (VHL: VHL gene), hereditary papillary renal

carcinoma (HPRC: c-Met gene), Birt-Hogg-Dube (BHD: BHD gene), and hereditary leiomyomatosis renal cell cancer (HLRCC: fumarate hydratase gene) provides an opportunity to develop pathway specific therapies

The complex relationship between folate/homocysteine metabolism and risk of Down syndrome.

Coppedè, Fabio *et al.*

Mutation Research/Reviews in Mutation Research, **682(1),** 54 (Aug., 2008)

Folates are essential nutrients that are required for one-carbon biosynthetic and epigenetic processes. A deficiency in cellular folates results in aberrant DNA methylation, point mutations, chromosome breakage, defective chromosome recombination and aneuploidy. In 1999 it was first reported that impairments in folate/homocysteine metabolism, due to genetic polymorphisms of metabolic enzymes, could increase the risk for having an infant with Down syndrome (DS). That paper stimulated considerable investigation into the possible role of folate/homocysteine metabolism in the risk of having a DS child and several studies have been performed so far in different countries to better address this issue. However, despite 10 years of active research, the question is still unsolved. Overall, both in vitro and in vivo studies indicate that an impaired folate/homocysteine metabolism can result in chromosome 21 nondisjunction; however, the birth of a DS child seems to be the result of the interplay of several factors of genetic, epigenetic, environmental, and stochastic origin, making it difficult to discriminate the single contribution of each of them. My opinion is that it is now time for the design of a collaborative study large enough to have the power to separate trisomy 21 into all its component parts and to test for the contribution of folate/homocysteine gene polymorphisms to each of them. This study should be paralleled by in vitro and in vivo studies aimed at clarifying the contribution, if any, of folate/homocysteine metabolism to the methylation pattern of regions involved in recombination and segregation of chromosome 21. Further studies are also required to address the possible contribution of both the paternal diet and the maternal grandmother dietary habits to chromosome 21 nondisjunction events.

The etiology of autoimmune thyroid disease: A story of genes and environment

Tomer, Yaron *et al.*

Journal of Autoimmunity, **32(3-4),** 231 (June 2009)

Autoimmune thyroid diseases (AITDs), including Graves' disease (GD) and Hashimoto's thyroiditis (HT) are prevalent autoimmune diseases, affecting up to 5% of the general population. Autoimmune thyroid diseases arise due to complex interactions between environmental and genetic factors. Significant progress has been made in our understanding of the genetic and environmental triggers contributing to AITD. However, the interactions between genes and environment are yet to be defined. Among the major AITD susceptibility genes that have been identified and characterized is the HLA-DR gene locus, as well as non-MHC genes including the CTLA-4, CD40, PTPN22, thyroglobulin, and TSH receptor genes. The major environmental triggers of AITD include iodine, medications, infection, smoking, and possibly stress. Recent data on the genetic predisposition to AITD lead to novel putative mechanisms by which the genetic-environmental interactions may lead to the development of thyroid autoimmunity.

The GAIT system: a gatekeeper of inflammatory gene expression.

Mukhopadhyay, Rupak *et al.*

Trends in Biochemical Sciences, **34(7),** 324 (July 2007).

Functionally related genes are coregulated by specific RNA-protein interactions that direct transcript-selective translational control.

In myeloid cells, interferon (IFN)-[gamma] induces formation of the heterotetrameric, IFN-[gamma]-activated inhibitor of translation (GAIT) complex comprising glutamyl-prolyl tRNA synthetase (EPRS), NS1-associated protein 1 (NSAP1), ribosomal protein L13a and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). This complex binds defined 3' untranslated region elements within a family of inflammatory mRNAs and suppresses their translation. IFN-[gamma]-dependent phosphorylation, and consequent release of EPRS and L13a from the tRNA multisynthetase complex and 60S ribosomal subunit, respectively, regulates GAIT complex assembly. EPRS recognizes and binds target mRNAs, NSAP1 negatively regulates RNA binding, and L13a inhibits translation initiation by binding eukaryotic initiation factor 4G. Repression of a post-transcriptional regulon by the GAIT system might contribute to the resolution of chronic inflammation.

The genetic and molecular basis of Fanconi anemia.

de Winter, Johan P.

Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis (2009), **668(1-2),** 11-19

The capacity to maintain genomic integrity is shared by all living organisms. Multiple pathways are distinguished that safeguard genomic stability, most of which have originated in primitive life forms. In human individuals, defects in these pathways are typically assocd. with cancer proneness. The Fanconi anemia pathway, one of these pathways, has evolved relatively late during evolution and exists - in its fully developed form - only in vertebrates. This pathway, in which thus far 13 distinct proteins have been shown to participate, appears essential for error-free DNA replication. Inactivating mutations in the corresponding genes underlie the recessive disease Fanconi anemia (FA). In the last decade the genetic basis of this disorder has been uncovered by a variety of approaches, including complementation cloning, genetic linkage anal. and protein assocn. studies. Here we review these approaches, introduce the encoded proteins, and discuss their possible role in ensuring genomic integrity.

The genetic basis of cancer of the kidney.

Grubb, Robert L., III *et al.*

Genetic Diseases of the Kidney (2009), 497-508.

A review discusses the role of genetics in renal cancer in the last two decades which has led to the identification of at least four distinct hereditary syndromes assocd. with increased risk of renal cell cancer (RCC): von Hippel-Lindau, hereditary papillary renal cancer, Birt-Hogg-Dube, and hereditary leiomyomatosis and RCC The characteristic histopathol. of each syndrome has aided in phenotypic differentiation and gene discovery with resultant elucidation of underlying mol. mechanisms.

The genetic basis of malignant arrhythmias and cardiomyopathies

Campuzano, Óscar *et al.*

Revista Española de Cardiología (English Edition), **62(4),** 422(Apr., 2009)

The remarkable advances that have taken place in biomedicine over the past 50 years have resulted in dramatic improvements in the prevention, diagnosis, and treatment of many diseases. Although cardiology has adopted these advances at a relatively slow pace, today it is fully immersed in this revolution and has become one of the most innovative medical specialties. Research is continuing to give rise to new developments in genetics and molecular biology that lead, almost daily, to innovative ways of preventing, diagnosing, and treating the most severe forms of heart disease. Consequently, it is essential that clinical cardiologists have some basic knowledge of genetics and molecular biology as these disciplines are having an increasing influence on clinical practice.

The genetics of human autoimmune disease

Invernizzi, Pietro *et al.*

Journal of Autoimmunity(In Press)

Autoimmune diseases are known to have a multifactorial pathogenesis, with both environmental and inherited components. Wide technical progresses together with the completion of the sequencing of human genome have recently allowed the identification of new genetic risk variants in many autoimmune disorders. While part of these studies confirmed previous knowledge, most of the data has disclosed novel and unsuspected roles in the development of autoimmunity for molecules involved in various pathogenic pathways. After the current first wave of data from high-density genome-wide studies, we now need to further characterize these genetic factors and find additional ones, possibly among rare variants. In addition, a role for sex chromosomes in the development of autoimmune diseases has also been suggested. This review covers the recent discoveries related to genetics of autoimmunity.

Unravelling the genetic basis of variable clinical expression in neurofibromatosis 1.

Sabbagh, Audrey *et al.*

Human Molecular Genetics (2009), **18(15),** 2768-2778

Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder which displays considerable inter-and intra-familial variability in phenotypic expression. To evaluate the genetic component of variable expressivity in NF1, the phenotypic correlations between affected relatives in 750 NF1 patients from 275 multiplex families collected through the NF-France Network was examined. Twelve NF1-related clinical features, including five quant. traits (no. of cafe-au-lait spots of small size and of large size, and number of cutaneous, s.c. and plexiform neurofibromas) and seven binary ones, were scored. All clinical features studied, with the exception of neoplasms, showed significant familial aggregation after adjusting for age and sex. For most of them, patterns of familial correlations indicated a strong genetic component with no apparent influence of the constitutional NF1 mutation. Heritability ests. of the five quant. traits ranged from 0.26 to 0.62. Moreover, we investigated for the first time the role of the normal NF1 allele in the variable expression of NF1 through a family-based assocn. study. Nine tag SNPs in NF1 were genotyped in 1132 individuals from 313 NF1 families. No significant deviations of transmission of any of the NF1 variants to affected offspring was found for any of the 12 clinical features examd., based on single marker or haplotype anal. Taken together, our results provided evidence that genetic modifiers, unlinked to the NF1 locus, contribute to the variable expressivity of the disease.

Value of genetic profiling for the prediction of coronary heart disease

van der Net, Jeroen B. *et al.*

American Heart Journal, **158(1),** 105 (July 2009)

Advances in high-throughput genomics facilitate the identification of novel genetic susceptibility variants for coronary heart disease (CHD). This may improve CHD risk prediction. The aim of the present simulation study was to investigate to what degree CHD risk can be predicted by testing multiple genetic variants (genetic profiling). We simulated genetic profiles for a population of 100,000 individuals with a 10-year CHD incidence of 10%. For each combination of model parameters (number of variants, genotype frequency and odds ratio [OR]), we calculated the area under the receiver operating characteristic curve (AUC) to indicate the discrimination between individuals who will and will not develop CHD. The AUC of genetic profiles could rise to 0.90 when 100 hypothetical variants with ORs of 1.5 and genotype frequencies of 50% were simulated.

The AUC of a genetic profile consisting of 10 established variants, with ORs ranging from 1.13 to 1.42, was 0.59. When 2, 5, and 10 times as many identical variants would be identified, the AUCs were 0.63, 0.69, and 0.76. To obtain AUCs similar to those of conventional CHD risk predictors, a considerable number of additional common genetic variants need to be identified with preferably strong effects.

Whole-genome association study identifies STK39 as a hypertension susceptibility gene.

Wang, Ying *et al.*

Proceedings of the National Academy of Sciences of the USA (2009), **106(1),** 226-231

Hypertension places a major burden on individual and public health, but the genetic basis of this complex disorder is poorly understood. The authors conducted a genome-wide assocn. study of systolic and diastolic blood pressure (SBP and DBP) in Amish subjects and found strong assocn. signals with common variants in a serine/threonine kinase gene, STK39. The authors confirmed this assocn. in an independent Amish and 4 non-Amish Caucasian samples including the Diabetes Genetics Initiative, Framingham Heart Study, GenNet, and Hutterites (meta-anal. combining all studies: n = 7125, P < 10-6). The higher BP-assocd. alleles have frequencies >0.09 and were assocd. with increases of 3.3/1.3 mm Hg in SBP/DBP, resp., in the Amish subjects and with smaller but consistent effects across the non-Amish studies. Cell-based functional studies showed that STK39 interacts with WNK kinases and cation-chloride cotransporters, mutations in which cause monogenic forms of BP dysregulation. The authors demonstrate that in vivo, STK39 is expressed in the distal nephron, where it may interact with these proteins. Although none of the assocd. SNPs alter protein structure, the authors identified and exptl. confirmed a highly conserved intronic element with allele-specific in vitro transcription activity as a functional candidate for this assocn. Thus, variants in STK39 may influence BP by increasing STK39 expression and consequently altering renal Na^+ excretion, thus unifying rare and common BP-regulating alleles in the same physiol. pathway.

Association of IL-10 receptor 2 (IL10RB) SNP with systemic sclerosis.

Hikami, Koki *et al.*

Biochemical and Biophysical Research Communications, **373(3),** 403 (Aug., 29, 2008)

Interleukin-10 (IL-10) signaling has been suggested to play a role in systemic sclerosis (SSc). IL10RB codes for IL-10 receptor 2 (IL-10R2), a component shared in receptor complexes for IL-10, IL-22, IL-26 and interferon (IFN)-[lambda]. In this study, authors examined association of IL10RB polymorphism with susceptibility to SSc. Genotype A/A at rs2834167 (47K/K) was significantly increased in diffuse cutaneous SSc (dcSSc) (41.3% in dcSSc, 20.9% in controls, P = 0.0018, odds ratio = 2.67). A SNP in the 5' flanking region of IL10RB, rs999788, also showed association with dcSSc; however, this association was shown to be secondarily caused by linkage disequilibrium with rs2834167. Significant association was not observed in limited cutaneous SSc (lcSSc). Presence of anti-topoisomerase I antibody was also associated with rs2834167A/A genotype (P = 0.0019). Serum IL-10 level was significantly associated with the number of rs2834167A allele (P = 0.007). These findings suggested that signaling through IL-10R2 may play a causative role in dcSSc.

Brain mitochondria as a primary target in the development of treatment strategies for Alzheimer disease.

Aliev, Gjumrakch *et al.*

The International Journal of Biochemistry & Cell Biology, **41(10),** 1989 (Oct., 2009)

Alzheimer's disease (AD) and cerebrovascular accidents are two leading causes of age-related dementia. Increasing evidence supports the idea that chronic

hypoperfusion is primarily responsible for the pathogenesis that underlies both disease processes. In this regard, hypoperfusion appears to induce oxidative stress (OS), which is largely due to reactive oxygen species (ROS), and over time initiates mitochondrial failure which is known as an initiating factor of AD. Recent evidence indicates that chronic injury stimulus induces hypoperfusion seen in vulnerable brain regions. This reduced regional cerebral blood flow (CBF) then leads to energy failure within the vascular endothelium and associated brain parenchyma, manifested by damaged mitochondrial ultrastructure (the formation of large number of immature, electron-dense "hypoxic" mitochondria) and by overproduction of mitochondrial DNA (mtDNA) deletions. Additionally, these mitochondrial abnormalities co-exist with increased redox metal activity, lipid peroxidation, and RNA oxidation. Interestingly, vulnerable neurons and glial cells show mtDNA deletions and oxidative stress markers only in the regions that are closely associated with damaged vessels, and, moreover, brain vascular wall lesions linearly correlate with the degree of neuronal and glial cell damage. Authors summarize the large body of evidence which indicates that sporadic, late-onset AD results from a vascular etiology by briefly reviewing mitochondrial damage and vascular risk factors associated with the disease and cerebral microvascular changes reason for the energy failure that occurs in normal aging and, to a much greater extent, AD.

Cardiovascular disease and heritability of the prothrombotic state.

Ajjan, R.A. *et al.*

*Blood Reviews***23(2),** 67 (Mar., 2009)

Atherothrombotic disease remains a major cause of mortality worldwide, and family clustering suggests an important contribution of genetic factors to disease pathogenesis. Thrombus formation represents the final step in atherothrombosis, a process influenced by genetic and environmental factors. A major difficulty of investigating the genetic regulation of thrombotic conditions is the complexity of the phenotype and the relatively modest effects of individual genetic variations. We address in this review genetic aspects involved in regulating thrombosis potential and their impact on the development of atherothrombotic disease. The effects of common genetic polymorphisms in clotting factors are discussed and examples of complex gene-gene and gene-environment interactions are highlighted. Understanding the effects of genetic factors on predisposition to thrombotic disease and unravelling the complex gene-environment interactions will help to better understand the pathophysiology of this complex condition, which will enable the development of new preventative and treatment strategies.

Clinical and genetic study of Japanese patients with type 3 Gaucher disease.

Tajima, Asako *et al.*

Molecular Genetics and Metabolism, **97(4),** 272 (Aug., 2008)

Information on the phenotypic variations seen in patients with type 3 (chronic neuronopathic) Gaucher disease (GD) is still limited compared with type 1 GD. We retrospectively investigated the clinical features of 42 Japanese patients with type 3 GD. The 42 patients classified as type 3 fell into two groups: those diagnosed as having type 3 GD at diagnosis (group A; n = 24) and those thought to have type 1 at diagnosis but who later developed neurological symptoms (group B; n = 18). The genotype of group A patients varied widely; however, L444P/L444P and L444P/F213I genotypes accounted for 83% in group B. All the patients who did not receive enzyme replacement with alglucerase or imiglucerase (4 in group A, 2 in group B) died. Nineteen patients received enzyme replacement in group A; however, 7 of these died despite the therapy. On the other hand, 14 patients received enzyme replacement alone in

group B and 13 of them survived. Among the ERT-treated patients who survived, only one of 12 in group A and 12 out of 13 in group B can walk unaided. In conclusion, some Japanese GD patients who are thought to have type 1 at diagnosis develop neurological symptoms during their clinical course, and careful observation is essential for patients with characteristic genotypes. Moreover, enzyme replacement alone might not have a sufficient effect on the early onset neurological symptoms in type 3 patients. A different treatment strategy is needed to improve the prognosis of these patients.

DNA-driven nutritional therapy of inflammatory bowel disease.

Lee, Goo *et al.*

Nutrition, **25 (9),** 885(Sept., 2009)

Inflammatory bowel disease (IBD) consists of two main disorders, ulcerative colitis and Crohn's disease, that cause chronic, recurrent inflammation of the intestine. An inappropriate immune response to the enteric ecosystem has been postulated to cause IBD. Genomewide association studies provide the information of diverse genetic variations and susceptibilities to patients with IBD. Through the application of these studies, the pathogenesis of IBD may result in part from genetic abnormalities that regulate epithelial barrier function and innate and adaptive immune responses. Crohn's disease shows strong association with CARD15, ATG15L1, and IRGM, which are involved in the innate immunity. In the adaptive immune response, IL23R, MST1, IL12B, and STAT3 polymorphisms are associated with Crohn's disease and ulcerative colitis. Current pharmacologic treatment of IBD, including 5-aminosalycylate, steroids, and immunomodulator therapy, are mainly aimed at suppressing inflammation non-specifically, except biologic therapies such as anti-tumor necrosis factor molecule, which block specific proinflammatory molecules. For nutritional issues in IBD, the mainstay of therapy has been supportive, with particular attention paid to the prevention, recognition, and therapy of nutritional deficiencies, and individual outcomes to specific dietary factors have been controversial. Parenteral nutritional support and exclusionary diets have been investigated and are not the subject of this review. The emerging concepts of nutrition-gene interaction gave birth to unique scientific fields, nutrigenetics and nutrigenomics. These studies provide information about 1) the genetic variability that induces an individual's response to nutrition according to particular states of health and disease and 2) changes in gene expression that develop as a result of nutrition-gene interaction. For IBD, the role of diet in the regulation of the immune response against gut flora is the subject of current intensive evaluation. These approaches may lead clinicians to derive a personalized nutritional prescription based on individual genetic variations and may result in a significant impact on IBD treatment.

Evaluation of the association of genetic variants on the chromosomal loci 9p21.3, 6q25.1, and 2q36.3 with angiographically characterized coronary artery disease.

Muendlein, Axele *et al.*

Atherosclerosis, **205(1),** 174 (July 2009)

The chromosomal loci 9p21.3, 6q25.1, and 2q36.3, represented by their respective leading variants rs1333049, rs6922269 and rs2943634, have been linked with a history of coronary artery disease (CAD) by genome-wide association studies. Whereas the association of variant rs1333049 with CAD was analysed in several subsequent studies, replication studies of variants rs6922269 and rs2943634 are missing. Furthermore, no direct association with coronary atherosclerosis has been established. We therefore aimed at investigating the association of the above variants with coronary atherosclerosis. We performed genotyping in two large cohorts of consecutive Caucasian patients undergoing coronary angiography for the evaluation of

suspected or established stable CAD, comprising 671 and 940 patients, respectively, with a total of 1611 subjects. In models of dominant inheritance, variant rs1333049 conferred a significantly increased risk of significant coronary stenoses with lumen narrowing >=50% in both study cohorts, with adjusted odd ratios (OR) of 1.71 (1.15-2.52); p = 0.007 and 1.55 (1.10-2.18); p = 0.012, respectively. Variant rs6922269 in neither cohort was significantly associated with CAD. Although carriers of the A allele of variant rs2943634 were at an increased risk of significant coronary stenoses in the second cohort (OR = 1.41 (1.06-1.88); p = 0.018), no such association was found for the first cohort nor for both cohorts combined. Our data from two populations show that variant rs1333049 is significantly associated with angiographically characterized CAD. In contrast, variant rs6922269 did not show any impact on coronary atherosclerosis. The association between variant rs2943634 and CAD warrants further investigation.

Genetic contributions to clinical pain and analgesia: avoiding pitfalls in genetic research.

Kim, Hyungsuk *et al.*

The Journal of Pain, **10(7)** 663(July 2009)

Understanding the genetic basis of human variations in pain is critical to elucidating the molecular basis of pain sensitivity, variable responses to analgesic drugs, and, ultimately, to individualized treatment of pain and improved public health. With the help of recently accumulated knowledge and advanced technologies, pain researchers hope to gain insight into genetic mechanisms of pain and eventually apply this knowledge to pain treatment. Authors have critically reviewed the published literature to examine the strength of evidence supporting genetic influences on clinical and human experimental pain. Based on this evidence and the experience of false associations that have occurred in other related disciplines, authors have given recommendations for avoiding pitfalls in pain genetic research.

Genetic determinants of ageing processes and diseases in later life.

Bostock, Clare V. *et al.*

Maturitas, **62(3),** 225 (Mar., 20, 2009)

To investigate the role of genetic factors in diseases of later life. Review of literature relevant to the role of genetic factors in variation of incidence of age-related diseases of later life using Medline, Web of Science, to search publications in English since 1980 and citations found in relevant publications. The identity of ageing and longevity genes remains unknown despite lively interest in lipoprotein metabolism, genomic instability, oxidative stress, cellular response to damage, inflammatory processes, insulin/IGF1-signalling and Sirtuins. Genes involved in lifespan appear remarkably conserved across species but genes that convey increased susceptibility to specific age-dependent diseases are not yet identified. Individual differences in rates of ageing and incidence of the common diseases of later life require explanation. The Sirtuins and the field of epigenetics are emerging as potentially informative research priorities. Further research includes the development of biomarkers and a greater understanding of the interaction between genes and the environment. The hypothetical treatment of ageing could retard or prevent age-associated diseases resulting in widespread health, social and economic benefit.

Genetic modulation of the pharmacological treatment of pain.

Lötsch, Jörn *et al.*

Pharmacology & Therapeutics (In Press)

Inadequately treated acute and chronic pain remains a major cause of suffering and dissatisfaction in pain therapy. A cause for the variable success of pharmacologic pain therapy is the different genetic disposition of patients to develop pain or to respond to analgesics. The patient's phenotype may be

regarded as the result of synergistic or antagonistic effects of several genetic variants concomitantly present in an individual. Variants modulate the risk of developing painful disease or its clinical course (e.g., migraine, fibromyalgia, low back pain). Other variants modulate the perception of pain (e.g., OPRM1 or GCH1 variants conferring modest pain protection by increasing the tone of the endogenous opioid system or decreasing nitric oxide formation). Other polymorphisms alter pharmacokinetic mechanisms controlling the local availability of active analgesic molecules at their effector sites (e.g., decreased CYP2D6 related prodrug activation of codeine to morphine). In addition, genetic variants may alter pharmacodynamic mechanisms controlling the interaction of the analgesic molecules with their target structures (e.g., opioid receptor mutations). Finally, opioid dosage requirements may be increased depending on the risk of drug addiction (e.g., DRD2 polymorphisms decreasing the functioning of the dopaminergic reward system). With the complex nature of pain involving various mechanisms of nociception, drug action, drug pharmacology, pain disease and possibly substance addiction, a multigenic or even genome wide approach to genetics could be required to base individualized pain therapy on the patient's genotype.

