REVIEW

Role of genomics in cardiovascular medicine

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Abstract

As all branches of science grow and new experimental techniques become readily accessible, our knowledge of medicine is likely to increase exponentially in the coming years. Recently developed technologies have revolutionized our analytical capacities, leading to vast knowledge of many genes or genomic regions involved in the pathogenesis of congenital heart diseases, which are often associated with other genetic syndromes, coronary artery disease and non-ischemic cardiomyopathies and channelopathies. The knowledge-base of the genesis of cardiovascular diseases is likely going to be further revolutionized in this new era of genomic medicine. Here, we review the advances that have been made over the last several years in this field and discuss different genetic mechanisms that have been shown to underlie a variety of cardiovascular diseases.

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INTRODUCTION

Cardiovascular disease is considered the primary cause of death in developed countries and is becoming a major cause of death in the developing world. As with all fields of medicine, much attention must be paid to classification of the disease and, consequently, to its genetic characterization. The field of cardiovascular medicine involves a broad spectrum of abnormalities that are characterized by a host of clinical and etiological features that range from simple congenital diseases related to metabolic defects to complex diseases that manifest in adulthood.

In congenital heart disease, chromosomal aberrations or mutations in genes regulating cardiac development are usually the cause of the disease[1-25]. The environment can have a small "teratogenic" effect in that some substances (e.g. early exposure to angiotensin converting-enzyme inhibitors, alcohol abuse and *Rubella virus*) alter the function of certain genes during embryogenesis[1].

A rough categorization of the remaining adulthood cardiovascular diseases is ischemic and non-ischemic. Non-ischemic cardiomyopathies are usually associated

with heart failure and sudden cardiac death (SCD) in the young, and ischemic cardiomyopathies are usually related to atherosclerosis and its sequelae, which include stroke and myocardial infarction (MI) in older populations[26,27].

SCD in the young is believed to be mainly due to non-ischemic cardiomyopathy (often involving structures of the heart or tissues) and channelopathies (in which ion channels are malfunctioning and cause conduction defects). In these cases, a distinction between primary and secondary cardiomyopathy must be made. In fact, these diseases can also be the consequence of other clinical phenotypes, such as hypertension and peri-myo-endocarditis. Furthermore, they can also be related to excessive physical activity and illicit drug and alcohol abuse. In all, these cases are defined as "secondary"[27]. When there is no clear etiology, or there is familial recurrence, they are defined as "primary."

Several mutations in different genes have been identified and functionally defined in both sporadic and familial forms of these diseases, and different models of inheritance; e.g. Mendelian and non-Mendelian, have been observed[28-37]. Despite the low frequency of functional mutations that lead to these phenotypes and inheritance models that can be applied to such variations, geneticists are reluctant to define any of them as "simple." Due to environmental influence and the modulation of common single nucleotide polymorphisms (SNPs) on the effect of disease causing mutations[38,39], most of these diseases should be considered as "complex."When over the age of 35-40 years, cardiac death is usually related to ischemic cardiomyopathy, which is secondary to the atherosclerotic process that leads to occlusion of the coronary artery resulting in acute myocardial ischemia or infarction. Except for very rare and clear cases of Mendelian mutations that cause premature coronary artery disease (CAD), atherosclerosis is a complex phenotype, in which environmental factors play a major role interacting with one another and with the biological background of each individual in determining the insurgence of the disease. The biological background of each individual can be considered unique, as one or more of the risk factors interplay with environmental factors. At least seven processes are involved in atherogenesis, leading to approximately 6 lesion stages as classified by the American Heart Association[40]. In the early stages, mainly endothelial dysfunction, endothelial cell activation and inflammation are involved, whereas proteolysis and apoptosis are essential in the formation of the lipid core and fibrous cap of each lesion. Finally, platelet aggregation, angiogenesis and thrombosis are major players in the last stages of the disease that involve plaque growth and rupture[40] (Figure 1). In each of these stages, hundreds of genes and proteins are believed to be involved. Nonetheless, genetic variants can have a major and diverse role in different stages of the disease, and risk variants in each stage may have no effect or even a protective role in other stages. The "mutation theory of atherosclerosis," which underlines the similarity between atherosclerotic and carcinogenic processes, is currently under study. This theory remains to be defined, but there is already significant evidence, in the form of microsatellite instability and loss of heterozygosity in smooth muscle cells of human plaques, that supports the hypothesis of genetic aberrations in atherogenesis[41,42]. Recent studies have also correlated chromosome telomere length and coronary heart disease (CHD), suggesting that, in atherosclerosis, as in other complex phenotypes such as type 2 diabetes and cancer, telomere length probably contributes as a primary abnormality[43].

CHD and MI are characterized by a high level of genetic and clinical heterogeneity. As mentioned earlier, the study of such diseases is complicated by the considerable impact of the environment on disease development, by the multiplicity of pathways involved in the response to environmental stress in different phases of disease evolution, and the multiplicity of clinical sub-phenotypes, such as hypertension and hypercholesterolemia.

ROLE OF GENETICS IN THE PATHOGENESIS OF CARDIOVASCULAR DISEASE

In 1964, Detweiler *et al*[44] investigated whether heart diseases, such as atherosclerosis, non-ischemic cardiomyopathy, congenital malformations, arrhythmias, conduction disturbances, congestive heart failure and hypertension, could be genetically determined in different species and breeds within species. Since then, much effort has gone into developing knowledge of the genetics of cardiovascular diseases. We briefly report the information that has accumulated over the last 4 decades. A schematic summary of cardiovascular defects and involved genes, which is far from being exhaustive, is shown in Table 1.

Congenital heart disease
Although biases related to recruitment methods must be considered, early studies on dogs comparing mongrels and purebreds, summarized in Detweiler's review, suggested a correlation between consanguinity and congenital heart disease[44].

Most types of congenital heart disease are usually associated with other syndromes, and are caused by chromosomal aneuploidies or mutations usually located in genes that have been implicated in cardiac development. The heritability of congenital heart disease depends on the disease and on the underlying genetic cause.

These defects can be caused by errors in meiosis (and the predisposition to such errors can be due to external variables, such as teratogens), by the heritability of a parental chromosomal translocation, or may be due to *de novo* mutations[1-25]. Since there is complexity in the classification of such diseases and their association with diverse genetic phenotypes, a rough scheme of the involved genes is presented in Table 1.

Non-ischemic cardiomyopathy
As mentioned previously, only the primary forms of these
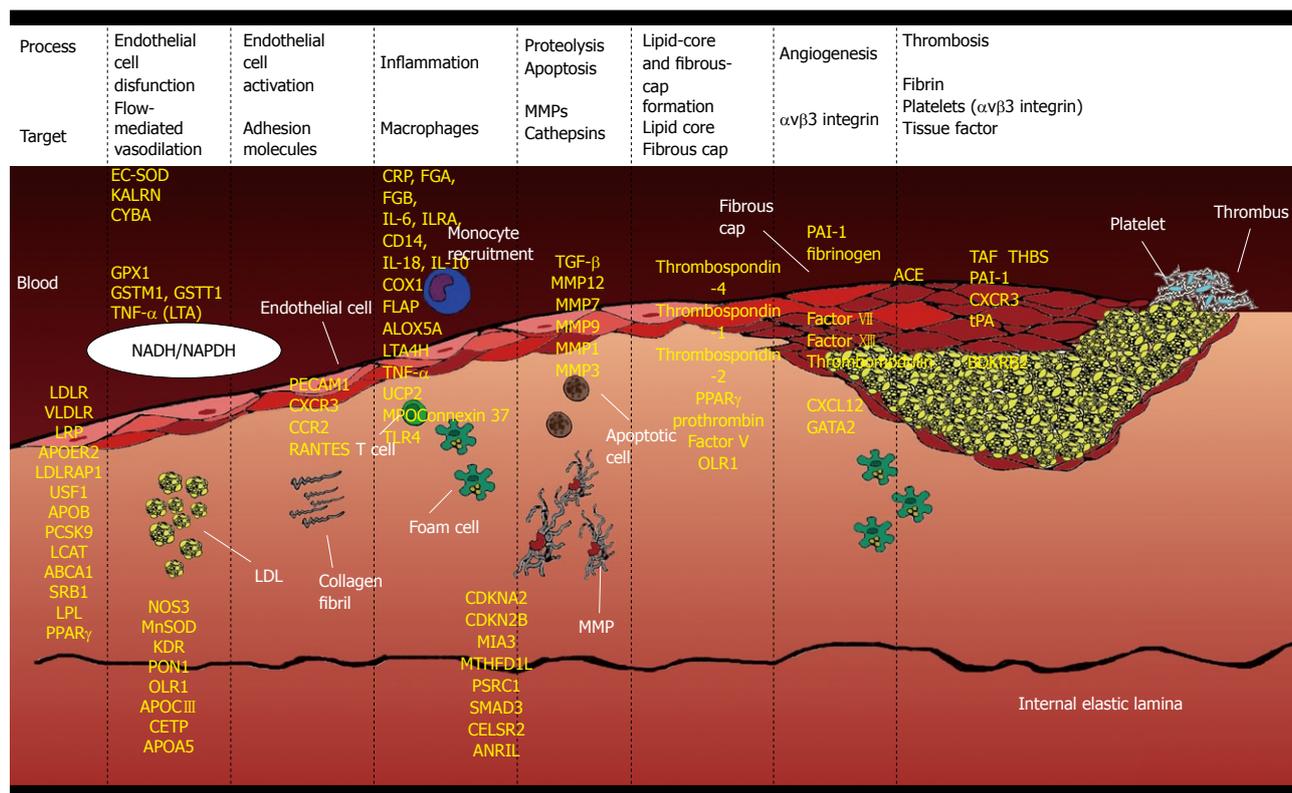
Figure 1 Clinical, biological and hypothesized genetic complexity of atherosclerosis as a model of complex disease. Modified from Watkins *et al*[40], 2006. MMPs: Matrix metalloproteinases; IL: Interleukin; LDL: Low density lipoprotein; TNF: Tumor necrosis factor; TGF: Transforming growth factor; PPAR: Peroxisome proliferator-activated receptor.