Genetic polymorphisms in non-alcoholic fatty liver disease: Interleukin-6-174G/C polymorphism is associated with non-alcoholic steatohepatitis.

Carulli, Lucia *et al.*

Digestive and Liver Disease (In Press)

Environmental and genetic factors play a role in the pathogenesis and natural history of non-alcoholic fatty liver disease (NAFLD). In 114 subjects with NAFLD we report the prevalence and correlation with clinical parameters of three polymorphisms: interleukin-6 (-174G/C), plasma cell differentiation antigen (K121Q) and microsomal transfer protein (-493G/T). In 59 biopsied patients with NAFLD the polymorphisms were also related to histological features. IL-6 -174C variant was more prevalent ($p<0.01$) in NAFLD compared to controls. In the NAFLD group, C carriers had higher HOMA-IR and fasting insulin than G carriers ($p<0.05$). The prevalence of IL-6/C variant was higher (83%) in biopsied than in not biopsied subjects (66%) ($p<0.05$). In biopsied subjects, C carriers had higher HOMA and fasting insulin ($p<0.05$) compared than those with G allele. The prevalence of IL-6 -174G/C polymorphism was significantly higher in NASH than in NAFLD ($p=0.048$). At logistic regression analysis IL-6 -174C was an independent predictor of both NAFLD (OR 4.116, C.I. 1.126-15.048) and NASH (OR 7.035, C.I. 1.167-42.394). Conversely, the distribution of PC-1 and MTP polymorphisms was not significantly different compared to the control group, nor associated with clinical or histological characteristics. Our data suggest that IL-6 -174C genetic polymorphisms, involved in inflammation and insulin resistance, are associated with NASH. These data may contribute to the understanding of the genetic susceptibility to NAFLD.

Genetic susceptibility in Parkinson's disease.

Bras, Jose Miguel *et al.*

Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, **1792(7),** 597 (July 2009)

It is hoped that an understanding of the genetic basis of Parkinson's disease (PD) will lead to an appreciation of the molecular pathogenesis of disease, which in turn will highlight potential points of therapeutic intervention. It is also hoped that such an understanding will allow identification of individuals at risk for disease prior to the onset of motor symptoms. A large amount of work has already been performed in the identification of genetic risk factors for PD and some of this work, particularly those efforts that focus on genes implicated in monogenic

forms of PD, have been successful, although hard won. A new era of gene discovery has begun, with the application of genome wide association studies; these promise to facilitate the identification of common genetic risk loci for complex genetic diseases. This is the first of several high throughput technologies that promise to shed light on the (likely) myriad genetic factors involved in this complex, late-onset neurodegenerative disorder.

Genetic variation in the ABCA1 gene, HDL cholesterol, and risk of ischemic heart disease in the general population.

Frikke-Schmidt, Ruth *et al.*

Atherosclerosis (In Press)

Epidemiological studies consistently demonstrate a strong inverse association between low levels of high-density lipoprotein (HDL) cholesterol and increased risk of ischemic heart disease (IHD). This review focuses on whether both rare and common genetic variation in ABCA1 contributes to plasma levels of HDL cholesterol and to risk of IHD in the general population, and further seeks to understand whether low levels of HDL cholesterol per se are causally related to IHD. Studies of the ABCA1 gene demonstrate a general strategy for detecting functional genetic variants, and show that both common and rare ABCA1 variants contribute to levels of HDL cholesterol and risk of IHD in the general population. The association between ABCA1 variants and risk of IHD appears, however, to be independent of plasma levels of HDL cholesterol. With the recent identification of the largest number of individuals heterozygous for loss-of-function mutations in ABCA1 worldwide, population studies suggests that genetically low HDL cholesterol per se does not predict an increased risk of IHD, and thus questions the causality of isolated low levels of HDL cholesterol for the development of IHD.

Impaired mitochondrial dynamics and function in the pathogenesis of Parkinson's disease.

Büeler, Hansruedi *et al.*

Experimental Neurology, **218(2),** 235 (Aug., 2008)

Parkinson's disease (PD), the most frequent movement disorder, is caused by the progressive loss of the dopamine neurons within the substantia nigra pars compacta (SNc) and the associated deficiency of the neurotransmitter dopamine in the striatum. Most cases of PD occur sporadically with unknown cause, but mutations in several genes have been linked to genetic forms of PD ([alpha]-synuclein, Parkin, DJ-1, PINK1, and LRRK2). These genes have provided exciting new avenues to study PD pathogenesis and the mechanisms underlying the selective dopaminergic neuron death in PD. Epidemiological studies in humans, as well as molecular studies in toxin-induced and genetic animal models of PD show that mitochondrial dysfunction is a defect occurring early in the pathogenesis of both sporadic and familial PD. Mitochondrial dynamics (fission, fusion, migration) is important for neurotransmission, synaptic maintenance and neuronal survival. Recent studies have shown that PINK1 and Parkin play crucial roles in the regulation of mitochondrial dynamics and function. Mutations in DJ-1 and Parkin render animals more susceptible to oxidative stress and mitochondrial toxins implicated in sporadic PD, lending support to the hypothesis that some PD cases may be caused by gene-environmental factor interactions. A small proportion of [alpha]-synuclein is imported into mitochondria, where it accumulates in the brains of PD patients and may impair respiratory complex I activity. Accumulation of clonal, somatic mitochondrial DNA deletions has been observed in the substantia nigra during aging and in PD, suggesting that mitochondrial DNA mutations in some instances may pre-dispose to dopamine neuron death by impairing respiration. Besides compromising cellular energy production, mitochondrial dysfunction is associated with the generation of oxidative stress, and

dysfunctional mitochondria more readily mediate the induction of apoptosis, especially in the face of cellular stress. Collectively, the studies examined and summarized here reveal an important causal role for mitochondrial dysfunction in PD pathogenesis, and suggest that drugs and genetic approaches with the ability to modulate mitochondrial dynamics, function and biogenesis may have important clinical applications in the future treatment of PD.

Influence of C-159T SNP of the CD14 gene promoter on lung function in smokers.

Zhou, Haibo *et al.*

Respiratory Medicine, **103(9),** 1358 (Sept., 2009)

CD14, a co-receptor for endotoxin, plays a significant role in regulating the inflammatory response to this agent. The C-159T single nucleotide polymorphism (SNP) in the CD14 gene promoter is an important regulator of CD14 expression, with TT homozygotes having increased expression of CD14. This SNP has been linked to pathogenesis of asthma and with cardiovascular diseases in smokers. We hypothesize that CD14 also plays a role in the pathophysiology of COPD in smokers who are exposed to endotoxin contained in cigarette smoke as well as endotoxin derived from Gram-negative microbes colonizing their airways. To assess the effect of the C-159T SNP of the CD14 gene promoter on lung function, we recruited 246 smokers 40 years of age or older with a range of 10-156 pack-year smoking exposures. The TT genotype was associated with lower lung function in smokers with a moderate smoking history. However, the CC genotype was associated with decreased lung function in heavy smokers (>56 pack years). The effect of CC genotype on severity of COPD is analogous with the effect of this genotype in risk for asthma. CD14 may be a factor in the pathophysiology of COPD, as it is in asthma and smoking-related cardiovascular diseases.

Interaction between *Helicobacter pylori,* diet, and genetic polymorphisms as related to non-cancer diseases.

Izzotti, Alberto *et al.*

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, **667(1-2)**, 142 (July 10, 2009)

Helicobacter pylori is a Gram-negative bacterium that infects the stomach of more than half of the world's population. *H. pylori* infection is an established risk factor for gastric cancer, although it is not sufficient cause for the appearance of cancer, per se. Several studies have investigated the role of this bacterium in non-cancer diseases, including gastritis ulcer, duodenal ulcer, gastroesophageal reflux, cardiovascular diseases, neurodegenerative diseases, ocular diseases, and dermatological disorders. DNA damage and failure in antioxidant defences is a common denominator of many among these pathological conditions. The clinical outcome of *H. pylori* infection is dependent on many variables, including H. pylori genotype, host health status, host genotype, and host exposure to environmental factors. The role of genetic and environmental factors is reviewed in this paper. Among non-cancer diseases, idiopathic thrombocytopenic purpura appears to show the strongest link with *H. pylori*. There is an evidence for a role of CagA-positive *H. pylori* infection in atherosclerosis and ischemic heart disease. On the whole, the major factors playing a pathogenic role in *H. pylori*-related non-cancer diseases are: (a) host polymorphisms in genes involved in inflammation and protection against oxidative damage, (b) host exposure to dietary genotoxic agents, and (c) bacterial genetic polymorphisms. In conclusion, there is an evidence that mutagenesis-related mechanisms play a pathogenic role in the appearance of non-cancer diseases following *H. pylori* infection.

Is the ornithine transcarbamylase gene a genetic determinant of Alzheimer's disease?

Hansmannel, Franck *et al.*

Neuroscience Letters, **449(1),** 76(Jan., 2, 2009)

Expression of ornithine transcarbamylase (OTC) is strongly induced in the brain of individuals suffering from Alzheimer's Disease (AD). Association studies in a population from northern France have revealed that two SNPs -389 G/A (rs5963409) and -241 A/G (rs5963411) located in the promoter of the OTC gene are associated with the risk of developing AD. In the present work, these association studies were extended to a population of 2113 AD cases and 1580 controls from northern France, western France, the United Kingdom and Italy. The rs5963409 minor allele was weakly but significantly associated with an increased risk of developing AD (OR = 1.19, p = 0.004). This association was independent of age and ApoE status. Our results support that the OTC gene may be a minor genetic determinant of AD.

Recently, VEGF gene promoter polymorphisms have been associated with an increased risk for AD in the Italian population. Conversely, two subsequent studies failed to find a positive association between VEGF variability and greater risk for AD. To better clarify this issue, a meta-analysis of all published association studies has been performed. Overall, polymorphic variants within VEGF gene promoter confer greater risk for AD at least in the Italian population; the meta-analysis provides evidence of a role of the functional variant C(-2578)A in the pathogenesis of the disease, although the pooled odds ratio obtained represents a modest effect. These findings provide new evidence for an additional candidate genetic risk factor for AD that can be tested in further studies.

Regenerative pharmacology in the treatment of genetic diseases: The paradigm of muscular dystrophy.

Mozzetta, Chiara *et al.*

The International Journal of Biochemistry & Cell Biology, **41(4),** 701(Apr., 2009)

Current evidence supports the therapeutic potential of pharmacological interventions that counter the progression of genetic disorders by promoting regeneration of the affected organs or tissues. The rationale behind this concept lies on the evidence that targeting key events downstream of the genetic defect can compensate, at least partially, the pathological consequence of the related disease. In this regard, the beneficial effect exerted on animal models of muscular dystrophy by pharmacological strategies that enhance muscle regeneration provides an interesting paradigm. In this review, we describe and discuss the potential targets of pharmacological strategies that promote regeneration of dystrophic muscles and alleviate the consequence of the primary genetic defect. Regenerative pharmacology provides an immediate and suitable therapeutic opportunity to slow down the decline of muscles in the present generation of dystrophic patients, with the perspective to hold them in conditions such that they could benefit of future, more definitive, therapies.

Relationship between Fc[gamma] receptor and interleukin-1 gene polymorphisms and post-treatment apical periodontitis.

Siqueira Jr., José F *et al.*

Journal of Endodontics (In Press)

Genetic polymorphisms have been reported to act as modifiers of diverse diseases and, as such, might theoretically influence the severity and response to treatment of apical periodontitis. The purpose of this study was to investigate the association of Fc[gamma] receptor and interleukin (IL)-1 gene polymorphisms with post-treatment apical periodontitis in Brazilian individuals. The study population consisted of 18 patients with post-treatment apical periodontitis and 44 individuals with root canal-treated teeth exhibiting healthy/healing periradicular tissues (controls). Patients were typed for the following genes (alleles): Fc[gamma]RIIA

(R131 or H131), Fc[gamma]RIIIB (NA1 or NA2), IL-1A (1 or 2), and IL-1B (1 or 2). No significant statistical differences were observed for all specific genotypes and almost all allele carriage rates of the test genes as well as combinations thereof with regard to association with disease (P > .05). Actually, only 2 genetic conditions were found to be associated with post-treatment apical periodontitis: carriage of allele H131 of the Fc[gamma]RIIa gene (P = .04) and a combination of this allele with allele NA2 of the Fc[gamma]RIIIb gene (P < .01). Data from the present study suggest that some conditions associated with polymorphism of Fc[gamma] receptor genes might influence the patient's response to endodontic treatment of teeth with apical periodontitis.

Relationships among molecular genetic and respiratory properties of Parkinson's disease cybrid cells show similarities to Parkinson's brain tissues.

Borland, M. Kathleen *et al.*

Biochimica et Biophysica Acta (BBA) – *Molecular Basis of Disease,* **1792 (1),** 68 (Jan., 2009)

We have studied sporadic Parkinson's disease (sPD) from expression of patient mitochondrial DNA (mtDNA) in neural cells devoid of their own mtDNA, the "cybrid" model. In spite of reproducing several properties of sPD brain, it remains unclear whether sPD cybrid cells reflect more complex sPD brain bioenergetic pathophysiology. We characterized and correlated respiration of intact sPD cybrid cells with electron transport chain (ETC) protein assembly, complex I ETC gene expression and ETC protein levels in sPD brain. We also assayed expression for multiple ETC genes coded by mtDNA and nuclear DNA (nDNA) in sPD cybrid cells and brain. sPD cybrid cells have reduced levels of mtDNA genes, variable compensatory normalization of mitochondrial gene expression and show robust correlations with mitochondrial ETC gene expression in sPD brains. Relationships among ETC protein levels predict impaired complex I-mediated respiration in sPD brain. That sPD cybrid cells and sPD brain samples show very correlated regulation of nDNA and mtDNA ETC transcriptomes suggests similar bioenergetic physiologies. We propose that further insights into sPD pathogenesis will follow elucidation of mechanisms leading to reduced mtDNA gene levels in sPD cybrids. This will require characterization of the abnormalities and dynamics of mtDNA changes propagated through sPD cybrids over time.

SNP 668C (-44) alters a NF-[kappa]B1 putative binding site in non-coding strand of human [beta]-defensin 1 (DEFB1) and is associated with lepromatous leprosy.

Prado-Montes de Oca, Ernesto et al.

Infection, Genetics and Evolution, **9(4),** 617 (July 2009)

Leprosy is an infectious disease caused by Mycobacterium leprae. The peptide human [beta]-defensin 1 is an antimicrobial effector of innate epithelial immunity. A study was done on the association of three single nucleotide polymorphisms (SNPs) in the [beta]-defensin 1 gene (DEFB1) - 668 C/G (-44 C/G or rs1800972; in 5' UTR), 692 A/G (-20 A/G or rs11362; in 5' UTR) and A1836G (rs1800971; in 3' UTR) - with leprosy susceptibility per se and clinical leprosy variants. The SNPs were genotyped by real-time polymerase chain reaction (rt-PCR) and PCR-restriction fragment length polymorphisms. Subjects were of Mexican mestizo ethnicity from Sinaloa state, México. Analysis was done on borderline leprosy, lepromatous leprosy (L-lep) and indeterminate leprosy subgroups compared with healthy controls. The genotypes associated with L-lep and no other leprosy subgroup after Bonferroni correction were those that contain 668C in a dominant model (OR = 3.06, 95% CI 1.47-6.4, p = 0.024). Estimated haplotype CGA is over-represented in L-lep (p = 0.009; OR = 2.25, 1.23-4.03). Five NF-[kappa]B1 putative

binding sites (NPBSs) were identified with JASPAR software in non-coding strand spanning the 5' UTR and intron 1 of DEFB1, including one which is altered when SNP 668C is present. SNP 668C probably abrogates NF-[kappa]B-dependent DEFB1 upregulation leading to L-lep variant.

Stem cell and genetic therapies for the fetus.

Roybal, Jessica L. *et al.*

Seminars in Fetal and Neonatal Medicine (In Press)

Advances in prenatal diagnosis have led to the prenatal management of a variety of congenital diseases. Although prenatal stem cell and gene therapy await clinical application, they offer tremendous potential for the treatment of many genetic disorders. Normal developmental events in the fetus offer unique biologic advantages for the engraftment of hematopoietic stem cells and efficient gene transfer that are not present after birth. Although barriers to hematopoietic stem cell engraftment exist, progress has been made and preclinical studies are now underway for strategies based on prenatal tolerance induction to facilitate postnatal cellular transplantation. Similarly, in-utero gene therapy shows experimental promise for a host of diseases and proof-in-principle has been demonstrated in murine models, but ethical and safety issues still need to be addressed. Here we review the current status and future potential of prenatal cellular and genetic therapy.

Tumor necrosis factor alpha - 308 gene locus promoter polymorphism: An analysis of association with health and disease.

Elahi, Maqsood M. *et al.*

Biochimica et Biophysica Acta (BBA) - *Molecular Basis of Disease,* **1792(3),** 163(Mar., 2009)

Tumor necrosis factor-alpha (TNF-[alpha]) is a potent immunomediator and proinflammatory cytokine that has been implicated in the pathogenesis of a large number of human diseases. The location of its gene within major histocompatibility complex and biological activities has raised the possibility that polymorphisms within this locus may contribute to the pathogenesis of wide range of autoimmune and infectious diseases. For example, a bi-allelic single nucleotide substitution of G (TNFA1 allele) with A (TNFA2 allele)} polymorphism at -308 nucleotides upstream from the transcription initiation site in the TNF-[alpha] promoter is associated with elevated TNF-[alpha] levels and disease susceptibilities. However, it is still unclear whether TNF-[alpha] - 308 polymorphism plays a part in the disease process, in particular whether it could affect transcription factor binding and in turn influence TNF-[alpha] transcription and synthesis. Several studies have suggested that TNFA2 allele is significantly linked with the high TNF-[alpha]-producing autoimmune MHC haplotype HLA-A1, B8, DR3, with elevated serum TNF-[alpha] levels and a more severe outcome in diseases.

VEGF genetic variability is associated with increased risk of developing Alzheimer's disease.

Del Bo, Roberto *et al.*

Journal of the Neurological Sciences, **283(1-2),** 66(Aug., 15, 2009)

Specific polymorphisms within the vascular endothelial growth factor (VEGF) gene promoter region are of particular interest: VEGF variability has been associated with increased risk of developing a wide variety of disorders from diabetes to neurodegenerative diseases, suggesting functions not confined to its originally described vascular effects. A hypothetical loss of the VEGF-mediated neuroprotective effect has been proposed as a cause of neurodegenerative disorders. An impaired regulation of VEGF expression has been also reported in Alzheimer's disease (AD) pathogenesis.

Genetic Testing for Healthcare

Chromosomes – which are units of heredity inside cells - were first discovered in the late 1800s. In the early 1900s, inherited diseases were first linked to chromosomes. Discoveries from the 1950s through the 1980s helped scientists to develop genetic tests for genetic conditions such as Down syndrome, cystic fibrosis, and Duchenne muscular dystrophy. Genetic testing was initially used to make or confirm a diagnosis of a genetic condition, and to screen newborns for conditions such as phenylketonuria (PKU), so that early interventions and treatments could be administered.

There were few research laboratories capable of conducting genetic testing and few commercial genetic testing laboratories. Individuals with the following family history backgrounds may inquire about genetic testing:

Family history of an uncommon inherited disease such as Duchenne Muscular Dystrophy or sickle cell anemia;

A history of more common diseases such as cancer or Parkinson's Disease that affect multiple relatives in several generations of a family;

Couples considering having a baby who have a family history of inherited diseases, or who belong to an ethnic group with a higher risk of a specific inherited disease.

There are a number of different types of genetic tests available today, including:

Diagnostic testing - identifies a genetic condition or disease that is making or in the future will make a person ill. The results of diagnostic testing can help in treating and managing the disorder.

Predictive and pre-symptomatic genetic testing - finds genetic variations that increase a person's chance of developing specific diseases. This type of genetic testing may help provide information about a person's risk of developing a disease, and can help in decisions about lifestyle and health care.

Carrier testing–tells people if they "carry" a genetic change that can cause a disease. Carriers usually show no signs of the disorder; however, they can pass on the genetic variation to their children, who may develop the disorder or become carriers themselves.

Prenatal testing - is offered during pregnancy to help identify fetuses that have certain diseases.

Pre-implantation genetic testing –is done in conjunction with *in vitro* fertilization to determine if embryos for implantation carry genes that could cause disease.

Newborn screening - is used to test babies one or two days after birth to find out if they have certain diseases known to cause problems with health and development.

Pharmacogenomic testing - gives information about how certain medicines are processed in a person's body. This type of testing can help a healthcare provider choose the medicines that work best with a person's genetic makeup. For example, genetic testing is now available to guide treatments for certain cancers.

Research genetic testing – helps scientists learn more about how genes contribute to health and disease, as well as develop gene-based treatments. Sometimes the results do not directly help the research participant, but they

may benefit others in the future by helping researchers expand their understanding of the human body.

Direct-to-consumer genetic testing is a new approach that allows people to order certain genetic tests by sending a sample of their saliva or tissue to a laboratory. The laboratory returns the results only to the individual who sent the sample. Often, no healthcare provider is involved in this process. Many people are concerned about whether or not their health insurance will cover the cost of a genetic test. Not all insurance companies cover all types of genetic testing. Other concerns about genetic tests include the privacy of genetic test results, especially from insurers or employers.

In the future, genetic testing will be used to scan all of a person's genetic material, so that disease riskvariants can be identified and early intervention and treatment can be planned. The cost of testing an individual's entire genome will be less than $1,000. We will live in a time of "personalized medicine," when many treatments for medical conditions will be chosen based upon what genetic testing indicates about a patient's specific genetic makeup.

(Source: In house compilation)

RDTABSTRACTS

Agreement among type 2 diabetes linkage studies but a poor correlation with results from genome-wide association studies.

Lillioja S *et al.*

Diabetologia (2009), **52(6),** 1061-74

Little of the genetic basis for type 2 diabetes has been explained, despite numerous genetic linkage studies and the discovery of multiple genes in genome-wide association (GWA) studies. To begin to resolve the genetic component of this disease, authors searched for sites at which genetic results had been corroborated in different studies, in the expectation that replication among studies should direct us to the genomic locations of causative genes with more confidence than the results of individual studies. Authors have mapped the physical location of results from 83 linkage reports (for type 2 diabetes and diabetes precursor quantitative traits [QTs, e.g. plasma insulin levels]) and recent large GWA reports (for type 2 diabetes) onto the same human genome sequence to identify replicated results in diabetes genetic 'hot spots'. Genetic linkage has been found at least ten times at 18 different locations, and at least five times in 56 locations. All replication clusters contained study populations from more than one ethnic background and most contained results for both diabetes and QTs. There is no close relationship between the GWA results and linkage clusters, and the nine best replication clusters have no nearby GWA result. Many of the genes for type 2 diabetes remain unidentified. This analysis identifies the broad location of yet to be identified genes on 6q, 1q, 18p, 2q, 20q, 17pq, 8p, 19q and 9q. The discrepancy between the linkage and GWA studies may be explained by the presence of multiple, uncommon, mildly deleterious polymorphisms scattered throughout the regulatory and coding regions of genes for type 2 diabetes.

Apolipoprotein E4 allele influences the response of plasma triglyceride levels to tamoxifen in breast cancer patients.

Chang, Nai-Wen *et al.*

Clinica Chimica Acta, **401(1-2),** 144(Mar., 2009)

Apolipoprotein E4 (APOE4) allele is an important risk factor for breast cancer and affects clearance of chylomicron remnants. Tamoxifen therapy increases serum triglyceride levels and sometimes inducing severe hyper-triglyceridemia in breast cancer patients. Thirty-three women with breast cancer were recruited to examine the APOE polymorphism and fasting plasma lipid profiles before and after tamoxifen treatment for 6 months. Authors found that plasma lipid profiles changed in accordance with the

APOE4 allele after tamoxifen treatment for 6 months. Especially plasma triglyceride levels significantly decreased in the APOE4-positive patients ($p = 0.025$), while there was no change in APOE4-negative patients ($p = 0.189$). The total plasma cholesterol levels were reduced in APOE-4 positive patients after 6-month tamoxifen treatment ($p = 0.014$). The levels of plasma low density lipoprotein cholesterol and high density lipoprotein cholesterol significantly decreased in both APOE4-negative and APOE4-positive patients. These findings indicate that the effects of tamoxifen on plasma triglyceride levels are modified by APOE polymorphism. Breast cancer patients with APOE4 allele have low plasma triglyceride levels when receiving tamoxifen therapy. Therefore, authors suggest that APOE gene polymorphism is a critical validation before tamoxifen treatment in breast cancer patients.

Association of functionally important polymorphisms in cytochrome P4501B1 with lung cancer.

Shah, Parag P. *et al.*

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, **643(1-2),** 4 (Aug., 25, 2008)

In the present study, genotype and haplotype frequencies of four polymorphisms of cytochrome P450 1B1 (CYP1B1) that cause amino acid changes (Arg-Gly at codon 48, Ala-Ser at codon 119, Leu-Val at 432 and Asn-Ser at codon 453) were studied in 200 patients suffering from lung cancer and equal number of controls. A significant difference was observed for the distribution of variant genotypes of CYP1B1Arg48Gly and Ala119Ser polymorphisms (CYP1B1*2) in cases when compared to the controls. No significant difference was observed for the distribution of variant genotypes of CYP1B1Leu432Val (CYP1B1*3) and CYP1B1Asn453Ser (CYP1B1*4) polymorphism. When the four SNPs were analyzed using a haplotype approach, SNPs at codon 48 (Arg48Gly) and codon 119 (Ala119Ser) exhibited complete linkage disequilibrium (LD) in all the cases and controls. Significant differences in the distribution of the three haplotypes (G-T-C-A, G-T-G-A and G-T-C-G) were observed in the cases when compared to controls. Tobacco use in the form of smoking as well as chewing was found to significantly increase the risk of lung cancer in patients by interacting with CYP1B1Ala119Ser genotypes demonstrating the role of gene-environment interaction in lung cancer. Further, the risk of lung cancer increased several fold in the patients carrying the genotype combinations of CYP1B1Ala119Ser and CYP1B1Leu432Val with GSTM1, a phase II enzyme suggesting the importance of gene-gene interactions in enhancing the susceptibility to lung cancer.

Association of polymorphism in MDM-2 and p53 genes with breast cancer risk in Indian women.

Singh, Virendra *et al.*

Annals of Epidemiology, **18(1),** 48 (Jan., 2008)

Single nucleotide polymorphism (SNP) at position -309 (T309G) in MDM-2 promoter induces tumor formation in the individuals possessing inherited p53 mutations. The present study was undertaken to investigate the association of MDM-2 SNP309, p53 Arg72Pro, and p53 intron-6 G/A polymorphism with total, premenopausal, and postmenopausal breast cancer risks in Indian women. Genotyping of MDM-2 SNP309, p53 Arg72Pro, and p53 intron-6 G/A in 104 patients and 105 controls was performed either by ARMS-PCR or by polymerase chain reaction and direct sequencing. The p53 Arg72Pro heterozygous variant and in combination with its homozygous variant exhibited a significant protective association with total (odds ratio [95% confidence interval]: 0.42 [0.22-0.81] and 0.46 [0.25-0.85], p value; 0.007 and 0.012) and postmenopausal breast cancer risk (odds ratio

[95% confidence interval]: 0.25 [0.07-0.73] and 0.27 [0.08-0.77], p value; 0.009 and 0.013]. Neither combined nor homozygous/heterozygous MDM-2 SNP309G was associated with total, premenopausal, or postmenopausal breast cancer risk; however, MDM-2 SNP309G, along with p53 Arg72Pro heterozygous variant, showed a significant protective association with premenopausal breast cancer risk (odds ratio [95% confidence interval]: 0.18 [0.02-1.20], p value; 0.041 for homozygous + heterozygous MDM-2 SNP309G). The results indicate protective associations of p53 Arg72Pro heterozygous variant with postmenopausal and MDM-2 SNP309G along with p53 Arg72Pro heterozygous variant with premenopausal breast cancer risk.

Bilateral renal-cell carcinoma associated with an acquired VHL mutation and long-term trichloroethylene exposure.

Wells, G. M.; Schroth, W.

Clinical Nephrology (2009), **71(6),** 708-713

The genetic basis for clear-cell renal carcinomas has been established in familial and many sporadic forms. Whether the latter can be induced by environmental carcinogens remains controversial, with concern over solvents such as trichloroethylene (TCE). To study this putative relationship, authors analyzed the VHL gene from a patient with long-term TCE exposure. PCR amplification and sequencing of VHL exons 1-3 were performed on peripheral blood and tumor tissue. The tumor alone had a previously undescribed mutation in exon 1 of the VHL gene: deletion of a cytidine residue at position 291 relative to the first ATG start codon of the wild-type sequence. This deletion causes a frameshift and predicts an altered protein sequence from position 98 onwards. The affected amino acids are in the functionally important α-domain of the VHL protein that is implicated in substrate binding for ubiquitylation, and authors hypothesize the mutation lowers that affinity. There is loss of suppressor function when substrates such as hypoxia-inducible factor have impaired degradation; they accumulate and ultimately cause uncontrolled cell turnover. This association of a proposed occupational cause and occurrence of renal-cell carcinoma emphasizes the availability and use of VHL sequencing for both studying the pathophysiology of malignant transformation and potentially playing a clinical role in genetic counseling or risk assessment.

Computational and structural analysis of deleterious mutations in functional SNPs of CREBBP and ARHGEF12 oncogene in acute leukemia.

Doss, C. George Priya

Journal of Computational and Theoretical Nanoscience (2009), **6(7),** 1596-1604.

The vol. of known genetic variations lends itself well to an informatics approach. Predicting and understanding the downstream effects of genetic variation using computational methods are becoming increasingly important for single nucleotide polymorphism (SNP) selection in genetics studies and understanding the molecular basis of diseases, such as cancer. In this study using computational methods, authors analyzed the genetic variations that can alter the expression and the function of CREBBP and ARHGEF12 genes responsible for causing acute myeloblastic leukemia (AML). Out of the total 360 SNPs in CREBBP gene, 3 were non synonymous (nsSNPs) and also found to be damaged by both SIFT and PolyPhen server. The mutation that occurred in the native protein (1JSP) coded by CREBBP gene is at amino acid position Y1175C for nsSNPs with id (rs28937315). Out of 418 SNPs in ARHGEF12 gene, 5 were non synonymous (nsSNPs) and 20% of them were found to be damaged by both SIFT and PolyPhen. The major mutations that occurred in the native protein (1TXD) coded by ARHGEF12 gene were at amino acid positions I864M and

Y973F for nsSNPs with ids (rs686830 and rs2305013). The native and mutant structures of these genes were further analyzed by NetASA, DSSP and normal mode analysis. From the overall results obtained in this work, authors propose Y1175C in CREBBP and Y973F in ARHGEF12 genes could be considered deleterious and important mutation causing acute myeloid leukemia.

CREB is a key regulator of striatal vulnerability in chemical and genetic models of Huntington's disease.

Choi, Yun-Sik *et al.*

Neurobiology of Disease (In Press)

Evidence of dysregulation of the CREB/CRE transcriptional pathway in animal models of Huntington's disease (HD) suggests that strategies designed to augment CRE-mediated transcription may be of therapeutic value. Here, we investigated the consequences of CREB activation and repression in chemical and transgenic mouse models of HD. In the 3-nitropropionic acid (3-NP) model, CREB phospho-activation in the striatum was potently repressed within the neurotoxic "core" region prior to cell death. Conversely, marked expression of phospho-CREB, as well the CREB-regulated cytoprotective gene Bcl-2, was detected in the "penumbral" region. To examine potential contributory roles for the CREB/CRE transcriptional pathway in striatal degeneration, we used both CREB loss- (A-CREB) and gain- (VP16-CREB) of-function transgenic mouse strains. 3-NP-induced striatal lesion size and motor dysfunction were significantly increased in A-CREB mice compared to controls. Conversely, striatal damage and motor deficits were diminished in VP16-CREB mice. Furthermore, transgenic A-CREB significantly accelerated motor impairment in the YAC128 mouse model of HD. Together, these results indicate that CREB functionality is lost during the early stages of striatal cell stress and that the repression of CREB-mediated transcription contributes to the pathogenic process.

Evaluation of MLH1 and MSH2 gene mutations in a subset of Iranian families with hereditary nonpolyposis colorectal cancer (HNPCC).

Salehi, M. *et al.*

Journal of Sciences, Islamic Republic of Iran (2009), **20(1),** 7-12

Hereditary nonpolyposis colorectal cancer is the most common form of hereditary colorectal cancers accounting for 5 to 10% of all colon carcinoma. It is inherited in an autosomal dominant mode and caused by hereditary mutations in mismatch repair genes (MMR) chiefly MLH1 and MSH2. The lifetime risk of colon cancer in affected persons is 80%. Screening, prevention strategies and consequently treatment options will be improved by understanding of the genetic basis of this disorder. The aim of this study was to assess mutations in MLH1 and MSH2 genes in a subset of Iranian HNPCC patients. The families that fulfill Amsterdam criteria were selected as HNPCC families. Genomic DNA was extd. from the peripheral blood of the samples and mutations of MLH1 and MSH2 were detected by PCR-single strand conformation polymorphism (PCR-SSCP) and DNA sequencing techniques. Hereditary mutations were found in 20 cases. Of these mutations, 14 were found in MLH1 and 6 in MSH2 genes thus MLH1 gene had higher mutation rate than MSH2. Eighteen out of 20 detected mutations in our population were previously reported and two were novel. Our results demonstrated that mutation range as well as genes involved in HNPCC is different from one region to other and characterizing mutations could be very helpful in diagnosis of the at risk individuals.

Evidence for various 20q status using allelotyping, CGH arrays, and quantitative PCR in distal CIN colon cancers.

Nicolet, Celine

Cancer Letters (Shannon, Ireland) (2009), **282(2),** 195-204

The genomic aberration profile of

chromosome 20q in distal CIN colon carcinomas was analyzed using allelotyping and CGH arrays. Allelotyping revealed carcinomas with allelic imbalance along the full long arm, and carcinomas with fully non-aberrant 20q. Oligonucleotide-based CGH showed that among the carcinomas without allelic imbalance, 47% had in fact a gain. In this subgroup, quant. PCR for the TOPI gene (20q12) confirmed this gain, and fluorescence in situ hybridization showed that the chromosome 20q gain resulted from tetra/polysomy instead of aneusomy. The 20q gain correlated with a high frequency of aberrations, with allelic imbalance at TP53 locus but not at APC locus, and carcinomas with a disomic 20q showed low frequency of genomic aberrations and were significantly assocd. to mucinous phenotype. The prognostic value of 20q amplification was not demonstrated in this study. These results indicate that on the basis of aberration frequency, chromosome 20q and TP53/APC locus status, distal CIN carcinomas harbor a high degree of genetic heterogeneity suggesting several pathways for carcinogenesis. This study also indicates that allelotyping needs to be carried out with a complementary technique, such as quant. PCR.

Genetic classification of oral and oropharyngeal carcinomas identifies subgroups with a different prognosis.

Smeets, Serge J. *et al.*

Cellular Oncology (2009), **31(4),** 291-300

The common risk factors for oral and oropharyngeal cancer are tobacco smoking and alc. consumption, and recently the human papillomavirus (HPV) was shown to be involved in a subgroup. HPV-positive and -negative carcinomas can be distinguished on basis of their genetic profiles. Aim of this study was to investigate patterns of chromosomal aberrations of HPV-negative oral and oropharyngeal squamous cell carcinomas (OOSCC) in order to improve stratification of patients regarding outcome. Thirty-nine OOSCCs were classified on basis of their genetic pattern determined by array comparative genomic hybridization (aCGH). Resulting groups were related to patient and tumor characteristics using the Fisher's exact test and in addition to survival with the Kaplan-Meier and log rank tests. Classification distinguished three groups, one characterized by hardly any chromosomal aberration (N=8) and another by a relatively high level (N=26), and one with a very high level (N=5) of chromosomal aberrations. This classification was significantly ($p=0.003$) assocciated with survival, with the best survival in the genetically silent' group and the worst survival in the most aberrant group. The silent profile was significantly ($p<0.05$) assocciated with wild-type TP53, an absence of alcohol consumption and a female gender. These carcinomas were negative for microsatellite instability. This classification of OOSCC was confirmed in an independent set of 89 oral carcinomas. In conclusion, the discovery of these new classes of oral and oropharyngeal cancer with unique genetic and clinical characteristics has important consequences for future basic and clinical studies.

Genetic models of Parkinson disease.

Lim, Kah-Leong *et al.*

Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, **1792(7),** 604 (July 2009)

To date, a truly representative animal model of Parkinson disease (PD) remains a critical unmet need. Although toxin-induced PD models have served many useful purposes, they have generally failed to recapitulate accurately the progressive process as well as the nature and distribution of the human pathology. During the last decade or so, the identification of several genes whose mutations are causative of rare familial forms of PD has heralded in a new dawn for PD modelling. Numerous mammalian as well as non mammalian models of genetically-linked PD have since been created. However, despite

initial optimism, none of these models turned out to be a perfect replica of PD. Meanwhile, genetic and toxin-induced models alike continue to evolve towards mimicking the disease more faithfully. Notwithstanding this, current genetic models have collectively illuminated several important pathways relevant to PD pathogenesis. Here, the article provides a comprehensive discussion on existing genetic models of PD.

Genetic polymorphism of glutathione S-transferase M1 and T1 in Delhi population of Northern India.

Singh, Satyender *et al.*

Environmental Toxicology and Pharmacology, **28(1),** 25 (July 2009)

Glutathione S-transferases (GSTs), protect cells from reactive chemical intermediates and oxidative stress. Among different classes of GSTs, GSTM1 (Mu) and GSTT1 (theta) are found to be genetically deleted. Present study was intended to genotype homozygous null distribution of GSTM1 and GSTT1 in healthy individuals of Delhi, located in Northern India. Out of 309 healthy individuals included in this study, we have found genetic deletion in 21% and 27.4%, GSTM1 and GSTT1 genes, respectively. A small proportion (0.7%) population showed deletion of both the genes. The prevalence of the GSTM1*0/0 and GSTT1*0/0 genotypes varied within India compared to communities in Chinese, Japanese, Korean and Caucasian.

Genetic polymorphisms in cytochrome P4501B1 and susceptibility to head and neck cancer.

Singh, Arvind P. *et al.*

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, **639(1-2),** 11 (Mar., 1, 2008)

Cytochrome P4501B1 (CYP1B1), a polycyclic aromatic hydrocarbon (PAH) metabolizing CYP, is genetically polymorphic in humans and may be involved in the individual susceptibility to chemical-induced cancer. In the present study, genotype and haplotype frequencies of four single nucleotide polymorphisms (SNPs) in CYP1B1 that cause amino acid changes (Arg-Gly at codon 48, Ala-Ser at codon 119, Leu-Val at codon 432 and Asn-Ser at codon 453) were studied in 150 cases suffering from head and neck squamous cell carcinoma (HNSCC) and in an equal number of controls. A significant difference was observed for the distribution of variant genotypes of Arg48Gly (CYP1B1*2) and Ala119Ser (CYP1B1*2) polymorphisms of CYP1B1 in cases versus controls. No significant differences were observed for the distribution of variant genotypes-Leu432Val (CYP1B1*3) and Asn453Ser (CYP1B1*4), respectively. When the four SNPs were analyzed using a haplotype approach, SNPs at codon 48 (Arg48Gly) and codon 119 (Ala119Ser) exhibited complete linkage disequilibrium (LD) in all the cases and controls. Significant differences in the distribution of the two haplotypes (G-T-C-A and G-T-G-A) were observed both in the cases and in controls. Furthermore, our data indicates a several fold increase in risk in the cases who use tobacco (cigarette smoking or tobacco chewing) or alcohol with the variant genotypes of CYP1B1 (CYP1B1*2 and CYP1B1*3) suggesting the role of gene-environment interaction in the susceptibility to HNSCC.

Genetic risk profiling and prediction of disease course in Crohn's disease patients.

Henckaerts, Liesbet *et al.*

Clinical Gastroenterology and Hepatology, **7(9),** 972 (Sept., 2009)

Clinical presentation at diagnosis and disease course of Crohn's disease (CD) are heterogeneous and variable over time. Early introduction of immunomodulators and/or biologicals might be justified in patients at risk for disease progression, so it is important to identify these patients as soon as possible. Authors examined the influence of recently discovered CD-associated susceptibility loci on changes in disease behavior and evaluated

whether a genetic risk model for disease progression could be generated. Complete medical data were available for 875 CD patients (median follow-up time, 14 years; interquartile range, 7-22). Fifty CD-associated polymorphisms were genotyped. Kaplan-Meier survival analyses, multiple logistic regression, and generalized multifactor dimensionality reduction analyses (GMDR) were performed, correcting for follow-up time. Homozygosity for the rs1363670 G-allele in a gene encoding a hypothetical protein near the IL12B gene was independently associated with stricturing disease behavior (odds ratio [OR], 5.48; 95% confidence interval [CI], 1.60-18.83; P = .007) and with shorter time to strictures (P = .01), especially in patients with ileal involvement (P = .0002). Male patients carrying at least one rs12704036 T-allele in a gene desert had the shortest time to non-perianal fistula (P < .0001). The presence of a C-allele at the CDKAL1 single nucleotide polymorphism rs6908425 and the absence of NOD2 variants were independently associated with development of perianal fistula (OR, 8.86; 95% CI, 1.13-69.78; P = .04 and OR, 0.56; 95% CI, 0.38-0.83; P = .004, respectively), particularly when colonic involvement and active smoking were present. CD-associated polymorphisms play a role in disease progression and might be useful in identifying patients who could benefit from an early top-down treatment approach.

Genetic testing: considerations for pediatric nephrologists.

Guay-Woodford, Lisa M. *et al.*

Seminars in Nephrology, **29(4),** 338 (July 2009)

With the completion of the Human Genome Project and the associated advances in genomic technologies, clinicians have at their disposal an increasing repertoire of tools to provide accurate and efficient diagnosis, assess disease predisposition and risk factors, and personalize therapeutic management. To date, more than 2,000 human disease genes have been identified, including genes involved in single-gene disorders that disrupt the structure and/or function of the kidney and developing urinary tract. The use of genetic tests for diagnostic purposes increasingly is being integrated into general medical practice and therefore it is important for clinicians to be familiar with the technical approaches and ethical implications of these methods. Here, we provide an overview of the utility and limitations of current genetic tests for diagnosis, prenatal examination, carrier detection, and presymptomatic testing of hereditary disorders, with emphasis on pediatric renal disorders. In addition, we describe new technical advances that are expected to be introduced into clinical practice in the coming years.

Genetic variants in MUTYH are not associated with endometrial cancer risk.

Ashton Katie A

Hereditary cancer in clinical practice (2009), **7(1),** 3

Hereditary non-polyposis colorectal cancer (HNPCC), also known as Lynch syndrome, is an autosomal dominant inherited predisposition to a number of epithelial cancers, most notably colorectal and endometrial cancer. Outside of the context of Lynch syndrome there is little evidence for an autosomal dominant or recessive condition that predisposes to endometrial cancer. Recently, genetic variants in MUTYH have been associated with a recessive form of colorectal cancer, known as MUTYH associated polyposis or MAP. MUTYH is involved in base excision repair of DNA lesions and as such a breakdown in the fidelity of this process would necessarily not be predicted to result in a specific disease. At present there is little information about the role of MUTYH in other types of cancer and only one report indicating a possible relationship with endometrial cancer.Similar to a previous study, we investigated a series of endometrial cancer patients to determine if MUTYH variants were

over-represented compared to a series of healthy control subjects and to assess whether or not endometrial cancer risk could be explained by an autosomal recessive model of inheritance.Two MUTYH mutations, Y165C and G382D, and three common MUTYH polymorphisms, V22M, Q324H and S501F, were genotyped in 213 endometrial cancer patients and 226 controls from Australia using real time PCR. Differences in genotype frequencies were compared using Chi-squared analysis and by calculating odds ratios and 95% confidence intervals.Three endometrial cancer patients were identified with heterozygous MUTYH mutations (two G382D and one Y165C). No bi-allelic mutation carriers were identified. Two of the three patients' clinical characteristics were similar to those commonly identified in HNPCC and lend support to the notion that MUTYH mutations increase the risk of developing HNPCC related diseases. There was no difference in the five genotype frequencies of the endometrial cancer patients compared to the controls. The results of our study suggest that MUTYH is unlikely to be involved in the genetic basis of endometrial cancer but a possible association of MUTYH variants with HNPCC related diseases cannot be excluded.