diseases, which are not the consequence of other phenotypes, such as hypertension, myocarditis and environmental factors like drug consumption or physical activity, can be ascribed to genetic factors. Familial cardiomyopathy and ion channelopathies are often described as single gene disorders. However, even in these disorders there are modifier genes that have a significant influence on phenotype, which may not be detected by conventional genetic techniques such as linkage analysis. Nonetheless, there is some suggestive evidence that arrhythmias, such as atrial fibrillation occurring in association with structural heart disease, are more prevalent in individuals with a certain genetic predisposition.

Quantification of the influence of genetics in these pathologies is quite difficult due to the complexity of aetiologies. To date, adult onset hypertrophic cardiomyopathy, which is the most common cardiomyopathy, is considered a genetically linked condition caused by inheritance or new mutations in genes that encode sarcomeric proteins. These include the cardiac β-myosin heavy chain (*MYH7*, 14q11.2), the cardiac myosin-binding protein C (*MYBPC3*, 11p11.2), cardiac troponin T (*TNNT2*, 1q32.1), cardiac troponin I (*TNNI3*, 19q13.42), essential myosin light chain (*MYL3,* 3p21.31), regulatory myosin light chain (*MYL2*, 12q24.11), α-tropomyosin (*TPM1*, 15q22.2), cardiac actin (*ACTC*, 15q14), and titin (*TTN*, 15q31.2). Mutations in the γ2 regulatory subunit of AMP-activated protein kinase (*PRKAG2*, 7q36.1) result in early-onset left ventricular hypertrophy with arrhythmias and, more rarely, fatal infantile cardiac glycogenesis. Mutations in the

gene encoding lysosome-associated membrane protein 2 (*LAMP2*, Xq24) cause massive left ventricular hypertrophy in male subjects in whom systemic manifestations (phenotype known as Danon's disease) may also develop. In 2008, Morita *et al*[28] sequenced 9 genes known to cause adult onset disease in 84 children with idiopathic cardiac hypertrophy diagnosed at an early age (under 15 years). The authors concluded that cardiac hypertrophy in children and adults has a common genetic basis; the cause of half of the presumed sporadic cases and of nearly two-thirds of familial cases of childhood-onset hypertrophy was mutations predominantly in *MYH7* and *MYBPC3*[28].

Other mutations in structural protein or ion channel subunit coding genes have been identified as underlying factors for other forms of cardiopathy, channelopathies (Brugada, LQT, SQT) and atrial fibrillation (Table 1).

Although intense efforts have been made to qualify and quantify the role of genetics in victims of SCD, it is still not possible to explain the role of mutations, modifier polymorphisms and environmental factors in a vast majority of cases[26-39].

Ischemic cardiopathy

Over the past 4-5 decades, information on the role of environmental and genetic factors predisposing to atherosclerosis and to its clinical sub-phenotypes has accumulated. The classical environmental risk factors are well established and are mainly associated with lifestyle (diet and smoking) and family history of early CAD[45,46].

With the exception of disease causing mutations that

Table 1 Summary of defects affecting the cardiovascular system and list of involved genes