Genetic variation and haplotype structures of innate immunity genes in eastern India.

Bairagya, Bijan B. *et al.*

Infection, Genetics and Evolution, **8(3),** 360 (May 2008)

This study reports results of an extensive and comprehensive study of genetic diversity in 12 genes of the innate immune system in a population of eastern India. Genomic variation was assayed in 171 individuals by resequencing ~75 kb of DNA comprising these genes in each individual. Almost half of the 548 DNA variants discovered was novel. DNA sequence comparisons with human and chimpanzee reference sequences revealed evolutionary features indicative of natural selection operating among individuals, who are residents of an area with a high load of microbial and other pathogens. Significant differences in allele and haplotype frequencies of the study population were observed with the HapMap populations. Gene and haplotype diversities were observed to be high. The genetic positioning of the study population among the HapMap populations based on data of the innate immunity genes substantially differed from what has been observed for Indian populations based on data of other genes. The reported range of variation in SNP density in the human genome is one SNP per 1.19 kb (chromosome 22) to one SNP per 2.18 kb (chromosome 19). The SNP density in innate immunity genes observed in this study (>3 SNPs kb-1) exceeds the highest density observed for any autosomal chromosome in the human genome. The extensive genomic variation and the distinct haplotype structure of innate immunity genes observed among individuals have possibly resulted from the impact of natural selection.

Genetic variations in esophageal cancer risk and prognosis.

Cheung, Winson Y. *et al.*

Gastroenterology Clinics of North America, **38(1),** 75 (Mar., 2009)

Investigations into inherited genetic variations in the DNA code (known as polymorphisms) in the field of oncology have provided preliminary support for an association with cancer risks and outcomes. Early studies have highlighted several genes with this potential predictive and prognostic power. However, these studies have had methodological limitations and have produced inconsistent results, making impractical as yet the routine evaluation of such genetic polymorphisms in general clinical practice. Continued research in this area is essential if we are to be able to soon use genetic polymorphisms to better select patients for targeted anticancer interventions. This review discusses the role of genetic polymorphisms

and their association with esophageal cancer risk and prognosis. The article also highlights future directions in this new, emerging field of molecular epidemiology.

Hypermethylation analysis of mismatch repair genes (hmlh1 and hmsh2) in locally advanced breast cancers in Indian women.

Naqvi, Raza Ali *et al.*

Human Pathology, **39(5),** 672 (May 2008)

Alterations in protooncogenes and tumor-suppressor genes at the DNA and/or protein level, which indicate the biological properties of individual breast cancers, led us to design a study encompassing the dilemma of "epigenetic silencing-driven genomic instabilities." In this study, we analyzed the promoter methylation of potent mismatch repair genes (hmlh1 and hmsh2) for the first time in 232 Indian patients with primary breast cancer (using methylation-specific polymerase chain reaction and expressional analysis). The study evaluates the gamut of epigenetic aberrations as well as genomic instabilities (microsatellite instabilities and loss of heterozygosity) and includes analysis of BAT-25, BAT-26, D2S123, D5S346, and D17S250. We observed hypermethylation of the hmlh1 gene in 43.5% of patients with primary breast cancer, of whom 66.9% had locally advanced breast cancer (stage IIIA, IIIB, and IIIC) ($P < .0001$). Similarly, authors also found hypermethylation of the hmsh 2 gene in 16% of primary breast cancer cases. Of these patients, 21.3% had locally advanced breast cancer ($P = 01$). To determine the effect of methylation, authors also performed expressional studies using reverse transcriptase polymerase chain reaction and Northern blotting, but authors were unable to get any significant expression in the presence of hypermethylation of either gene (hmlh1 and hmsh2). Interestingly, statistical analysis revealed that hypermethylation of the hmlh1 gene is one of the peculiar attributes of locally advanced breast cancer. In addition, this study indicates that for more sensitive stage-specific diagnosis or prognosis, both methylation of promoter and expression studies must be considered in the analyses in a reproducible manner. Therefore, pinpointing the methylation fingerprints (5'CpG island methylation) of potent DNA repairing genes not only shows the specific attributes of locally advanced breast cancer but also provides important insight into the mode of therapy to be used by clinical oncologists.

Identification and *in silico* analysis of functional SNPs of the BRCA1 gene.

Rajasekaran, R. *et al.*

Genomics, **90(4),** 447 (Oct., 2007)

Single-nucleotide polymorphisms (SNPs) play a major role in the understanding of the genetic basis of many complex human diseases. Also, the genetics of human phenotype variation could be understood by knowing the functions of these SNPs. It is still a major challenge to identify the functional SNPs in a disease-related gene. In this work, authors have analyzed the genetic variation that can alter the expression and the function of the BRCA1 gene using computational methods. Of the total 477 SNPs, 65 were found to be nonsynonymous (ns) SNPs. Among the 14 SNPs in the untranslated region, 4 were found in the 5' and 10 were found in the 3' untranslated region (UTR). It was found that 16.9% of the nsSNPs were damaging, by both the SIFT and the PolyPhen servers. The UTR Resource tool suggested that 2 of 4 SNPs in the 5' UTR and 3 of 10 SNPs in the 3' UTR might change the protein expression levels. We identified major mutations from proline to serine at positions 1776 and 1812 of the native protein of the BRCA1 gene. From a comparison of the stabilizing residues of the native and mutant proteins, we propose that an nsSNP (rs1800751) could be an important candidate for the breast cancer caused by the BRCA1 gene.

Identifying disease associations via genome-wide association studies.

Huang Wenhui *et al.*

BMC Bioinformatics (2009), **10 Suppl 1** S68.

Genome-wide association studies prove to be a powerful approach to identify the genetic basis of different human diseases. Researchers studied the relationship between seven diseases characterized in a previous genome-wide association study by the Wellcome Trust Case Control Consortium. Instead of doing a horizontal association of SNPs to diseases, we did a vertical analysis of disease associations by comparing the genetic similarities of diseases. Our analysis was carried out at four levels - the nucleotide level (SNPs), the gene level, the protein level (through protein-protein interaction network), and the phenotype level. Results of the study show that Crohn's disease, rheumatoid arthritis, and type 1 diabetes share evidence of genetic associations at all levels of analysis, offering strong molecular support for the current grouping of the diseases. On the other hand, coronary artery disease, hypertension, and type 2 diabetes, despite being considered as a natural group with potential aetiological overlap, do not show any evidence of shared genetic basis at all levels. Our study is a first attempt on mining of GWA data to examine genetic associations between different diseases. The positive result is apparently not a coincidence and hence demonstrates the promising use of our approach.

P53 codon 72 polymorphism and ovarian cancer risk: a meta-analysis.

Zhang, Zhizhong *et al.*

Journal of Nanjing Medical University

p53 is a tumor suppressor gene and is involved in the etiology of ovarian cancer. Studies investigating the associations between the p53 codon 72 polymorphism and ovarian cancer risk showed conflicting results. Authors performed this meta-analysis from eligible studies to evaluate this purported relationship. This meta-analysis was performed from 9 case-control studies, including 825 ovarian cases and 1073 controls. T he fixed and random effect models were used to estimate the odds ratios(ORs) for various contrasts of this polymorphism. The combined results based on all studies showed that a significantly decreased risk was associated with the variant Pro/Pro genotype, compared with Arg/Pro+Arg/Arg genotypes (OR, 0.70; 95%CI, 0.51~0.95). When stratifying the studies by ethnicity, authors found that individuals with the variant genotype Pro/Pro had a significantly decreased risk of ovarian cancer compared with Arg/Arg genotype (OR, 0.43; 95%CI, 0.20~0.89) and Arg/Pro+Arg/Arg genotypes(OR, 0.61; 95%CI, 0.37~0.99) among Africans. This meta-analysis suggests that the p53 codon 72 polymorphism may contribute to genetic susceptibility to ovarian cancer. More studies based on larger sample size should be performed to confirm the findings.

Pro-inflammatory genetic background and zinc status in old atherosclerotic subjects.

Giacconi, Robertina *et al.*

Ageing Research Reviews, **7(4),** 306 (Dec., 2008)

Inflammation and genetics are prominent mechanisms in the pathogenesis of atherosclerosis (AT) and its complications. In this review we discuss the possible impact on AT development of several genetic determinants involved in inflammation, oxidative stress and cytoprotection (IL-6, TNF-[alpha], IL-10, CD14, TLR4, MT, HSP70). Genetic polymorphisms of these genes may affect a differential inflammatory response predisposing to AT. However, allelic polymorphisms of genes which increase the risk of AT frequently occur in the general population but, only adequate gene-environment-polymorphism interactions promote the onset of the disease. Zinc deficiency has been suggested as an environmental risk factor for AT. With advancing age, the incidence of zinc deficiency increases for several reasons.

Among them, dietary intake, malabsorption and genetic background of inflammatory markers may be involved. A crucial contribution may also be played by increased oxidative stress which may lead to the appearance of dysfunctional proteins, including metallothioneins (MT) that are in turn involved in zinc homeostasis. The detection of candidate genes related to inflammation and promoting AT and their reciprocal influence/interaction with zinc status might allow earlier appropriate dietary interventions in genetically susceptible subjects.

Role of genetic variant A-204C of cholesterol 7[alpha]-hydroxylase (CYP7A1) in susceptibility to gallbladder cancer.

Srivastava, Anvesha *et al.*

Molecular Genetics and Metabolism, **94(1),** 83 (May 2008)

Gallbladder carcinoma (GBC) usually arises in the background of gallstone disease. Cholesterol 7[alpha]-hydroxylase (CYP7A1) is a rate-limiting enzyme for cholesterol catabolism and bile acid synthesis. A-204C genetic polymorphism in CYP7A1 may influence gene expression and thus affect the risk of gallstone disease and GBC. We aimed to study the association of A-204C variation of CYP7A1 gene promoter polymorphism in GBC patients, gallstone patients and healthy subjects. The study included 141 histopathologically proven GBC patients, ultrasonographically proven 185 symptomatic gallstone patients and 200 gallstone-free healthy subjects. Genotyping was done by PCR-RFLP method. CYP7A1 A-204C genotypes in control population were in Hardy-Weinberg equilibrium. The CC genotype conferred marginally significant risk for gallstone disease ($p = 0.051$; OR = 1.54; 95% CI = 0.9-3.4). In GBC patients, the CYP7A1 A-204C polymorphism conferred high risk for GBC at genotype ($p = 0.005$; OR = 2.78; 95% CI: 1.3-5.6) as well as allele levels ($p = 0.008$; OR = 1.58 and 95% CI: 1.1-2.2). After stratification of GBC patients on the basis of presence or absence of gallstones, CC genotype imparted higher risk for GBC without stones ($p = 0.002$; OR = 4.44: 95% CI = 1.7-11.3). The association of the polymorphism with GBC was more pronounced in female GBC patients, and also in cancer patients who developed GBC at advanced age. The CC genotype of CYP7A1 is an independent genetic risk factor for GBC but plays a modest role in susceptibility to gallstone disease. The GBC pathogenesis by CYP7A1 polymorphism appears to be independent of gallstone pathway and probably involves genotoxicity due to lipid peroxidation mechanisms.

TET2 mutations in myelodysplasia and myeloid malignancies.

Mullighan, Charles G.

Journal; General Review written in English. CAN 151:144720

AN 2009:777036 CAPLUS (A review. The genetic basis of myelodysplasia has long been enigmatic, with few common targets of mutation known. A new study reports common mutations in the TET2 gene in myelodysplasia and related myeloid malignancies, suggesting that TET2 has an important role in hematopoiesis and in the pathogenesis of this disease.

TP53 codon 72 polymorphism and cervical cancer: a pooled analysis of individual data from 49 studies.

Klug, Stefanie J *et al.*

The Lancet Oncology, **10(8),** 772 (Aug., 2009)

Cervical cancer is caused primarily by human papillomaviruses (HPV). The polymorphism rs1042522 at codon 72 of the TP53 tumour-suppressor gene has been investigated as a genetic cofactor. More than 80 studies were done between 1998 and 2006, after it was initially reported that women who are homozygous for the arginine allele had a risk for cervical cancer seven times higher than women who were heterozygous for the allele.

However, results have been inconsistent. Here we analyse pooled data from 49 studies to determine whether there is an association between TP53 codon 72 polymorphism and cervical cancer. Individual data on 7946 cases and 7888 controls from 49 different studies worldwide were reanalysed. Odds ratios (OR) were estimated using logistic regression, stratifying by study and ethnic origin. Subgroup analyses were done for infection with HPV, ethnic origin, Hardy-Weinberg equilibrium, study quality, and the material used to determine TP53 genotype. The pooled estimates (OR) for invasive cervical cancer were 1·22 (95% CI 1·08-1·39) for arginine homozygotes compared with heterozygotes, and 1·13 (0·94-1·35) for arginine homozygotes versus proline homozygotes. Subgroup analyses showed significant excess risks only in studies where controls were not in Hardy-Weinberg equilibrium (1·71 [1·21-2·42] for arginine homozygotes compared with heterozygotes), in non-epidemiological studies (1·35 [1·15-1·58] for arginine homozygotes compared with heterozygotes), and in studies where TP53 genotype was determined from tumour tissue (1·39 [1·13-1·73] for arginine homozygotes compared with heterozygotes). Null results were noted in studies with sound epidemiological design and conduct (1·06 [0·87-1·29] for arginine homozygotes compared with heterozygotes), and studies in which TP53 genotype was determined from white blood cells (1·06 [0·87-1·29] for arginine homozygotes compared with heterozygotes). Subgroup analyses indicated that excess risks were most likely not due to clinical or biological factors, but to errors in study methods. No association was found between cervical cancer and TP53 codon 72 polymorphism when the analysis was restricted to methodologically sound studies.

New Leads

NEWLEADS

A genetic variation in the ADORA2A gene modifies age at onset in Huntington's disease.

Dhaenens, Claire-Marie *et al.*

Neurobiology of Disease Based on the pathophysiological role of adenosine A2A receptors in HD, authors have evaluated the association of the 1976C/T single-nucleotide polymorphism in the ADORA2A gene (rs5751876) with residual age at onset (AAO) in HD. The study population consisted of 791 unrelated patients belonging to the Huntington French Speaking Network. The variability in AAO attributable to the CAG repeats number was calculated by linear regression using the log (AAO) as the dependent variable, and the respective rs5751876 genotypes as independent variables. Authors show that the rs5751876 variant significantly influences the variability in AAO. The R2 statistic rose slightly but significantly ($p = 0.019$) when rs5751876 T/T genotype was added to the regression model. Patients harbouring T/T genotype have an earlier AAO of 3.8 years as compared to C/C genotype ($p = 0.02$). Our data thus strengthens the pathophysiological role of A2A receptors in Huntington's disease.

Association between ADAMTS13 polymorphisms and risk of cardiovascular events in chronic coronary disease.

Isolmar T. *et al.*

Thrombosis Research

Association between ADAMTS13 levels and cardiovascular events has been described recently. However, no genetic study of ADAMTS13 in coronary patients has been described. Based on related populations frequencies and functional studies, authors tested three ADAMTS13 polymorphisms: C1342G (Q448E), C1852G (P618A) and C2699T (A900V) in a group of 560 patients enrolled in the Medical, Angioplasty, or Surgery Study II (MASS II), a randomized trial comparing treatments for patients with coronary artery disease (CAD) and preserved left ventricular function. The incidence of the 5-year end-points of death and death from cardiac causes, myocardial infarction, refractory angina requiring revascularization and cerebrovascular accident was determined for each polymorphim's allele, genotype and haplotype. Risk was assessed with the use of logistic regression and Cox proportional-hazards model and multivariable adjustment was employed for possible confounders. Clinical characteristics and received treatment of each genotype group were similar at baseline. In an adjusted model for cardiovascular risk variables, we were able to observe a significant association between ADAMTS13 900V variant and an increased risk of death (OR: 1,92 CI: 1,14-3,23, $p = 0,015$) or death from cardiac cause (OR:2,67, CI: 1,59-4,49, $p = 0,0009$). No association between events and ADAMTS13 Q448E or P618A was observed. This first report studying the association between ADAMTS13 genotypes and cardiovascular events provides evidence for the association between ADAMTS13 900V variant and an increased risk of death in a population with multi-vessel CAD.

Functional promoter variant in zinc finger protein 202 predicts severe atherosclerosis and ischemic heart disease.

Stene, Maria C.A *et al.*

Journal of the American College of Cardiology, **52(5),** 369 (July 29, 2008)

The study was designed to test the hypotheses that single nucleotide polymorphisms (SNPs), in zinc finger protein 202 (ZNF202), predict severe atherosclerosis and ischemic heart disease (IHD).ZNF202 is a transcriptional repressor controlling promoter elements in genes involved in vascular maintenance and lipid metabolism. We first determined genotype association for 9 ZNF202 SNPs with severe atherosclerosis (ankle

brachial index >0.7 vs. <=0.7) in a cross-sectional study of 5,355 individuals from the Danish general population. We then determined genotype association with IHD in 10,431 individuals from the Danish general population, the CCHS (Copenhagen City Heart Study), including 1,511 incident IHD events during 28 years of follow-up. Results were verified in 2 independent case-control studies including, respectively, 942 and 1,549 cases with IHD and 8,998 controls. Finally, we determined whether g.-660A>G altered transcriptional activity of the ZNF202 promoter in vitro. Cross-sectionally, ZNF202 g.-660 GG versus AA homozygosity predicted an odds ratio for severe atherosclerosis of 2.01 (95% confidence interval [CI]: 1.34 to 3.01). Prospectively, GG versus AA homozygosity predicted a hazard ratio for IHD of 1.21 (95% CI: 1.02 to 1.43). In the 2 case-control studies, the equivalent odds ratios for IHD were 1.29 (95% CI: 1.02 to 1.62) and 1.60 (95% CI: 1.34 to 1.92), confirming the results from the prospective study. Only 2 other SNPs, which were highly correlated with g.-660A>G, also predicted risk of severe atherosclerosis and IHD. Finally, ZNF202 g.-660G versus g.-660A was associated with a 60% reduction in transcriptional activity *in vitro,* whereas none of the 2 correlated SNPs were predicted to be functional. Homozygosity for a common functional promoter variant in ZNF202 predicts severe atherosclerosis and an increased risk of IHD.

G allele of the SNP -1149G/T of the extrapituitary prolactin promoter is involved in the SLE pathogenesis.

Fojtíková, M. *et al.*

Joint Bone Spine, **75(2),** 245 (Mar., 2008)

Prolactin (PRL) is a polypeptide hormone produced by pituitary lactotrophs and extrapituitary tissues including immune cells. PRL acts as a cytokine and influences immune cells maturation and differentiation. Association between high serum PRL levels and systemic lupus erythematosus (SLE) has been demonstrated. Extrapituitary PRL synthesis is directed by an alternative promoter, which contains single nucleotide polymorphism (SNP) -1149 G/T. Higher PRL mRNA expression in lymphocytes has been associated with G allele. We investigated the role of this G allele in relation to clinical and laboratory features of SLE. We investigated -1149 G/T SNP in 156 SLE patients and 123 healthy individuals (control group). SLE patients: 134 (85.9%) females and 22 (14.1%) males, average age 43.4 years. Control group: 40 (32.5%) females and 83 (67.5%) males, average age 38.7. PCR-RFLP methodology was used for -1149 G/T SNP detection. PCR: The 137 base pairs (bp) region of the PRL extrapituitary promoter was amplified by employing the following primers: forward 5'-GCAGGTCAAGATAACCTGGA and reverse 5'-CATCTCAGAGTTGAATTTATTTCCTT. RFLP: ApoI restriction endonucleasis was used. The genotypes we identified were:TT homozygote characterized by 120bp+17bp, GG homozygote by 85bp+35bp+17 bp and GT heterozygote by 120bp+85bp+35bp+17bp DNA fragments. Results were evaluated by [chi]2 test with Bonferroni correction. In the SLE group there was no difference in genotype and allele frequencies compared to healthy individuals. With respect to specific organ manifestation of SLE we detected an association between G allele and arthritis (Pc=0.0086;OR 2.56,CI 1.51-4.33). According to age when SLE was diagnosed we observed GG genotype frequency in 21-to-40-years subgroup in 44.8% compared to 15.8% and 24.0% in the <20 years and >40 years subgroup, respectively (Pc=0.023;OR 2.94,CI 1.43-5.96). The presence of G allele and GG genotype of the PRL extrapituitary promoter -1149 G/T SNP is associated with certain clinical features of SLE namely arthritis and age of SLE onset.

Genetic association analysis of COPD candidate genes with bronchodilator responsiveness.

Kim, Woo Jin *et al.*

Respiratory Medicine,**103(4),** 552 (Apr., 2009)

Airflow limitation in COPD patients is not fully reversible. However, there may be large variability in bronchodilator responsiveness (BDR) among COPD patients, and familial aggregation of BDR suggests a genetic component. Therefore, we investigated the association between six candidate genes and BDR in subjects with severe COPD. A total of 389 subjects from the National Emphysema Treatment Trial (NETT) were analyzed. Bronchodilator responsiveness to albuterol was expressed in three ways: absolute change in FEV1, change in FEV1 as a percent of baseline FEV1, and change in FEV1 as a percent of predicted FEV1. Genotyping was completed for 122 single nucleotide polymorphisms (SNPs) in six candidate genes (EPHX1, SFTPB, TGFB1, SERPINE2, GSTP1, ADRB2). Associations between BDR phenotypes and SNP genotypes were tested using linear regression, adjusting for age, sex, pack-years of smoking, and height. Genes associated with BDR phenotypes in the NETT subjects were assessed for replication in 127 pedigrees from the Boston Early-Onset COPD (EOCOPD) Study. Three SNPs in EPHX1 ($p = 0.009$-0.04), three SNPs in SERPINE2 ($p = 0.004$-0.05) and two SNPs in ADRB2 (0.04-0.05) were significantly associated with BDR phenotypes in NETT subjects. One SNP in EPHX1 (rs1009668, $p = 0.04$) was significantly replicated in EOCOPD subjects. SNPs in SFTPB, TGFB1, and GSTP1 genes were not associated with BDR. In conclusion, a polymorphism of EPHX1 was associated with bronchodilator responsiveness phenotypes in subjects with severe COPD.

Genetic polymorphism of angiotensin-converting enzyme (ACE) is not associated with the development of parkinson's disease and of l-dopa-induced adverse effects.

Esterina *et al.*

Journal of the Neurological Sciences, **276(1-2),** 18(Jan., 15, 2009)

Sporadic Parkinson's disease (PD) is a frequent neurodegenerative movement disorder. Both environmental and genetic factors have been studied in the etiology of PD. Among genetic factors, increasing evidences suggest that deletion/insertion (D/I) gene polymorphism of the angiotensin I-converting enzyme (ACE) may be involved in the pathogenesis of PD and in the occurrence of the adverse effects of chronic l-dopa therapy. Authors investigated this hypothesis by evaluating the frequency of the ACE gene D/I polymorphism in 120 Italian PD patients and 132 controls. Out of the 120 PD patients, 91 were under chronic l-dopa treatment. Our results revealed no difference in ACE I/D genotype ($[chi]2 = 0.79$, $p = 0.66$) and allele ($[chi]2 = 0.34$, $p = 0.56$) frequencies between PD and controls. Authors also failed to observe any significant association with the occurrence of l-dopa-induced adverse effects in long-term treated PD patients, thereby excluding the presence of an association between ACE I/D genotypes and the genetic susceptibility to PD and the development of adverse effect of chronic l-dopa therapy.

Genetic risk factors and markers for Alzheimer's disease and/or depression in the VITA study.

Grünblatt, Edna *et al.*

Journal of Psychiatric Research, **43(3),** 298 (Jan., 2009)

In ageing population, both Alzheimer's disease (AD) and depression are common. Significant depressive symptoms are often co-morbid with cognitive impairment and dementia. In this study, authors attempted to find various factors and markers for both AD and depression in a longitudinal cohort, the Vienna-Transdanube-Aging (VITA)-study. The VITA-Study consisted of 305 healthy subjects, 174 subjects with depression only, 55 subjects diagnosed with AD only and 72 subjects with depression as well as AD. Associations between AD and/or depression to

gene polymorphisms APO E ([epsilon]4), choline acetyltransferase (ChAT) 4G to A, serotonin-transporter gene promoter-length, dopamine-D4-receptor, ciliary-neurotrophic-factor-null mutation and brain-derived neurotrophic factor (C270T) and to various known factors were analyzed. AD and depression were significant associated. Significant risk factors found for AD were low education, low folic acid and depressive-symptoms, while for depression were low education and higher nonsteroidal anti-inflammatory drugs (NSAID) consume. Moreover, the ChAT polymorphism associated significant to depression. Gender, education, and ChAT significantly associated with the combination AD and/or depression. Such studies must be conducted cautiously, as co-morbidities and gene-environmental-social influences may sway the results dramatically. Authors found in the VITA-study significant association between depression and AD and between ChAT polymorphism and depression.

Genetic classification of oral and oropharyngeal carcinomas identifies subgroups with a different prognosis.

Smeets, Serge J. *et al.*

Cellular Oncology (2009), **31(4),** 291-300.

The common risk factors for oral and oropharyngeal cancer are tobacco smoking and alc. consumption, and recently the human papillomavirus (HPV) was shown to be involved in a subgroup. HPV-pos. and -neg. carcinomas can be distinguished on basis of their genetic profiles. Aim of this study was to investigate patterns of chromosomal aberrations of HPV-neg. oral and oropharyngeal squamous cell carcinomas (OOSCC) in order to improve stratification of patients regarding outcome. Thirty-nine OOSCCs were classified on basis of their genetic pattern detd. by array comparative genomic hybridization (aCGH). Resulting groups were related to patient and tumor characteristics using the Fisher's exact test and in addn. to survival with the Kaplan-Meier and log rank tests. Classification distinguished three groups, one characterized by hardly any chromosomal aberration (N=8) and another by a relatively high level (N=26), and one with a very high level (N=5) of chromosomal aberrations. This classification was significantly (p=0.003) assocd. with survival, with the best survival in the genetically silent' group and the worst survival in the most aberrant group. The silent profile was significantly (p<0.05) assocd. with wild-type TP53, an absence of alcohol consumption and a female gender. These carcinomas were neg. for microsatellite instability. This classification of OOSCC was confirmed in an independent set of 89 oral carcinomas. In conclusion, the discovery of these new classes of oral and oropharyngeal cancer with unique genetic and clinical characteristics has important consequences for future basic and clinical studies.

Genome-wide SNP-based linkage scan identifies a locus on 8q24 for an Age-related hearing impairment trait.

Huyghe, Jeroen R *et al.*

The American Journal of Human Genetics, **83(3),** 401 (Sept., 12, 2009)

Age-related hearing impairment (ARHI), or presbycusis, is a very common multifactorial disorder. Despite the knowledge that genetics play an important role in the etiology of human ARHI as revealed by heritability studies, to date, its precise genetic determinants remain elusive. Here we report the results of a cross-sectional family-based genetic study employing audiometric data. By using principal component analysis, we were able to reduce the dimensionality of this multivariate phenotype while capturing most of the variation and retaining biologically important features of the audiograms. We conducted a genome-wide association as well as a linkage scan with high-density SNP microarrays. Because of the presence of genetic population substructure, association testing was stratified after which evidence was

combined by meta-analysis. No association signals reaching genome-wide significance were detected. Linkage analysis identified a linkage peak on 8q24.13-q24.22 for a trait correlated to audiogram shape. The signal reached genome-wide significance, as assessed by simulations. This finding represents the first locus for an ARHI trait.

Germline SDHB mutations and familial renal cell carcinoma.

Kibel, Adam S.

Urologic Oncology: Seminars and Original Investigations (2009), **27(4),** 463-464.

Familial renal cell carcinoma (RCC) is a heterogeneous disorder that is most commonly caused by germline mutations in the VHL, MET, and FLCN genes or by constitutional chromosome 3 translocations. However, for many patients with familial RCC, the genetic basis of the disease is undefined. We investigated whether germline mutations in fumarate hydratase (FH) or succinate dehydrogenase subunit genes (SDHB, SDHC, SDHD) were assocd. with RCC susceptibility in 68 patients with no clinical evidence of an RCC susceptibility syndrome. No mutations in FH, SDHC, or SDHD were identified in probands, but 3 of the 68 (4.4%) probands had a germline SDHB mutation. Patients with a germline SDHB mutation presented with familial RCC (n = 1) or bilateral RCC (n = 2), and no personal or family history of pheochromocytoma or head and neck paraganglioma. Age at diagnosis of RCC in SDHB mutation carriers ranged from 24 to 73 years. These findings (1) demonstrate that patients with suspected inherited RCC should be examd. for germline SDHB mutations, (2) suggest that all identified SDHB mutation carriers should be offered surveillance for RCC, and (3) provide a further link between familial RCC and activation of hypoxic-gene response pathways.

Hemoglobin binding to A[beta] and HBG2 SNP association suggest a role in Alzheimer's disease.

Perry, Rodney T. *et al.*

Neurobiology of Aging, **29(2),** 185 (Feb., 2008)

From a normal human brain phage display library screen authors identified the gamma (A)-globin chain of fetal hemoglobin (Hb F) as a protein that bound strongly to A[beta]1-42. Authors showed the oxidized form of adult Hb (metHb A) binds with greater affinity to A[beta]1-42 than metHb F. MetHb is more toxic than oxyhemoglobin because it loses its heme group more readily. Free Hb and heme readily damage vascular endothelial cells similar to Alzheimer's disease (AD) vascular pathology. The XmnI polymorphism (C --> T) at -158 of the gamma (G)-globin promoter region can contribute to increased Hb F expression. Using family-based association testing, it was found a significant protective association of this polymorphism in the NIMH sibling dataset (n = 489) in families, with at least two affected and one unaffected sibling (p = 0.006), with an age of onset >50 years (p = 0.010) and >65 years (p = 0.013), and families not homozygous for the APOE4 allele (p = 0.041). Authors hypothesize that Hb F may be less toxic than adult Hb in its interaction with A[beta] and may protect against the development of AD.

HLA-DQ and risk gradient for celiac disease.

Megiorni, Francesca *et al.*

Human Immunology,**70(1),** 55 (Jan., 2009)

Celiac disease (CD) is a rare example of multifactorial disorder in which a genetic test is of great clinical relevance, as the disease rarely develops in the absence of specific HLA alleles. Researchers typed DR-DQ genes in 437 Italian children with celiac disease, 834 first-degree relatives, and 551 controls. Of patients, 91% carried DQ2 and/or DQ8 heterodimers, 6% only had [beta]2 chain, 2% was [alpha]5 positive, and four were DQ2/DQ8/[beta]2/[alpha]5 negative. Only the presence of [alpha]5 resulted negatively

associated to disease (p = 2 × 10-4), whereas we confirmed the effect of the [beta] half of DQ2 dimer on CD predisposition (p = 4 × 10-12). Considering 1:100 disease prevalence, we obtained a risk gradient ranging from 1:7 for DQ2 and DQ8 individuals down to 1:2518 for subjects lacking all predisposing factors. The DQB1*02 and DQB1*0302 concurrence (p = 9 × 10-4), besides the DQB1*02/*02 homozygosity, had an additional role in disease genetic determination. The CD prevalence rose to 17.6% in sisters, 10.8% in brothers, and 3.4% in parents. In the three groups, the subjects carrying high-risk HLA molecules were 57%, 71%, and 58%; among them, 29%, 15%, and 6% respectively had CD. Those siblings and parents with no susceptible factors were not affected. These findings indicate the impact of the HLA test for CD in clinical practice.

Identification of genes that confer tumor cell resistance to the Aurora B kinase inhibitor, AZD1152.

Guo, J. *et al.*

Pharmacogenomics Journal (2009), **9(2),** 90-102

AZD1152 is a highly selective Aurora B kinase inhibitor currently undergoing Phase I and II clin. evaluation in patients with acute myelogenous leukemia and advanced solid malignancies. Authors have established two AZD1152-resistant cell lines from SW620 colon and MiaPaCa pancreatic carcinoma lines, which are >100-fold resistant to the active metabolite of AZD1152, AZD1152 HQPA and interestingly, cross-resistant to the pan-Aurora kinase inhibitor, VX-680/ MK0457. Using whole-genome microarray analysis and comparative genomic hybridization, authors were able to identify MDR1 and BCRP as the causative genes that underlie AZD1152 HQPA-resistance in these models. Furthermore, the upregulation of either of these genes is sufficient to render in vivo tumor growth insensitive to AZD1152. Finally, the upregulation of MDR1 or BCRP is predictive of tumor cell sensitivity to this agent, both *in vitro* and *in vivo.* The data provide a genetic basis for resistance to Aurora kinase inhibitors, which could be utilized to predict clinical response to therapy.

Microtubule-associated protein tau (MAPT) influences the risk of Parkinson's disease among Indians.

Das, Gautami *et al.*

Neuroscience Letters, **460(1),** 16 (Aug., 21, 2009)

Parkinson's disease (PD) is a neurodegenerative disease of the central nervous system and its prevalence increases with age. Microtubule-associated protein tau (MAPT), a neuronal protein is involved in the pathogenesis of several neurodegenerative diseases including PD. To determine the broader significance of this association with PD, replicative studies in distinct ethnic populations are required. In this study, we investigated MAPT for its potential association with PD using five haplotype-tagging SNPs and the del-In9 polymorphism of MAPT in 301 PD patients and 243 healthy controls from eastern India. Our case-control analysis did not show a significant association with any of the markers and PD. However, a risk haplotype [GAC + G] for PD was identified (OR = 1.563; 95% CI = 1.045-2.337; p = 0.03). In addition, haplotype AAC + A (OR = 2.787; 95% CI = 1.372-5.655; p = 0.004) was strongly associated with early onset PD (age at onset <=40 years) and AAC + G haplotype showed a weak association (OR = 2.233; 95% CI = 1.018-4.895; p = 0.045) with late onset PD (age at onset >40 years). This observation highlights the significance of rs7521 in modifying the age at onset of PD under a common haplotype background. Authors also identified AGC + A as a risk haplotype for sporadic cases (OR = 2.773, 95% CI = 1.198-6.407, p = 0.016). This is the first association study from India conducted on MAPT among PD patients and provides valuable information for

comparison with other ethnic groups.

The golden gene (SLC24A5) differentiates US sub-populations within the ethnically admixed Y-SNP haplogroups.

Sims, Lynn M. *et al.*

Legal Medicine, **10(2),** 72 (Mar., 2008)

Y-SNPs are currently being investigated for their potential to predict the ethnogeographic origin of the donor of a crime scene sample. Unfortunately, due to the presence of genetically admixed individuals within ethnic sub-populations within a particular haplogroup (hg), it is sometimes difficult to predict the ethnogeographic ancestry of an individual using only Y-SNPs. In the present work the feasibility of using a combination of the golden pigmentation gene (SLC24A5) SNP and recently described high resolution Y-SNP markers to distinguish some of the different ethnic groups within particular Y-SNP hgs was determined. Four hundred twenty-four individuals (128 African, 206 European, 50 Hispanic/Latin, 20 Pakistan, 20 E.Asian/Indian) were typed for a SNP within the golden gene. The Y-SNP hg was determined for all males and it was found that many of the European derived hg possessed a significant amount of ethnic admixture, with R1b3 having the most. Authors show the use of the golden gene, in combination with more informative Y-SNPs (U152, U106, and M222) and those that define the major hg, can differentiate between most of the African vs. European and African vs. E. Asian members of these heterogeneous populations.

TPH2 -703G/T SNP may have important effect on susceptibility to suicidal behavior in major depression.

Yoon, Ho-Kyoung *et al.*

Progress in Neuro-Psychopharmacology and Biological Psychiatry, **33(3),** 403 (Apr., 30, 2009)

Serotonergic system-related genes can be good candidate genes for both major depressive disorder (MDD) and suicidal behavior. In this study, we aimed to investigate the association of serotonin 2A receptor gene -1438A/G SNP (HTR2A -1438A/G), tryptophan hydroxylase 2 gene -703G/T SNP (TPH2 -703G/T) and serotonin 1A receptor C-1019G (HTR1A C-1019G) with suicidal behavior. One hundred and eighty one suicidal depressed patients and 143 non-suicidal depressed patients who met DSM-IV criteria for major depressive disorder were recruited from patients who were admitted to Korea University Ansan Hospital. One hundred seventy six normal controls were healthy volunteers who were recruited by local advertisement. Patients and normal controls were genotyped for HTR2A -1438A/G, TPH2 -703G/T and 5-HT1A C-1019G. The suicidal depressed patients were evaluated by the lethality of individual suicide attempts using Weisman and Worden's risk-rescue rating (RRR) and the Lethality Suicide Attempt Rating Scale-updated (LSARS-II). In order to assess the severity of depressive symptoms of patients, Hamilton's Depression Rating Scale (HDRS) was administered. Genotype and allele frequencies were compared between groups by [chi]2 statistics. Association of genotype of the candidate genes with the lethality of suicidal behavior was examined with ANOVA by comparing the mean scores of LSARS and RRR according to the genotype. There were statistically significant differences in the genotype distributions and allele frequencies of TPH2 -703G/T between the suicidal depressive group and the normal control group. The homozygous allele G (G/G genotype) frequency was significantly higher in suicidal depressed patients than in controls. However, no differences in either genotype distribution or in allele frequencies of HTR2A -1438A/G and HTR1A C-1019G were observed between the suicidal depressed patients, the non-suicidal depressed patients, and the normal controls. There were no differences in the lethality of suicidal behavior in suicidal depressed patients according to the genotypes of three polymorphisms. Furthermore, an increased frequency of G

allele of TPH2 SNP may be associated with elevated suicidal behavior itself rather than with the diagnosis of major depression and may increase risk of suicidality, independent of diagnosis.

Links between DNA double strand break repair and breast cancer: Accumulating evidence from both familial and nonfamilial cases.

Ralhan, Ranju *et al.*

Cancer Letters, **248(1),** 17, (Apr., 8, 2007)

DNA double strand break (DSB) repair dysfunction increases the risk of familial and sporadic breast cancer. Advances in the understanding of genetic predisposition to breast cancer have also been made by screening naturally occurring polymorphisms. These studies revealed that subtle defects in DNA repair capacity arising from low-penetrance genes, or combinations thereof, are modified by other genetically determined or environmental risk factors and correlate to breast cancer risk. Overexpression of DSB repair enzymes, absence of surveillance factors and mutation or loss of heterozygosity in any of these genes contributes to the pathogenesis of sporadic breast cancers. The results identifying DSB repair defects as a common denominator for breast cancerogenesis focus attention on functional assays in order to assess DSB repair capacity as a diagnostic tool to detect increased breast cancer risk and to enable therapeutic strategies specifically targeting the tumor.

p53 genotypes and haplotypes associated with risk of breast cancer.

Buyru, Nur *et al.*

Cancer Detection and Prevention, **31(3),** 207(2007)

The biological significance of sequence variants in form of SNPs needs to be carefully evaluated, as conflicting associations with cancer predisposition have been reported. Haplotypes, the combination of closely linked alleles on a chromosome, play key roles in the study of the genetic basis of disease. There is strong evidence that different polymorphisms within a single gene in cis position can interact to create a large effect on the observed phenotype. Several polymorphisms have been reported in the p53 gene. Some of these are within the coding region and may affect the function of the p53 protein, others are within introns or non-coding regions, and their significance is unclear. In this study, we investigated the association of specific p53 genotypes and haplotypes with risk of breast cancer. One hundred and fifteen patients with breast cancer and 63 healthy individuals were analyzed. DNA was isolated by salting out. The polymorphic sites were analyzed by PCR RFLP. Pearson's [chi]2 and Kolmogorof Simirnow tests were used for statistical analyses. Extended haplotype frequencies were estimated. The distribution of the genotypes was similar for all three polymorphisms in the cases and the controls. Our estimated haplotype results indicate that the intron 3 (+16 bp) exon 4 (Arg) diplotype and the intron 3 (+16 bp) exon 4 (Arg) intron 6 (G) haplotype combinations are overrepresented in the breast cancer group, suggesting that the intron 3 (+16 bp) exon 4 (Arg) alleles may play a role in breast carcinogenesis. We conclude that two haplotypes harboring the intron 3 polymorphic (+16 bp) allele are associated with a higher risk of breast cancer in the Turkish population.

Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors

Kotronen, Anna *et al.*

Gastroenterology, **137(3),** 865 (Sept., 1, 2009)

Our aims were to develop a method to accurately predict non-alcoholic fatty liver disease (NAFLD) and liver fat content based on routinely available clinical and laboratory data and to test whether knowledge of the recently discovered genetic variant in the PNPLA3 gene (rs738409) increases accuracy of the prediction. Liver fat content was measured using proton magnetic resonance

spectroscopy in 470 subjects, who were randomly divided into estimation (two thirds of the subjects, n = 313) and validation (one third of the subjects, n = 157) groups. Multivariate logistic and linear regression analyses were used to create an NAFLD liver fat score to diagnose NAFLD and liver fat equation to estimate liver fat percentage in each individual. The presence of the metabolic syndrome and type 2 diabetes, fasting serum (fS) insulin, fS-aspartate aminotransferase (AST), and the AST/alanine aminotransferase ratio were independent predictors of NAFLD. The score had an area under the receiver operating characteristic curve of 0.87 in the estimation and 0.86 in the validation group. The optimal cut-off point of -0.640 predicted increased liver fat content with sensitivity of 86% and specificity of 71%. Addition of the genetic information to the score improved the accuracy of the prediction by only <1%. Using the same variables, we developed a liver fat equation from which liver fat percentage of each individual could be estimated. The NAFLD liver fat score and liver fat equation provide simple and noninvasive tools to predict NAFLD and liver fat content.

Preoperative chemoradiotherapy in locally advanced rectal cancer: correlation of a gene expression-based response signature with recurrence.

Liersch, Torsten *et al.*

Cancer Genetics and Cytogenetics (2009), **190(2),** 57-65

Preoperative chemoradiotherapy is recommended for locally advanced rectal cancer (UICC stage II/III). We recently demonstrated that responsive and nonresponsive tumors showed differential expression levels of 54 genes. In this follow-up study, we investigated the relationship between this gene set and disease-free (DFS) and overall survival (OS). Pretherapeutic biopsies from 30 participants in the CAO/ARO/AIO-94 trial of the German Rectal Cancer Study Group were analyzed using gene expression microarrays. Statistical anal. was performed to identify differentially expressed genes between recurrent and nonrecurrent tumors and to correlate these changes with disease recurrence and outcome. After a median follow-up of 59 mo, seven of eight patients with recurrent disease was a nonresponder, and one responsive tumor recurred. Response to chemoradiotherapy was significantly correlated with an improved DFS (log rank P = 0.028), whereas OS did not differ significantly (P = 0.11). Applying a class comparison anal., we identified 20 genes that were differentially expressed between recurrent and nonrecurrent tumors (P < 0.001). Analyzing the first two principal components of the 54 genes previously identified to predict response, we obsd. that this response signature correlated with an increased risk of cancer recurrence. These data suggest that the genetic basis of local response also affects the genetic basis of tumor recurrence. Genes that are indicative of nonresponse to preoperative chemoradiotherapy might also be linked to an increased risk of tumor recurrence.

Single nucleotide polymorphisms of the tenomodulin gene (TNMD) in age-related macular degeneration.

Tolppanen, Anna-Maija *et al.*

Molecular Vision (2009), **15** 762-770

Tenomodulin (TNMD) is located in the X-chromosome encoding a putative angiogenesis inhibitor which is expressed in retina. Associations of single nucleotide polymorphisms of TNMD with the prevalence of age-related macular degeneration (AMD) were examined. Six markers covering 75% of the common sequence variation in the coding region of TNMD and 10 kb up-and downstream were genotyped in a sample consisting of 89 men and 175 women with exudative AMD, 18 men and 25 women with atrophic AMD, and 55 men and 113 women without AMD. All participants were over 65 years old and did not have diabetes mellitus. Due to the chromosomal locus, the assoccation

of genotypes with AMD was assessed genderwise. Three markers, rs1155974, rs2073163, and rs7890586, were associated with a risk of AMD in women. In comparison to women with other genotypes, the women who were homozygous for the minor allele (genotypes rs1155974-TT or rs2073163-CC) had 2.6 fold (p = 0.021) or 1.9 fold (p = 0.067) risk for having AMD, respectively. These differences were due to the unequal prevalence of exudative AMD. In comparison to women who were homozygous for the major alleles, the women with rs1155974-TT genotype had a 2.8 fold risk (p = 0.021 in additive model; p = 0.022 in recessive model) for exudative AMD, and the women with rs2073163-CC genotype had a 1.8 fold risk (p = 0.09 in additive model; p = 0.038 in recessive model). Furthermore, women carrying the rare rs7890586-AA genotype had a significantly smaller risk for having AMD than women with the other genotypes (odds ratio 0.083; p = 0.001 in recessive model), but due to the low frequency of this genotype, this finding must be interpreted cautiously. The false discovery rate was <10% for all of the aforementioned results. On the basis of the putative antiangiogenic role of TNMD and the present genetic assocns. of TNMD with AMD in women, authors suggest that TNMD could be a novel candidate gene for AMD. These results should be confirmed in further studies.

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Genetic Basis of Diabetes & Ayurveda

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Introduction & Background

According to *Ayurveda*, the Indian system of medicine, each individual has different body composition, built & mental strength known as the constitution *(prakruti)*. Individuals are comprised of three vital forces (body humors) or *Gunas (Viita, Pitta, Kapha)* in unique combinations such that no two persons are alike. The constitutional determination provides insight about an individual. The foods, spices, medicines, emotions, thoughts, climate and activities etc, tend to either equilibrate or vitiate some particular vital force of an individual resulting in either to subdue or aggravate the various types of humors which are thereby termed as *Doshas* or *'Vikara(s)'* or disorders. Furthermore, it is possible to outline the disease tendencies of different constitutions *(prakruti),* so that a preventive lifestyle may be worked out; for positive health and individuals can plan it with a scientific, rational, time tested natural approach. In an era of individualized patient care or personalized/customized medicine, this postulate of traditional medicine has acquired greater significance which requires different medicines or set of medicines for their ailments.

The same disorder(s) are manifested in individuals of different constitutions with different etiologies. This has been broadly classified into three groups for understanding, an individual with a predominantly *'Vataja'* constitution will experience symptoms that are different than those for *'Pittaja'* or *'Kaphaja',* even though all of them have been diagnosed with the same disease/disorder. Since drug sensitivity responses are not all alike, hence priority is on the individual's constitution based treatment. There is an intricate interplay between hereditary, nutritional, environmental, emotional and lifestyle factors that affect the risk severity and age of onset of complex disorders, since no single agent by itself causes disease/disorder or brings health. Better insight into the genetic and epigenetic factors affecting complex disorders would greatly facilitate the implementation of personalized medicine that emphasizes the clinical use of Individualized, genotype based pharmacotherapy.

Individual response to the pharmacotherapy is determined by pharmacokinetic and pharmacodynamic factors that often depend on the individual's constitution. The factors influencing drug efficacy for complex disorders such as asthma, diabetes and hypertension are the myriad and unlikely to be deciphered from studies in small patient cohorts even with hundreds of patients. Many studies of complex disorders report very small genetic effects for some gene alleles, and only a large contribution to disease prevalence and severity by a single allele. Such individual variation in health and disease has been recognized by Ayurveda about 5000 years ago as follows: "Every individual is different from one another and hence should be considered as

a different entity, as many variations are there in universe, all are seen in human being" (Charaka Samhita, 1995). Likewise, natural plants are not alike to each other in quality & action. Ayurgenomics (Patwarthan, 2003) describe the basis of individual variation and it has been shown to have remarkable similarities with the pharmacogenomics, which is expected to become the basis of designer medicine. Understanding the possible relationship between *Prakruti* and genome will be important for designing customized drugs/functional foods. As described above, there are different human constitutions (genotypes), disease constitutions (phenotypes) and drug constitution. Nearly 5800 clinical signs and symptoms are available in Ayurvedic texts. According to the Ayurvedic chronobiology principles, the effect of season, time and environmental conditions also needs to be considered to advice lifestyle modification followed by dietary advices (Patwardhan *et al.,* 2005). The individuals who vary in their constitution *(Prakruti)* need different kinds of medicines, both in terms of the Posology as well as in the form of alteration in the medication. Charaka Samhita also classifies the plants into three groups of *'Vata', 'Pitta'* and *'Kapha'* and prescribes that different types of plant compositions are required for a specific patient.

Ayurveda, Diabetes and the Modern Context

While the incidence of Type I Diabetes (T1DM) is about 10-20 million worldwide, the type II diabetes (T2DM) is a major and fast growing global health problem with about 150 million (The DIAMOND Project Group, 2006). As per estimates, the number of adults afflicted with diabetes will increase to 300 million by the year 2025, out of which 75% will be in developing countries. There is high prevalence of TII DM, Obesity and coronary artery disease among urban and migrant Asian Indians, despite the absence of traditional risk factors. Evidence exists that Asian Indians are more insulin resistant than Europeans, which may play an important role in the pathogenesis of the disease. Increased visceral fat in Asian Indians is associated with increased generalized obesity, which is not apparent from their non obese body mass index. Increased visceral fat is related to dyslipidemia and increased frequency of insulin resistance. In addition, early protein energy deprivation, as indicated by low weight at birth and at 1 year of age, may induce a state of vulnerability to the development of type II diabetes at later stage of the life, besides nutrition altered lifestyles pose additional challenge.

T1DM is known to be an auto immune disorder (Gariani et al., 2009). According to modern understanding of genetics, the first degree relatives are reported to have a higher risk of developing T1DM than unrelated individuals (Dorman & Bunker, 2000). The highest risk has been reported for the HLA gene cluster at chromosome 6, comprising several hundred genes known to be involved in the immune response. Presently, the more compelling theory is 'Accelerator Hypothesis', which suggests that increasing body weight in the younger children acts as the accelerator for inducing T1DM, indicating that being overweight accelerates insulin resistance, leading to development of T1DM in genetically predisposed children (Wilkin 2006). However, recent temporal increase in T1DM incidence also points to a changing global environment, rather than mere variation in the gene pool. While viral infections, as early as *in utero,* have been shown to increase a child's subsequent risk of developing the disease (Dahlquist et al., 1995), shorter period of breast feeding has also been shown to be associated with increased incidence of T1DM (Borch-Johnsen, 1984). It is postulated that breast feeding not only provides better immunological protection but also helps in gut maturation leading to proper colonization of the gut with protective flora (Kolb & Pozzili, 1999).

Except for the maturity onset diabetes of the young, in whom the specific gene mutations have been identified, viz., of the glucokinase gene (Williams & Pickup, 1999), genetic determinants of T2DM are not yet well understood, because it is reported to be polygenic in nature (Flores et al., 2003). Though it may not yet be clear as to how TCF7L2 inhibits insulin secretion, da Silva Xavier et al., 2009 have clearly shown that this gene affects insulin secretion.

While there are known cases of stress induced transient diabetes, the mechanism of onset or disappearance of such diabetes is not understood in modern medicine. Ayurveda, however, ascribes it to one of the etiological factors responsible for activating diabetes.

Etio-pathogenesis of *Madhumeha (Diabetes) in Ayurved*

The word *madhumeha* corresponds to 'diabetes' comprising two words: *"Madhu"* (meaning honey i.e., sweet/sweetness) and *"Meha"* (excessive urination), is a type of *Prameha. Madhumeha* is thus, classified in the group of urination disorders known as *prameha.* While Charaka Samhita (10th century BC), one of the earliest texts of Ayurveda, describes a diabetic to pass astringent, sweet and rough (dry-Rooksha) urine. However, Vagbhatta (5th Century AD) further added that the sweetness is found have increased all over the body. Etiologically *Madhumeha* is multifactorial, caused by either inherited tendencies at birth or to derangements acquired afterwards. Specifically, if the three major *doshas* become imbalanced, this may lead to *Madhumeha.*. Likewise, disorder may arise directly from abnormalities in the tissues of the body, such as fat *(medas),* muscle *(mansa),* and muscle fat *(vasa),* or from the action of imbalanced *doshas* on these tissues. External causes which are believed to contribute include excesses of *doshas* like sleep, excessive appetite (especially for sweet food), lack of physical exercise, excessive sexual intercourse, suppressing of natural urges, uneven body postures etc.

Based on the main imbalance of the bodily humor (*dosha*) involved, these disorders are further classified as *kaphaja, pittaja,* and *vataja* (from the three doshas: *kapha, pitta,* and *vata,* respectively). There are at least 20 types of *Pramehas* (urinary disorders) described in traditional texts. These are also classified on the basis of *Tridosha* theory. In the progression of diabetes, in the initial stage of *Prameha,* the *Kapha dosha* is predominantly at high level. *Madhumeha* normally falls under the category of *Vataja Pramehas.* Ultimatly all types of *Pramehas,* directly or indirectly progress into *Madhumeha,* if properly not taken care off. Even as early as in Susruta's time, varied presentation of diabetes was appreciated.

Two types of *Vataja Pramehas* are described for diabetes. The first, called *sahaja,* is thought to be due to a defect in genetic content of either the mother or father. This form corresponds to juvenile-onset diabetes, and such patients are often described as thin and are thought to have more serious disease. It may be further subdivided as *Kulaj* i.e., having familial history but not congenital. The second type, is *apathyanimittaja,* is believed to be acquired later in life due to excessive habits, such as overindulgence in food or sweets. This corresponds to adult-onset diabetes, and the patients are described as obese.

Charaka describes that all *Pramehas* (urinary disorders) start with a derangement of the bodily humor *kapha.* The vitiated *kapha* spreads throughout the body and mixes with fat *(medas)* because the fat has properties similar to those of *kapha.* The affected body fluids are passed in the urine, but they block the openings of the urinary tubules coming out of the bladder. This is believed to be the cause of the frequent urination observed in *Madhumeha.* Even though impaired *kapha*

plays a dominant role in diabetes, the other two bodily *humors - vata* and *pitta - are* also important in the development of different types of the disease. Ayurveda holds that prognosis of diabetic patients with predominance of vitiated *Pitta* or *Kapha* is better than those with aggravated Vata. It is observed that in all types of *Pramehas* (urinary disorders), *Kapha* is aggravated but in *Madhumeha* (Diabetes), *Vata* is also often aggravated. Charaka has also classified diabetics into two groups as obese and lean or weak. Both the groups are treated with different regimen and diet. Sthool pramehi and krish pramehi, both have their different genetic composition, etiology and treatment. Sthool pramehi (obese) is an outcome of sedentary lifestyle with over eating at mature age group whereas krish pramehi at early age group with dependency on insulin. This may be regarded as juvenile onset whereas the other one as maturity onset diabetes.

Treatment protocols are different for each type. Treatment for obese patients begins with a cleansing. Lean diabetic patients, however, are considered too frail to undertake radical cleansing; they and other frail diabetic patients customarily undergo milder cleansing procedures. Both groups are then treated with specific herbal therapy and diet.

Pharmacogenomics & Ayurveda

Methods for analyzing large datasets from a single experiment now provide the opportunity to simultaneously analyze the expression of thousands of genes in multifactorial diseases such as type-2 diabetes mellitus (DM). In human pancreas, muscle, fat, and liver tissue, mRNA levels of about 800 genes are modulated in DM. Many of these are targets of the insulin /insulin receptor signaling and belong to functional classes that can account for most of the biological and metabolic effects of diabetes. Furthermore, gene expression changes in peripheral blood cells (PBCs) also distinguishing variable diabetic states. Identification of candidate gene expression signature in PBCs raises the possibility of using easily accessible biomarkers to identify and monitor diabetes. (Rao *et al., 2005)*

The interplay between common variants in candidate genes and an affluent environment seems to explain most of the genetic component of the disease. Expression of many of these genes inf1uences energy metabolism and regulation of insulin secretion. At the moment, two genes, one inf1uencing insulin sensitivity (PPAR-y) and the other influencing beta-cell function (Kir 6.2), seem to be the most reproducible candidate genes for type 2 diabetes across the world, but certainly many more are out there to be identified. (Groop et al. 2004).

Metabolomics & Diabetes

Metabolomics and proteomics serve as biological markers and diagnostic tools for metabolic classification of individuals through quantitative, non-invasive analysis of easily accessible human body fluids like urine, blood and saliva. Metabolomics has been employed in preclinical and clinical research, for environmental, biomedical application, toxicology, coronary heart disease and on the relationship between serum metabolic profiles in diabetes and hypertension etc. Beyond disease diagnosis, it has also been successfully used to identify lifestyle-related biomarkers of health (Kussmann, 2006).

Obesity and diabetes are 'conditions resulting from a chronic imbalance between energy intake and energy expenditure. These are inheritable complex diseases involving genetic, environmental and behavioral factors. Most cases of chronic disease' like obesity, diabetes, cardiovascular disease (CYD) and cancer are due to complex interactions between many genes and environmental factors. Diabetes is associated with inflammatory state, increased cardiovascular mortality and several other associated

disorders (Kussmann, 2006; Poulsen, 2005; Rolo & Palmeira, 2006,).

Leukotrienes are arachidonic acid metabolites derived from the 5-1ipoxygenase pathway that possess vasoactive, chemotactic and pro-inflammatory properties. Urinary excretion of leukotriene E4 (LTE4) in type 1 diabetic subjects and healthy volunteers and the influence of glycemic control can be studied by HbAIC on LTE4 excretion. Urinary excretion of LTE4, can be measured by LC-MS. Its significantly increased levels are found in diabetic patients compared to healthy subjects and indicate a trend towards increased LTE4 excretion in patients with poor glycemic control, whereas negligible differences were reported in patients with good metabolic control and healthy subjects. This study suggested that increased LTE4 excretion in type 1 diabetic state might reflect systemic activation of the 5-lipoxygenase pathways. It could be a determinant of underlying inflammatory state and vascular disease (Hardy *et al.* 2005).

Obesity often causes insulin resistance, a decline in the ability of insulin to stimulate glucose uptake in the body, which leads to compensatory over-secretion of this hormone by the pancreatic beta-cells and, eventually, to beta-cell exhaustion and development of type-2 diabetes mellitus (T2DM). Over-consumption of energy, types of fats, proteins and carbohydrates absorbed and micronutrient deficiencies have been associated with obesity and T2DM. The discrimination of T2DM patients from healthy controls can be achieved by metabolomic profiling of serum fatty acids and plasma phospholipids (Yang *et al* 2004). Intake of saturated fatty acids (SFAs) correlates with higher levels of low-density lipoprotein (LDL), the principal target of intervention for coronary heart disease reductien. in addition, to obesity and diabetes. Carbohydrates practically all cohort studies ofT2DM and the glycemic index (GI, quantitative measure of food based on postparandial glucose response) showed an association between GI and T2DM (Hardy et al. 2005, Kussmann, 2006; Thomas et al. 2005).

Gestational diabetes mellitus (GDM), a transient metabolic disorder is a strong predictor of type 2 diabetes and cardiovascular disease is associated with reduced red cell long-chain omega-6 and omega-3 fatty acids. In GDM plasma triacylglycerols (TG), phosphatidylcholine (PC), sphingomyelin (SM), and red cell PC, phosphatidylethanolamine (PE) and SM fatty acids showed patients had lower total saturated fatty acids (SF A), TG and PC and higher omega-6 and omega-3 metabolites in the plasma PC than the controls. Conversely, the red cell PC and PE of the patient contained higher proportions of palmitic and SF A but lower arachidonic and docosahexaenoic acids compared with the controls (Hardy et al. 2005, Thomas *et al.*2005). The reduced membrane arachidonic and docosahexaenoic acids in patients might be attributed to the effect of the disease. Women with GDM and their neonates have lower levels of arachidonic (AA), docosahexaenoic (DHA) and dihomogamma-linolenic (DHGLA) fatty acids in red cell membranes and in choline phospho glycerides (CPG). They also had lower levels of AA, adrenic and osbond acids in triglycerides (TG) and cholesterol esters (CE). Mead acid, a marker of generalised shortage of derived and parent essential fatty acids, was higher in CPG and TG of the GDM group. The ratio of adrenic/osbond acid, a biochemical marker of DHA insufficiency, was reduced in CPG, TG and CE of the GDM group. Such findings, suggested that the neurovisual and vascular development and function of the offspring of GDM women might be adversely affected if the levels of AA and DHA are compromised further (Hardy *et al.* 2005, Thomas *et al.* 2005). Increased lipid peroxidantion in type- 2 diabetes mellitus (T2DM) as exhibited by elevated 8-epi-prostaglandin F_2 levels (Gopaul

et al. 1995) and 9-hydroxy linoleic acid (Inouye eL5l 1999) and quantities of acetone in plasma may be used as biomarker (Dong *et al.* 2006).

Dietary chemicals can affect gene expression, directly or indirectly. The number of nuclear receptors capable of binding fatty acids seems to increase daily and the genes are regulated by fatty acids. Metabolic conversion of dietary chemicals also serves as a control mechanism of gene expression: the levels of steroid hormones, ultimately derived from the dietary component cholesterol, are regulated by enzymatic activities in the steroid biosynthetic pathway (Kussmann, 2006; Rolo & Palmeira, 2006, Poulsen, 2005).

Diabetes, Oxidative Stress and DNA Damage

Oxidative modifications of DNA are abundant, mutagenic and thought to be important in Diabetes (Shin *et al.* 2001, Dandona *et a*l. 1996). The bodily defenses against oxidants include an extensive system of antioxidant enzymes and radical scavengers and chain breakers, of which many are nutritionally dependent. Failure of the system of enzymatic, endogenous and nutritional antioxidants may lead to mutagenic oxidative DNA damage as well as deregulation of cell cycle control, resulting in several diseases. DNA damages are considered as the most serious ROS-induced cellular modifications as DNA is not synthesized de novo but copied and hence the modifications can induce mutations and genetic instability (Rolo & Palmeira, 2006, Poulsen, 2005). Naturally occurring antioxidant phytochemicals such as plant phenols, vitamins, carotenoids and terpenoids, have revealed significant beneficial effects to control oxidative stress in diabetics (Kussmann, 2006).

Production of reactive oxygen species (ROS) and lipid peroxidation are increased in diabetic patients, especially in those with poor diabetic control. Increased ROS generation may result in oxidative damage of DNA. 8-hydroxydeoxyguanosine (8-0HdG) is an indicator of oxidative damage of DNA that can be measured in the urine through modern techniques like LC-MS/MS (Oyama *et al.* 2006, Shin et al 2001).

The Traditional Knowledge, Modern Medicine & Modern Science, the trio described as Golden Triangle by Dr. R A Mashelkar can be an important model to study a disease like diabetes. At this stage of development of Systems Biology, there is a need to correlate these facts and develop preventive, therapeutic as well as promotive tools involving an integrated approach.

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NDABSTRACTS

Whole genome expression and biochemical correlates of extreme constitutional types defined in Ayurveda

Bhavana Prasher *et al*

Journal of Translational Medicine 2008, **6:**48doi:10.1186/1479-5876-6-48

Ayurveda is an ancient system of personalized medicine documented and practiced in India since 1500 B.C. According to this system an individual's basic constitution to a large extent determines predisposition and prognosis to diseases as well as therapy and life-style regime. Ayurveda describes seven broad constitution types (*Prakriti*s) each with a varying degree of predisposition to different diseases. Amongst these, three most contrasting types, *Vata*, *Pitta*, *Kapha*, are the most vulnerable to diseases. In the realm of modern predictive medicine, efforts are being directed towards capturing disease phenotypes with greater precision for successful identification of markers for prospective disease conditions. In this study, we explore whether the different constitution types as described in Ayurveda has molecular correlates.

Normal individuals of the three most contrasting constitutional types were identified following phenotyping criteria described in Ayurveda in Indian population of Indo-European origin. The peripheral blood samples

of these individuals were analysed for genome wide expression levels, biochemical and hematological parameters. Gene Ontology (GO) and pathway based analysis was carried out on differentially expressed genes to explore if there were significant enrichments of functional categories among *Prakriti* types.

Individuals from the three most contrasting constitutional types exhibit striking differences with respect to biochemical and hematological parameters and at genome wide expression levels. Biochemical profiles like liver function tests, lipid profiles, and hematological parameters like haemoglobin exhibited differences between *Prakriti* types. Functional categories of genes showing differential expression among*Prakriti* types were significantly enriched in core biological processes like transport, regulation of cyclin dependent protein kinase activity, immune response and regulation of blood coagulation. A significant enrichment of housekeeping, disease related and hub genes were observed in these extreme constitution types.

Ayurveda based method of phenotypic classification of extreme constitutional types allows us to uncover genes that may contribute to system level differences in normal individuals which could lead to differential disease predisposition. This is a first attempt towards unraveling the clinical phenotyping principle of a traditional system of medicine in terms of modern biology. An integration of Ayurveda with genomics holds potential and promise for future predictive medicine.

Genetic Association Analysis of Copy-Number Variation (CNV) in Human Disease Pathogenesis

Among the many important insights derived from completion of the Human Genome Project was the recognition of the abundance of single nucleotide polymorphisms (SNPs) as a major source of genetic variation, leading to speculation that the bulk of phenotypic variability in human populations is due to single base changes. As a result, intense efforts were made to develop high-throughput sequencing and SNP genotyping platforms, SNP databases, detailed linkage disequilibrium maps and statistical methodologies for analyzing SNP genotype and haplotype data in mapping disease-susceptibility genes. Until recently, the overwhelming majority of gene-mapping studies have focused exclusively on the role of SNPs in human diseases. Indeed, using population-based studies to identify genetic determinants of common disease, dozens of SNP-based susceptibility variants have been identified for human diseases as diverse as diabetes, macular degeneration, cancer, asthma, and Crohn disease. However, studies over the past three years have resulted in increasing recognition of the critical role of structural genetic variation (most of which appear to be in the form of copy-number variation) in modulating gene expression and disease phenotype. In fact, copy-number variants (CNVs) are now known to be a prevalent form of common genetic variation and represent a substantial proportion of total genetic variability in human populations. Moreover, a few association studies have already demonstrated the importance of CNVs as disease-susceptibility variants, with specific CNVs found to confer differential risk to HIV infections, autoimmune disease, and asthma. Recently, genome-wide surveys have demonstrated that rare CNVs altering genes in neurodevelopmental pathways are implicated in autism spectrum disorder and schizophrenia. It is therefore becoming increasingly clear that genetic studies of complex diseases must pay closer attention to the contribution of CNVs.

In contrast to the well-developed resources available for SNP-association studies, we are still in the early phases of incorporating structural genetic variation in genome-wide association studies. Nevertheless, we anticipate a burgeoning focus on structural genetic variation in human disease over the next few years, and foresee the development of many tools needed for such studies. In this commentary, a brief description of the presently known landscape of structural genetic variation has been provided to review recent successes in identifying CNVs associated with human diseases, and then address the current challenges of CNV-association studies, including the limitations of current genotyping platforms and available statistical methods.

Presently Known Landscape of CNVs

Structural genetic variation refers to a class of genomic alterations of DNA that usually span more than 1000 bases. It includes quantitative (unbalanced) changes such as copy-number variants (CNVs), and less common balanced variations involving chromosomal inversions, insertions, and translocations. Here, the focus is on CNVs, the most prevalent type of structural genetic variation.

Table 1.

Replicated associations of DNA copy-number variants with common complex disease

Locus	CNV frequency	Clinical phenotype	CNV type	Risk estimate (odds ratio)	Comments
CCL3L1	10–20%	HIV/AIDS susceptibility	Deletion	0.67–0.90	CCL3L1 inhibits HIV cellular entry, Higher CCL3L1 number increases CCL3L1 expression
		Rheumatoid arthritis	Gain: > 2 copies	1.34	
FCGR3B	Deletion: ~ 25%	Systemic autoimmune disease	Deletion	1.58–2.56	CNV associated with glomerulonephritis in rats and humans
	Gain: ~ 15%				
C4	~ 40%	Systemic lupus erythematosus	Deletion	Absence: 5.27	> 75% of C4 or C1 deletion carriers have SLE-like disease Strongest SLE genetic risk factor thus far in blacks
				Carrier: 1.61	
				Gains: 0.57	
DEFB4	2–12 copies (median 4)	Colonic Crohn disease	Loss: < 4 copies	3.06	↓ number associated with ↓ mucosal gene expression.
		Psoriasis	Gain: > 5 copies	1.69	
GSTM1	Up to 50%	Asthma, lung function, allergic response	Deletion	1.59–1.89	Potent antioxidant. Deletion related to many adverse asthma-related outcomes

[a] FCGR3B demonstrates phenotypic pleiotropy: OR for lupus 2.21; for Wegener's granulomatosis 1.58–2.46; for microscopic polyangiitis 2.56.

Structural genetic variation has long been known to impact health, though until very recently this impact was thought to be limited to rare genomic disorders. A handful of Mendelian disorders, such as Williams–Beuren Syndrome (deletion at chromosome region 7q11.23) or Charcot–Marie Tooth neuropathy Type 1A (duplications of peripheral myelin

protein-22 at chromosome region 17p11.2), are caused exclusively by recurrent DNA copy-number changes at critical loci. However, with the realization of the existence of widespread common structural variation among otherwise healthy individuals greater attention is now being focused on whether this type of genetic variation influences more common human diseases.

The current map of structural variation in the human genome is far from complete. While several databases exist to catalog this newly-appreciated form of human genetic variation (notably the Database of Genomic Variants — and the Human Structural Variation Database — quality control is lacking, and studies have differed in technological approaches, precise boundary definition of CNVs, DNA quality, and even discrepancies in terminology Nevertheless, the latest compilation of data on structural genetic variation (The Database of Genomic Variants — November 29, 2007) from 46 different articles over the past three years indicates that as many as 4878 loci (comprised of 11,784 different CNV entries) have now been identified. We anticipate that our understanding of the location and extent of CNV in the human genome will improve markedly in the next few years. Emerging technologies are more sensitive for detection of CNVs and provide more precise definition of boundaries. Undoubtedly, as a clearer map of human structural genetic variation emerges, we will begin to more comprehensively include this type of genetic variation in genome-wide association studies that attempt to elucidate the role of CNVs in human disease.

CNVs in Health and Disease

Several distinguishing features of CNVs support their role in disease pathogenesis. First, though less abundant than SNPs, it has been suggested that CNVs account for more nucleotide variation than do SNPs, on account of their sheer size. By spanning thousands of bases, CNVs often encompass (and can sometimes disrupt) functional DNA sequences. Second, there appears to be an enrichment of currently known CNVs toward "environmental sensor" genes — i.e. genes that are not necessarily critical for early embryonic development, but rather help us to perceive and interact successfully with our ever-changing environment. This includes enrichment for olfactory receptors, immune and inflammatory response genes, cell signaling and cell adhesion molecules, structural proteins, and ion channels. Third, like other forms of genetic variation, both purifying and adaptive natural selective pressures appear to have influenced the frequency distribution of selective CNVs, suggesting their functional significance. Lastly, a recent comparison of the relative impact of SNPs and CNVs on gene expression noted that a substantial proportion (~18%) of gene expression variability was attributable to known CNVs greater than ~40 kb in size. Notably, 53% of genes whose expression was influenced by CNVs had the corresponding CNV outside of the actual gene, suggesting that many CNVs could affect important regulatory sequences that are situated at a distance from the actual target gene.

Given these features, it is perhaps not surprising that early genetic association studies of known CNVs have quickly produced promising results. Presented in Table 1 are recent examples of copy-number variable loci implicated in the pathogenesis of complex traits, where the association has been observed in at least two independent populations. These loci share several noteworthy features that may provide important insights into the role CNVs may play in complex diseases. First, the copy-number frequencies for all five loci are high – greater than 10% in all cases – confirming that the allelic spectrum of disease-related CNVs is not restricted to rare variants. Second, with the exception of the CCL3L1 HIV/AIDS protective alleles, the genetic risk conferred by

these variants is quite high (relative to SNPs, particularly in the context of polygenic, complex traits). Currently available data suggest that many CNVs confer greater disease risk than SNPs and in some cases these CNV-based disease-susceptibility variants appear to increase risk by as much as 30%. Although we caution over interpretation of these early estimates (given that risk tends to be overestimated in initial studies due to the so-called "winner's curse" these early returns do support an important role for CNVs in the genetic etiology of common diseases.

These early studies also suggest that copy-number variable loci may exhibit copy-number dependent genetic pleiotropy. It was observed that for two of the loci listed in Table 1, gains and losses are associated with distinct phenotypes (HIV and Rheumatoid Arthritis for CCL3L1; Crohn disease and Psoriasis for DEFB4). These observations are reminiscent of neuropathies associated with copy-number variation at the Peripheral Myelin Protein 22 (PMP22) locus, where PMP22 duplication confers Charcot–Marie Tooth Disease and PMP22 deletions cause hereditary neuropathy with liability to pressure palsies (also known as bulb diggers' palsy). Whether this phenomenon will be observed at other copy-variable loci is unclear.

Another striking feature shared by the loci listed in Table 1 is that all are immune or inflammatory-related genes. Though certainly a function of the diseases studied in these surveys, this enrichment is consistent with the distribution of functional gene classes in CNV regions, where inflammatory and immune-related genes were among the most overrepresented.

It is important to recognize that all of these loci were identified using candidate gene approaches rather than hypothesis-free, genome-wide surveys. It is therefore unclear whether the above observations (relating to allele frequency distribution, effect sizes and functional class representation) will continue to hold as novel loci are identified through genome-wide association studies not predicated on prior biological knowledge. In one recent genome-wide CNV-association study on autism spectrum disorder (ASD) 264 families (including 165 families with autistic children and 99 control families) were screened for *de novo* CNV mutations. In this study, the authors observed a disproportionate incidence of *de novo* mutations in families with ASD (12 deletions and 2 duplications among affected families compared to 2 gains among controls). This represented an approximate 3-fold increase in *de novo* mutation rate. All of the ASD-associated CNVs harbored at least one gene, several of which have been implicated in clinical contexts to overlap with autism. A similar study showed the importance of rare CNVs at multiple sites in schizophrenia. In this study, the authors observed that novel (that is, not present in the Database of Genomic Variants) microdeletions and microduplications (> 100 kilobases) were present in 15% of schizophrenia cases, a frequency three times that in controls. Notably, mutations in cases disproportionately affected genes from signaling networks controlling neurodevelopment, including neuregulin and glutamate pathways.

The early successes described above suggest that there will be a sharp increase in the number of published CNV-association studies over the coming years. In anticipation of this, we stress that there still remain considerable technical, methodological, and analytical challenges related to CNV-based association studies that must be recognized and carefully addressed.

Technical Challenges in CNV Studies

CNV-based association studies pose additional unique challenges, including choice of genotyping platform (for a recent review, please see Carter and DNA quality control.

Three broad platform classes are currently available for genome-wide copy-number surveys: (1) Large insert clone-based comparative genomic hybridization (CGH), where differentially-labeled test and reference samples compete for binding to DNA from large insert genomic clones — such as BACs (e.g., Fiegler *et al.* (2) long, isothermic oligonucleotide-based CGH arrays (where differentially-labeled test and reference samples compete for binding to 50–65mer oligonucleotides that are designed to have similar thermodynamic kinetics); and (3) SNP-based arrays (one-sample arrays where intensity values derived from genotyping assays are used to infer copy number).

Statistical Challenges in Analysis of CNV Associations with Human Disease

Genetic epidemiology of CNVs is still in its infancy — so too are the statistical methods for the analysis of CNV association with disease. As discussed above, genotyping platforms vary in their signal-to-noise ratio and also in their ability to define precise CNV genotypes (i.e. discrete copy number). Current CNV typing technologies produce quantitative measures meant to reflect total DNA amount in a given sample. In contrast to SNP genotyping, oftentimes the distribution of these measurements is continuous, making it difficult to accurately estimate DNA copy number. Currently only a small fraction of the known CNVs are genotypable.

Two main statistical methodologies can be employed for CNV analyses, and they differ in their need for precise CNV quantification. The first involves a two-step procedure, based on first inferring the underlying genotype and then performing a regular test of association. Because it depends on "genotypable" markers, this class of statistical methodologies is currently only applicable to a limited number of CNVs. Also, it is not immediately clear how the uncertainty of CNV genotype calling should be incorporated in the analysis; when raw measurements show a continuous distribution, forcibly classifying them into discrete copy-number classes (e.g. gain, no change, or loss) may result in the loss of substantial information and statistical power relative to the raw measurements The second methodology bypasses the genotype calling step and instead, directly analyzes the intensity measurements which are thought to reflect the true underlying copy number. This strategy has been advocated in several recent papers.

Conclusions

The study of structural genetic variation in human diseases is a new and rapidly evolving field. The main limitations of this field of study relate to the lack of technological and statistical tools dedicated to these efforts, and reliance on the "hammers" developed for the study of SNPs. Over the next few years, a much more thorough understanding of the extent and precise location of copy-number variation will likely be available as new platforms become available to accurately capture CNV information. This will allow us to more comprehensively understand the role of these genetic variants in the pathogenesis of human diseases. However, in addition to the development of better CNV genotyping platforms, it is stressed that rigorous attention to study design and statistical analysis is critical so as not to relive past experiences of early disease association studies that yielded "unreplicable" and all too often false-positive results. Whenever possible, initial reports of CNV-disease association should include independent evidence of replication in other studies and populations. Generating such data will frequently require collaboration between research groups, and may include sharing of DNA samples given the current technical challenges of CNV genotyping and operator-dependence of quantitative genotyping assays like qPCR. Despite the many obstacles yet to be overcome, we foresee CNVs quickly taking their place alongside SNPs in genetic epidemiology studies. Once identified, these

loci can then be evaluated experimentally using animal models that recapitulate the disease-associated molecular defect (e.g., knock-out mice for CNV losses and over-expressing transgenic models for CNV gains) and the development of specific molecular therapeutics, ultimately leading to novel therapies for our most common diseases.

(Based on the article published in Genomics 93 (2009) 22-26)

Identifying disease associations via genome-wide association studies.

Huang, Wenhui *et al.*

BMC Bioinformatics (2009), 10 (Suppl. 1), No pp. given

Genome-wide assocn. studies prove to be a powerful approach to identify the genetic basis of different human diseases. We studied the relationship between seven diseases characterized in a previous genome-wide assocn. study by the Wellcome Trust Case Control Consortium. Instead of doing a horizontal assocn. of SNPs to diseases, we did a vertical anal. of disease assocns. by comparing the genetic similarities of diseases. Our anal. was carried out at four levels - the nucleotide level (SNPs), the gene level, the protein level (through protein-protein interaction network), and the phenotype level.

Our results show that Crohn's disease, rheumatoid arthritis, and type 1 diabetes share evidence of genetic assocns. at all levels of anal., offering strong mol. support for the current grouping of the diseases. On the other hand, coronary artery disease, hypertension, and type 2 diabetes, despite being considered as a natural group with potential etiol. overlap, do not show any evidence of shared genetic basis at all levels. Our study is a first attempt on mining of GWA data to examine genetic assocns. between different diseases. The pos. result is apparently not a coincidence and hence demonstrates the promising use of our approach.

Low frequency variants in the exons only encoding isoform A of HNF1A do not contribute to susceptibility to Type 2 Diabetes.

Jafar-Mohammadi, Bahram *et al.*

PLoS One (2009), **4(8)**

There is considerable interest in the hypothesis that low frequency, intermediate penetrance variants contribute to the proportion of Type 2 Diabetes (T2D) susceptibility not attributable to the common variants uncovered through genome-wide assocn. approaches. Genes previously implicated in monogenic and multifactorial forms of diabetes are obvious candidates in this respect. In this study, we focussed on exons 8-10 of the HNF1A gene since rare, penetrant mutations in these exons (which are only transcribed in selected HNF1A isoforms) are assocd. with a later age of diagnosis of Maturity onset diabetes of the young (MODY) than mutations in exons 1-7. The age of diagnosis in the subgroup of HNF1A-MODY individuals with exon 8-10 mutations overlaps with that of early multifactorial T2D, and we set out to test the hypothesis that these exons might also harbor low-frequency coding variants of intermediate penetrance that contribute to risk of multifactorial T2D. Authors performed targeted capillary resequencing of HNF1A exons 8-10 in 591 European T2D subjects enriched for genetic etiol. on the basis of an early age of diagnosis (< 45 years) and/or family history of T2D (□1 affected sibling). PCR products were sequenced and compared to the published HNF1A sequence. We identified several variants (rs735396 [IVS9-24T>C], rs1169304 [IVS8+ 29T>C], c.1768+44C>T [IVS9+ 44C>T] and rs61953349 [c.1545G>A, p.T515T] but no novel non-synonymous coding variants were detected. It wasconcluded that low frequency, nonsynonymous coding variants in the terminal exons of HNF1A are unlikely to contribute to T2D-susceptibility in European samples. Nevertheless, the rationale for seeking low-frequency causal variants in genes known to contain rare, penetrant

mutations remains strong and should motivate efforts to screen other genes in a similar fashion.

Sequence-based advances in the definition of cancer-associated gene mutations.

Simpson Andrew J G *et al.*

Current opinion in oncology (2009), **21(1),** 47-52.

Recent rapid progress in DNA sequencing has permitted projects to be undertaken that are aimed at building unbiased genome-wide portraits of the underlying mutations in human tumors. This review sets out the highlights of the recent progress in this area and the rapidly evolving picture of the underlying genetic basis of human epithelial cancers. Individual tumors are estimated to contain around 80 point mutations in protein coding genes of which 15 are likely to be tumorigenic. It is likely that there are hundreds of different genes that when mutated contribute to human tumorigenesis most in only a small fraction of tumors. Mutations caused by large chromosomal rearrangements also appear to be common in tumors. In prostate and lung cancers, recurrent chromosomal translocations resulting in tumorigenic fusion proteins have been identified. The multitude of new mutated genes being identified in human tumors represent many new directions for experimental research into the molecular pathways that lead to tumor formation. These studies, in turn, are likely to lead to many novel approaches to targeted therapy useful in subsets of tumors with particular types of gene mutation.

SLC29A3 gene is mutated in pigmented hypertrichosis with insulin-dependent diabetes mellitus syndrome and interacts with the insulin signaling pathway.

Cliffe, Simon T. *et al.*

Human Molecular Genetics (2009), **18(12),** 2257-2265

Pigmented hypertrichotic dermatosis with insulin-dependent diabetes (PHID) syndrome is a recently described autosomal recessive disorder associated with predominantly antibody negative insulin-dependent diabetes mellitus. In order to identify the genetic basis of PHID and study its relationship with glucose metabolism authors performed homozygosity mapping in five unrelated families followed by candidate gene sequencing. Five loss-of-function mutations were identified in the SLC29A3 gene which encodes a member of a highly conserved protein family that transports nucleosides, nucleobases and nucleoside analog drugs, hENT3. It is shown that PHID is allelic with a related syndrome without diabetes mellitus, H syndrome. The interaction of SLC29A3 with insulin signaling pathways was then studied using an established model in Drosophila melanogaster. Ubiquitous knockdown of the Drosophila ortholog of hENT3, dENT1 is lethal under stringent conditions; whereas milder knockdown induced scutellar bristle phenotypes similar to those previously reported in the knockdown of the Drosophila ortholog of the Islet gene. A cellular growth assay showed a reduction of cell size/no. which could be rescued or enhanced by manipulation of the Drosophila insulin receptor and its downstream signaling effectors, dPI3K and dAkt. In summary, inactivating mutations in SLC29A3 cause a syndromic form of insulin-dependent diabetes in humans and in Drosophila profoundly affect cell size/no. through interactions with the insulin signaling pathway. These data suggest that further investigation of the role of SLC29A3 in glucose metabolism is a priority for diabetes research.

Patents

PATENTS

Genetic markers of schizophrenia

Brennan, Mark David and Philips D Kay

University of Louisville Research Foundation Inc., Louisville, KY, US

US 20090012371 January, 8, 2009

The invention includes method of determining if a subject has a genetic predisposition to clinically diagnosed schizophrenia (SZ), schizotypal personality disorder (SPD), and / or schizoaffective disorder (SD).

Genetic risk factor for cancer.

Van Loo *et al.*

Synthon B.V. Netherlands

WO/2008/061672 May 29, 2008

The invention relates to an isolated nucleic acid molecule that has been found to be implied in the susceptibility of an individual to develop cancer. Specifically, the present invention relates to an isolated nucleic acid molecule comprising a variant of the human *Aph-1b* gene that causes the amino acid residue in position 217 of the encoded *Aph-1b* to be an aliphatic amino acid, in particular a leucine.

Genetic variants on chromosome 5pl2 and 10q26 as markers for use in breast cancer risk assessment, diagnosis, prognosis and treatment

Stacey, Simon *et al.*

Decode, Genetics Reykjavik, Iceland, Inc

WO/2008/146309

The invention pertains to certain genetic variants on Chr5p12 and Chr10q26 as susceptibility variants of breast cancer. Methods of disease management, including diagnosing increased and/or decreased susceptibility to breast cancer, methods of predicting response to therapy and methods of predicting prognosis using such variants are described. The invention further relates to kits useful in the methods of the invention.

Methods for genetic analysis.

Cox, David R. *et al.*

Perlegen Sciences, Inc. Belmont, California, US

US 20070037198 Febuary 15, 2007

Methods are described for assessing an individual's likelihood of developing or exhibiting a multifactorial trait, and for predicting the effectiveness of a drug treatment regimen in an individual. The methods include determining a plurality of genotypes for the individual at a plurality of biallelic polymorphic loci, using the genotypes to compute a score for the individual, and comparing the score to at least one threshold value.Genetic tests are also described for assessing an individual's likelihood of developing or exhibiting a multifactorial trait.

Detecting kit for cardiovascular and cerebrovascular diseases predisposing gene chip

Haidong Mu *et al.*

Shanghai Yulong Biolog Science, China

CN101333561 Dec., 31, 2008

The invention provides a cardiovascular and cerebrovascular diseases susceptibility gene chip detection kit, which can be used to parallelly and economically detect thirteen genes related to the susceptibility of cardiovascular and cerebrovascular diseases. The kit comprises an extraction solution, a hybridization buffer solution, a washing liquor, an amplifying solution, Taq enzyme and a gene chip, the amplifying solution contains a primer for amplifying the thirteen genes containing mutant sites, and the thirteen genes respectively are: an Enos gene, an ADD1 gene, an APOB-2515 gene, an AT1R gene, an APOB-3500 gene, an APOB-4181 gene, an eNOS-894 gene, an FgB-148 gene, an APOE gene, a GNB3 gene, an AGT gene, an FVII gene and an MTHFR gene. A plurality of genes are related to the susceptibility of the cardiovascular and cerebrovascular diseases, and the detection kit can be used for detecting

the related thirteen genes parallelly.

Genetic susceptibility variants of type 2 diabetes mellitus

Steinthorsdottir Valgerdur and Thorleifeson G

Decode Genetics EHF Iceland

KR 20090087486, August 17, 2009

Association analysis has shown that certain genetic variants are susceptibility variants for Type 2 diabetes. The invention relates to diagnostic applications of such susceptibility variants, including methods of determining increased susceptibility to Type 2 diabetes, as well as methods of determining decreased susceptibility to Type 2 diabetes in an individual. The invention further relates to kits for determining a susceptibility to Type 2 diabetes based on the variants described herein.

Genetic brain tumor markers.

French Peter James *et al.*

Univ Erasmus Medical, Center Rotterdam, Rotteerdam, Netherlands

US20090215055 August, 27, 2009

The invention relates to method of genetic analysis for the prediction of treatment sensitivity and survival prognosis of patients with brain tumors, especially oligodendroglial tumors. The invention provides a method for producing a classification scheme for oligodendroglial tumors comprising the steps of a) providing a plurality of reference samples, said reference samples comprising cell samples from a plurality of reference subjects suffering from oligodendroglial tumors; b) providing reference profiles by establishing a gene expression profile for each of said reference samples individually; c) clustering said individual reference profiles according to similarity; and d) assigning an oligodendroglial tumor class to each cluster.

Reagent kit for detecting heart brain disease genetic susceptibility

Zuye Zou and Zhemin Feng

Shanghai Zhujian Biolog Engine, China

CN101354338 January, 28, 2009

The invention discloses a kit used for detecting the genetic susceptibility of cardio-cerebral diseases. The kit comprises a specific primer pair which detects genotypes of eighteen sites of single nucleotide polymorphism (SNP) in ABCA1, ACE, ADIPOQ, AGT, APOB, APOE, AT1R, CYP11B2, ENOS, LEP, LPL, MTHFR, PON1, PPARG, SOD3, UCP2 and VDR simultaneously and specified fluorescent probe pairs, conventional components used in fluorescence quantitative PCR detecting, and the like. The kit of the invention evaluates genetic susceptibility of cardio-cerebral diseases by detecting genotypes of eighteen sites of single nucleotide polymorphism which is closely related to genetic susceptibility of cardio-cerebral diseases.

Genetic risk assessment in heart failure: impact of genetic variation of beta 1 adrenergic receptor gly389arg polymorphism.

Worcel M and Sabolinski ML

Nitromed Inc; Univ Pittsburgh,US

US2009192128 July 30, 2009

The invention provides methods for (a) reducing mortality associated with heart failure; (b) improving oxygen consumption; (c) treating heart failure; (d) treating hypertension; (e) improving the quality of life in a heart failure patient; (f) inhibiting left ventricular remodeling; (g) reducing hospitalizations related to heart failure; (h) improving exercise tolerance; (j) increasing left ventricular ejection fraction; (k) decreasing levels of B-type natriuretic protein; (l) treating renovascular diseases; (m) treating end-stage renal diseases; (n) reducing cardiomegaly; (o) treating diseases resulting from oxidative stress; (p) treating endothelial dysfunctions; (q) treating diseases caused by endothelial dysfunctions; or (r) treating cardiovascular diseases; in a patient in need thereof, wherein the patient has a Arg389Arg polymorphism and/or a Gly389Gly polymorphism in the beta

1 adrenergic receptor gene, comprising administering to the patient (i) at least one antioxidant compound or a pharmaceutically acceptable salt thereof; (ii) at least one nitric oxide enhancing compound; and (iii) optionally the best current therapy for the treatment of cardiovascular diseases. In one embodiment the antioxidant is a hydralazine compound or a pharmaceutically acceptable salt thereof and the nitric oxide enhancing compound is isosorbide dinitrate and/or isosorbide mononitrate.

Method of determining anti-hypertensive drug therapy by genetic profiling

Huggins Gordon S

Tufts Medical CT Inc; US

WO2009082470 July 2, 2009

The invention comprises a method for determining an anti-hypertension therapy for an individual based upon the presence or absence of specific alleles affecting baseline blood pressure and sensititivity to different therapeutic formulations.

Genetic risk assessment in heart failure: impact of the genetic variation of NOS3

Worcel M and Sabolinski ML

Nitromed Inc; Univ Pittsburgh, US

US2009075956 March 19, 2009

The invention provides methods for (a) reducing mortality associated with heart failure; (b) improving oxygen consumption; (c) treating heart failure; (d) treating hypertension; (e) improving the quality of life in a heart failure patient; (f) inhibiting left ventricular remodeling; (g) reducing hospitalizations related to heart failure; (h) improving exercise tolerance; (j) increasing left ventricular ejection fraction; (k) decreasing levels of B-type natriuretic protein; (l) treating renovascular diseases; (m) treating end-stage renal diseases; (n) reducing cardiomegaly; (o) treating diseases resulting from oxidative stress; (p) treating endothelial dysfunctions; (q) treating diseases caused by endothelial dysfunctions; (r) treating cardiovascular diseases; in a patient in need thereof, wherein the patient has at least one polymorphism in the endothelial nitric oxide synthase (NOS3) gene, comprising administering to the patient (i) at least one antioxidant compound or a pharmaceutically acceptable salt thereof; (ii) at least one nitric oxide enhancing compound; and (iii) optionally the best current therapy for the treatment of cardiovascular diseases. In one embodiment the antioxidant is a hydralazine compound or a pharmaceutically acceptable salt thereof and the nitric oxide enhancing compound is isosorbide dinitrate and/or isosorbide mononitrate.

Use of genetic determinants in cardiovascular risk assessment

Johnson J and Beitelshees AL

Univ Florida; Johnson Julie, US

WO2008060618, May 22, 2009

The invention generally provides compositions and methods of using a subject's genetic information for the selection of prophylactic or therapeutic agents and treatment regimens, and related methods for assaying the risk of an adverse cardiovascular event in the patient.

Methods and compositions for correlating genetic markers with cardiovascular disease

Vance J M et al.

Duke University, Durham, North Carolina, US

US2009087844, April 12, 2009

The invention provides methods of identifying a subject having an increased or decreased risk of developing cardiovascular disease, comprising: a) correlating the presence of one or more genetic markers in chromosome 3q13.31 with an increased or decreased risk of developing cardiovascular disease; and b) detecting the one or more genetic markers of step (a) in the subject, thereby identifying the subject as having an increased or decreased risk of developing cardiovascular disease. Also provided are methods of identifying subjects with

cardiovascular disease as having a good or poor prognosis, as well as methods of identifying effective treatment regimens for cardiovascular disease, based on correlation with genetic markers in chromosome 3q13.31.

Genetic susceptibility variants associated with cardiovascular disease

Helgadottir Anna

Decode Genetics EHF, Iceland

WO2008102380, August 28, 2008

The invention relates to methods of diagnosing susceptibility to cardivascular disease, including coronary artery disease, MI, abdominal aorta aneurysm, intracranial aneurysm restenosis and peripheral arterial disease, by assessing the presence or absence of alleles of certain polymorphic markers found to be associated with cardiovascular disease. The invention further relates to kits encompassing reagents for assessing such markers, and methods for assessing the probability of response to therapeutic agents and methods using such markers.

Genetic marker for risk of cardiovascular disorder

Agah Ramtin and Murugesan G

Cleveland Clinic Foundation,US

WO2008098159, August 14, 2008

A method of identifying a subject at increased risk of developing cardiovascular disorder includes obtaining a biological sample containing a nucleic acid from the subject and determining the presence of at least one polymorphism in at least one of a TGFB 1 gene, TGFB R2 gene, or TLR2 gene. The presence of the at least one polymorphism is indicative of an increased risk of a cardiovascular disorder.

Method for evaluating genetic cardiovascular disease risk in sportsmen

Sidorenko BA and Zatejshchikov DA

Federal Noe G Uchrezhdenie UCH, Russia

RU2322193, April 20, 2008

Method involves determining polymorph markers of gene candidates. Genetic analysis of sportsman blood is done and predisposition and protection alleles and genotypes of polymorph markers of gene candidates are determined like T174M marker of AGT gene predisposition allele M and protection allele T, A(-153)G marker of AT2R1 gene predisposition genotypes AG, GG and allele G and protection genotype AA and allele A, A1298C marker of MTHFR gene predisposition genotype CC and allele C and protection allele A, C825T marker of GNB3 gene predisposition genotype CC, Ala(-9)Val marker of SOD2 gene predisposition genotype AA and allele A and protection genotype VV and allele V, T(-262)C marker of CAT gene predisposition genotype CC, Cys311Ser marker of PON2 gene predisposition genotype Ser/Ser and protection genotype Cys/Ser, I/D marker of gene ApoB predisposition genotype I/I and allele I and protecting genotypes I/I allele I and protection ID and DD and allele D for cardiac ischemia cases; Ser447Ter marker of LPL gene predisposition allele Ser and protection allele Ter, Pro12Ala marker of PPARG2 gene predisposition allele Ala and protection genotypes Pro/Pro and allele Pro, A1298C marker of MTHFR gene predisposition allele C and protection genotype AA and allele A, T(9282) marker of CAT gene predisposition genotype TC and protection genotype CC, Lys198Asn of END1 gene predisposition genotype Lys/Lys for hypertension disease cases; 4a/4b marker of NOS3 gene predisposition genotype 4b/4a and allele 4a and protection genotype 4b/4b and allele 4b, A(-153)G of AT2R1 gene predisposition genotypes AG and allele G and protection genotype AA and allele A, A1298C marker of MTHFR gene predisposition genotype CC and allele C and protection allele A, G7831A of gene ACE predisposition genotypes AA and GA and allele A and protection genotype GG and allele G for hypertension heart cases. It gives base for making conclusion about predisposition when quantitative predomination of predisposition

genotypes and alleles take place. Equal quantities of predisposition and protection genotypes and alleles or quantitative predomination of protection genotypes and alleles being the case, genetic protection against pathologic changes development is concluded to be available.

Use of genetic determinants in cardiovascular risk assessment

Johnson Julie

Univ Florida ; Johnson Julie

WO2008060618, May 22, 2008

The invention generally provides compositions and methods of using a subject's genetic information for the selection of prophylactic or therapeutic agents and treatment regimens, and related methods for assaying the risk of an adverse cardiovascular event in the patient.

Gene map of the human genes associated with asthma disease

Belouchi A and Allesad R

Genizon Biosciences Inc., Carolina, US

CA2667476, July 17, 2008

The invention relates to the selection of a set of poymorphism ma rkers for use in genome wide association studies based on linkage disequilib rium mapping. In particular, the invention relates to the fields of pharmaco genomics, diagnostics, patient therapy and the use of genetic haplotype info rmation to predict an individual's susceptibility to asthma disease and/or t heir response to a particular drug or drugs.

ANGE gene in atopy

Zhang Y and Moffatt M

Isis Innovation Ltd., UK

US2009007280, January 1, 2009

The invention relates to isolated nucleic acid sequences of ANGE, CLLD8 and CLLD7 or sequences complementary or substantially homologous thereto or fragments thereof. Also provided are sequences comprising hybrid nucleic acid sequences from two or more of the genes. Also provided are nucleic acid expression vectors, polypeptides, antibodies to the polypeptides, host cells, non-human transgenic animals and pharmaceutical compositions and agents. Also provided is the use of the nucleic acid sequence and/or protein in medicine and research, methods for diagnosing or determining predisposition to disease or severity of disease, methods for preventing or treating disease, and kits for use in the methods and the use of the nucleic acid sequence and protein in treating or preventing IgE mediated diseases and non-atopic asthma, and in screens for identifying new agents for use in the methods.

Pharmaceutical and therapeutic applications relating to a type 9 adenylyl cyclase polymorphism in asthma and reversible bronchial obstruction

Liggett Stephen B

US2009149376 , June 11, 2009

A pharmaceutical composition comprising an expression product of a Type Nine adenylyl cyclase gene polymorphism, along with a suitable physiological carrier, are provided. In addition, methods related to treating patients having asthma or a reversible bronchial obstruction, kits for determining the responsiveness of an individual to treatment regimens, and assays for screening pharmaceutical for efficacy in treatment are also provided.

Genomic sequence of the 5-lipoxygenase-activating protein (FLAP), polymorphic markers thereof and methods for detection of asthma

Blumenfeld M and Chumakov I

Serono Genetics Inst SA, France

US2009155806, June 8, 2009

The invention concerns the genomic sequence of the FLAP gene. The biallelic markers of a FLAP gene and the association established between these markers and diseases involving the leukotriene pathway such as asthma. The invention provides means to determine the predisposition of individuals to diseases involving the leukotriene pathway

as well as means for the diagnosis of such diseases and for the prognosis/detection of an eventual treatment response to agents acting on the leukotriene pathway.

Genetic variants in the TCF7L2 gene as diagnostic markers for risk of type 2 diabetes mellitus

Grant; Struan F.

deCODE genetics EHF, Reykjavik, Iceland

US 7,585,630 September 8, 2009

Polymorphisms in the gene TCF7L2 are shown by association analysis to be a susceptibility gene for type II *diabetes*. Methods of diagnosis of susceptibility to *diabetes,* of decreased susceptibility to *diabetes* and protection against *diabetes,* are described, as are methods of treatment for type II *diabetes*.

Bardet-Biedl susceptibility gene and uses thereof

Sheffield, Val C and Stone E

The University of Iowa Research Foundation, Iowa City, Iowa, US

US 7,332,591 February 19, 2008

The invention relates to the designation of ADP-ribosylation factor-like 6 as the BBS3 gene, that is involved in the genetic disease Bardet Biedl Syndrome (BBS), which is characterized by such diverse symptoms as obesity, diabetes, hypertension, mental retardation, renal cancer and other abnormalities, retinopathy and hypogonadism. Methods of use for the gene, for example in diagnosis and therapy of BBS and in drug screening, also are described.

Method of predicting genetic risk for hypertension

Yamada Y and Tokota M.

Nagoya Industrial Science Research Institute, Nagoya-shi, Japan

US 7,572,576 August 11, 2009

It is intended to provide a means of predicting genetic risk for hypertension at a high accuracy and high prediction possibility. Namely, risk for hypertension is predicted by a method involving the following steps: (i) the step of analyzing two or more polymorphisms selected from among 4 gene polymorphisms having been revealed as relating to hypertension; (ii) the step of determining the genotype of a nucleic acid sample based on the polymorphism data obtained in the above step; and (iii) the step of predicting the genetic risk for hypertension from the genotype thus determined.

CICKb mutation as a diagnostic and therapeutical target

Lang F and Waldegger S.

Eberhard-Karls-Universitaet Tuebingen Universitaetsklinikum Tuebingen, DE, Germany

US 7,235,364 June 26, 2007

The invention relates to a method for diagnosing hypertension, and/or allergy, and/or hair loss, and/or liabilty for infection, of a human being, or a predisposition therefor; to a nucleic acid molecule coding for a human ClCKb protein comprising a genetic alteration at amino acid position 481 compared to the wild type, as well as for corresponding segments thereof; to a nucleic acid molecule which binds to the before-mentioned nucleic acid molecule under stringent conditions, as well as to a nucleic acid molecule which binds to that nucleic acid molecule; to a (poly)peptide encoded by the afore-mentioned nucleic acid molecules; to a method for identifying substances modulating activity of a peptide derived from ClCKb protein that is genetically altered at amino acid position 481 compared to the wild type; to a substance for modulating activity of a peptide derived from ClCKb protein that is genetically altered at amino acid position 481 compared to the wild type; to methods for preparing a pharmaceutical composition for treatment of hypertension, and/or allergy, and/or hair loss, and/or liability for infection; to pharmaceutical compositions; and to a method for treating a human being affected by hypertension, and/or

allergy and/or hair loss, and/or liability for infection.

Methods for identifying subjects susceptible to Charcot-Marie-Tooth neuropathy type 1C

Chance , *et al.*

University of Washington, Seattle, Washington, US

US 7,449,291 November 11, 2008

In one aspect, the invention provides methods of identifying genetic mutations that are associated with peripheral neurological disease. The methods comprise identifying a difference between a nucleic acid sequence of a small integral protein of the lysosome/late endosome ("SIMPLE") gene from a mammalian subject exhibiting peripheral neuropathy and a nucleic acid sequence of a SIMPLE gene from a subject which is not exhibiting peripheral neuropathy, wherein the difference is a genetic mutation associated with peripheral neurological disease. In another aspect, isolated nucleic acid molecules encoding SIMPLE missense mutations are provided. In another aspect, a method of screening a subject to determine if the subject has a genetic predisposition to develop Charcot-Marie-Tooth type 1C neuropathy is provided. In another aspect, the invention provides kits for determining susceptibility or presence of Charcot-Marie-Tooth type 1C neuropathy in a mammalian subject.

Matching system

Tuck; Edward F *et al.*

Social Fabric Corporation, West Covina, California, US

US 7,592,910 September 22, 2009

Methods and apparatus for finding a match between or among persons (17a, 17b), characteristics and/or objects are disclosed. In one embodiment of the invention, an electronic device (10a, 10b), such as a handheld radio, is used to find a person who meets criteria specified by a user. In another embodiment, this electronic device (10a, 10b) is programmed with information regarding the genetic attributes (33) of individuals. These individuals are matched by computing a correlation of the *genetic* attributes of two individuals (33a, 33b). The information regarding these genetic attributes are determined by testing a tissue or fluid sample. In yet another embodiment of the invention, once biological or genetic attributes (33) are determined, a perfume (544) may be manufactured which is based on a unique set of genetic attributes, and which may either fortify the sexual self-confidence and broadcast the attributes of the person who wears the perfume, or may be used to enhance the attractiveness of another person.

Human diabetes susceptibility SEMA6D gene

Philippi, A and Hager J

Integragen , Evry, France

WO/2008/087205, July 24, 2008

The invention relates to a diagnostic method of determining whether a subject, preferably an obese subject, is at risk ofdeveloping type 2 diabetes, which method comprises detecting the presence of an alteration in the SEMA6D gene locus in a biological sample of said subject.

Human diabetes susceptibility BTBD9 gene.

Philippi, A and Hager J

Integragen, Evry, France

WO/2008/087204, July 24, 2008

The invention relates to a diagnostic method of determining whether a subject, preferably an obese subject, is at risk of developing type 2 diabetes, which method comprises detecting the presence of an alteration in the BTBD9 gene locus in a biological sample of said subject.

Test method for type-2 diabetes using gene polymorphism

Yasuda, K and Kasuga M

Japan Health Sciences Foundation, National University Corporation, Kobe University, Japan

Patents

WO/2009/078385, June 25, 2009

Disclosed is a method for testing the genetic sensitivity to type-2 diabetes in a subject, which comprises detecting at least one polymorphism occurring in KCNQ1 gene and/or EIF2KA4 gene in a DNA-containing sample collected from the subject. The method enables the precise, rapid and simple test of the genetic sensitivity to type-2 diabetes in a subject by using a definitive genetic factor for determining the genetic sensitivity to type-2 diabetes.

Method and apparatus for detection of the healthy carrier state for the 35delg mutation in the gjb2 gene as risk factor for hearing loss

Guastalla, P and Gasparini P

Universita' Degli Studi Di Trieste and Instituto Di Ricovero E Cura A, IT

WO/2009/080562, July 2, 2009

A method is described for detection of the healthy carrier state or the genetic mutation 35delG in the GJB2 gene based on the measurement of epidermal thickness. Since this mutation correlates with the occurrence of genetically based hearing loss, the screening of a general normal hearing population for a potential genetic mutation, performed by non-invasive, rapid and reliable measurement of epidermal thickness, represents an important tool for genetic screening and assessment for a potential associated deafness risk. In addition to the method, the invention describes the apparatus to perform said method.

Therapeutic effects of Math1 gene pairs forsensorineural hearing loss

Shiming Y and Suoqiang Z

Shiming Yang, China

CN101392267 March 25, 2009

The invention relates to a gland virus carrier for expressing Math1 albumen and a transformant thereof, and a construction method of the carrier. The gland virus for expressing Math1 albumen obtained by the method can overcome one or a plurality of defects of the existing medication route and is safely and effectively used for treating sensorineural hearing loss.

Method for detecting hereditary hearing loss relative connexin 26 gene GJB2 mutation and kit for detection

Yongyi Y and Pu Dai

Zhengce J, China

CN101363054 February 11, 2009

The invention relates to a method for testing gap linking protein 26 gene GJB2 mutation related to hereditary hearing impairment, which completely enlarges GJB2 gene base boot sector, exon 1, exon 2 and shear zone by polymerase chain reaction; and then, DNA sequence is measured to detect whether GJB2 genetic mutation exists; the method of the invention can completely cover all the genetic mutation of the GJB2 gene base boot sector, the exon 1, the exon 2 and the shear zone, so that detectable rate of the GJB2 genetic mutation and diagnosis rate of hereditary hearing impairment related to the GJB2 gene are improved; compared with that sequence measurement is separately carried out on a GJB2 code area, the method is more comprehensive and reliable, so as to be beneficial to the diagnosis of hereditary hearing impairment.

Modulation of the transcription of pro-inflammatory gene products

Hecker M and Wagner A, Gottingen, Germay

US20090221686, September 3, 2009

The invention refers to inhibitors of the transcription factors IRF-1, their use as therapeutic agents as well as their use for prevention and therapy of cardiovascular complications like re-stenosis after percutaneous angioplasty or stenosis of venous bypasses, chronic (transplant arteriosclerosis or vasculopathy) or acute transplant rejection, graft versus host disease (GVHD), immunological hypersensitivity reactions (allergies), particularly bronchial asthma and atopic dermatitis, chronic recurrent inflammatory diseases, particularly ulcerative

colitis and Crohn's disease, psoriasis and sarcoidosis, as well as autoimmune diseases, particularly diabetes mellitus, multiple sclerosis, collagenoses (e.g. systemic lupus erythematodes), rheumatoid arthritis and vasculotids.

Marker gene for examining psoriasis vulgaris

Inoko H and Oka A

Univ Tokai and Genodive Pharma Inc., Japan

WO2009093288, July 30, 2009

It is intended to identify a novel psoriasis vulgaris-sensitive gene by a mapping method using microsatellites at a high efficiency and a low cost. Namely, a novel psoriasis vulgaris-sensitive gene such as OR2J3 gene or IGSF4D gene in human genomic DNA sequence has been identified by conducting a case control correlation analysis relating to psoriasis vulgaris with the use of microsatellite polymorphism markers, which are designed at intervals of about 100 kb, narrowing down candidate regions and then conducting a correlation analysis and a chain analysis with the use of SPNs as markers.

Human autism susceptibility gene encoding a transmembrane protein and uses thereof

Phillippi A and Rousseau F

Integragen, France

US2009215040, August 27, 2009

The invention discloses the identification of a human autism susceptibility gene, which can be used for the diagnosis, prevention and treatment of autism and related disorders, as well as for the screening of therapeutically active drugs. The invention more specifically discloses that the ATP2B2 gene on chromosome 3 and certain alleles thereof are related to susceptibility to autism and represent novel targets for therapeutic intervention. The present invention relates to particular mutations in the ATP2B2 gene and expression products, as well as to diagnostic tools and kits based on these mutations. The invention can be used in the diagnosis of predisposition to, detection, prevention and/or treatment of Asperger syndrome, pervasive developmental disorder, childhood disintegrative disorder, mental retardation, anxiety, depression, attention deficit hyperactivity disorders, speech delay or language impairment, epilepsy, metabolic disorder, immune disorder, bipolar disease and other psychiatric and neurological diseases including schizophrenia.

Human autism susceptibility gene encoding a kinase and uses thereof

Philippi A and Rousseau F

Integragen, France

US2009001414, January 8, 2009

The present invention discloses the identification of a human autism susceptibility gene, which can be used for the diagnosis, prevention and treatment of autism and related disorders, as well as for the screening of therapeutically active drugs. The invention more specifically discloses that the MARK1 gene on chromosome 1 and certain alleles thereof are related to susceptibility to autism and represent novel targets for therapeutic intervention. The present invention relates to particular mutations in the MARKI gene and expression products, as well as to diagnostic tools and kits based on these mutations. The invention can be used in the diagnosis of predisposition to, detection, prevention and/or treatment of Asperger syndrome, pervasive developmental disorder, mental retardation, anxiety, depression, attention deficit hyperactivity disorders, speech delay, epilepsy, metabolic disorder, immune disorder, bipolar disease and other psychiatric and neurological diseases including schizophrenia.

Novel gene disruptions, compositions and methods relating thereto

de Sauvage Fred *et al.*

Genentech Inc., South San Francisco, California, US

US2009096876, August 6, 2009

The invention relates to transgenic animals, as well as compositions and methods

relating to the characterization of gene function. Specifically, the invention provides transgenic mice comprising disruptions in PRO194, PRO220, PRO241, PRO284, PRO331, PRO354, PRO355, PRO533, PRO541, PRO725, PRO937, PRO1014, PRO1120, PRO1182, PRO1325, PRO1382, PRO1410, PRO1555, PRO1556, PRO1760, PRO1787, PRO1868, PRO4326, PRO4332, PRO4346, PRO4400, PRO6003, PRO6094, PRO6244, PRO9820, PRO9828, PRO10274, PRO16090, PRO19644, PRO21340, PRO92165, PRO85143, PRO1124, PRO1026 or PRO23370 genes. Such in vivo studies and characterizations may provide valuable identification and discovery of therapeutics and/or treatments useful in the prevention, amelioration or correction of diseases or dysfunctions associated with gene disruptions such as neurological disorders; cardiovascular, endothelial or angiogenic disorders; eye abnormalities; immunological disorders; oncological disorders; bone metabolic abnormalities or disorders; lipid metabolic disorders; or developmental abnormalities. .

4th International Symposium on Current Trends in Drug Discovery Research (CTDDR-2010)

17-02-2010 to 21-02-2010

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Centre for Cellular & Molecular Biology, Hyderabad

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Compiled By : Wamiq F. Rahman
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Symposium
On
Recent Trends in Biophysics
February 13 – 15, 2010

And

Workshop on
Emerging Techniques of Biophysics
February 16, 2010

Organised by
Department of Physics
Banaras Hindu University, Varanasi

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