	Phenotype	Involved genes	Associated diseases
Congenital heart disease			
Cyanotic heart disease	Transposition of the great arteries	NKX2-5, THRAP2	
	Tetralogy of fallot	NKX2-5, NOTCH1, TBX1, JAG1, NOTCH2	DiGeorge syndrome, alagille syndrome
	Tricuspid atresia	NKX2-5	
	Pulmonary atresia	PTPN11, JAG1, NOTCH2	Alagille syndrome
	Ebstein's anomaly of the tricuspid valve	NKX2-5	
	Double outlet right ventricle	NKX2-5, THRAP2	
	Persistent truncus arteriosus	TBX1	DiGeorge syndrome
	Anomalous pulmonary venous connection		
Left-sided obstruction defects	Hypoplastic left heart syndrome	NOTCH1	
	Mitral stenosis		
	Aortic stenosis	NOTCH1, PTPN11	
	Aortic coarctation	NOTCH1, PTPN11	
	Interrupted aortic arch	TBX1	DiGeorge syndrome
Septation defects	Atrial septation defects	NKX2-5, GATA4, TBX20, MYH6, TBX5	HOS
	Ventricular septal defects	NKX2-5, GATA4, TBX20, TBX1, TBX5	HOS
	Atrioventricular septal defects	PTPN11, KRAS, SOS1, RAF1, CRELD1	Noonan syndrome
Other congenital heart defects	Bicuspid aortic valve	NOTCH1	
	Patent ductus arteriosus	TFAP2B	Char syndrome
Non ischemic cardiopathies			
Structural defects	CMH	MYH7, TNNT2, TPM1, MYBPC3, PRKAG2, TNNI3, MYL3, TTN, MYL2, ACTC1, CSRP3, LAMP2	CMH1, CMH2, CMH3, CMH4, CMH5, CMH6, CMH7, CMH8, CMH9, CMH10, CMH11, CMH12, Danon disease
	Dilated cardiomyopathy	ACTC, DES, SGCD, MYH7, TNNT2, TPM1, TTN, VCL, MYBPC, MLP, ACTN2, PLN, ZASP, MYH6, ABCC, TNNC1, TCAP, EYA4, LMNA, SCN5A, DMD, TAZ, TNNI3	Laminopathies, hypertension, ischemic disease
	Arrhythmogenic right ventricular dysplasia/cardiomyopathy	JUP, DSP, PKP2, DSG2, DSC2, RYR2, TGFB3	Naxos disease, Carvajal disease
Channelopathies	Long QT syndrome	SCN5A, SCN4B, KCNQ1, KCNH2, KNE1, KNE2, KCNJ2, ANK2, CAV3	Romano-Ward syndrome, Jervell Lange-Nielsen syndrome, Andersen-Tawil syndrome, Timothy syndrome
	Brugada syndrome	SCN5A, SCN1B, GPD1L, CACNA1C, CACNB2b	
	Sindrome di Lev-Lenègre	SCN5A	
	Short QT syndrome	KCNH2, KCNQ1, KCNJ2	
	Sindrome di Wolff-Parkinson-White	AMPK	
	Tachicardia ventricolare	ADRB1, ADRB2, ADRB3	
	Tachicardia ventricolare polimorfica catecolaminergica	RYR2, CASQ2	
	Atrial fibrillation	KCNQ1, KCNE2, KCNJ2, KCNH2	
Ischemic cardiopathy			
Coronary artery disease, myocardial infarction	Mendelian inheritance	LDLR, APOB, ABCG5, ABCG8, APOA1, ABCA1, CBS	Familial hypercholesterolemia
	Complex disease	9p21, SH2B3, MRPS6-SLC5A3-KCNE, PHACTR1, CELSR2-PSRC1-SORT, CXCL12, MIA3, PCSK9	

CMH: Hypertrophic cardiomyopathy; HOS: Holt-Oram syndrome.

lead to premature CAD, genetic factors leading to atherosclerosis are often addressed as polymorphisms, which are variants that show high frequencies in the general population and participate in individual susceptibility to develop the disease. Mendelian forms of CAD are caused by mutations in genes involved in sterol metabolism, HDL concentration regulation, cholesterol efflux in macrophages and homocysteine concentration regulation. These include the low density lipoprotein receptor (*LDLR*, 19p13.2), the apolipoprotein B and A1 (*APOB*, 2p24.1 and *APOA1*,

11q23.3), members 5 and 8 of the subfamily G of the ATP binding cassette (*ABCG5* and *ABCG8*, 2p21), member 1 of the subfamily of the ATP binding cassette (*ABCA1*, 9q31.1) and the cystathionine-beta-synthase (*CBS*, 21q22.3) genes. Identification of the *LDLR* gene in the pathogenesis of familial hypercholesterolemia advanced knowledge on the cholesterol metabolism pathway as a major player in atherogenesis[40]. Since this discovery, many studies, in particular large scale genome-wide association studies, identified several common variants in genes encoding for

proteins involved in cholesterol metabolism, inflammation and immunity that are associated with atherogenesis. In particular, an association between CAD and a region on chromosome 9 (9p21) was first identified in 2005[47]. This result was replicated in another 25 different studies. A recent meta-analysis of 16 of these 25 studies has confirmed a statistically significant association between 9p21 polymorphisms and CAD[48]. Nevertheless, this chromosomal region is devoid of protein-coding genes and a clear functional interpretation is still lacking. However, it is known that this region neighbours *CDKN2A/B* (encoding cyclin-dependent kinase inhibitors involved in cell cycle) genes. Recently, Visel *et al*[49] observed that deletion of the orthologous 70 kb non-coding region on *Mus musculus* chromosome 4 affects cardiac expression of the neighbouring genes, as well as proliferation properties of cells in the vessel wall. As a consequence, Chr4$^{\Delta70/\Delta70}$ mice showed rapid weight gain and increased mortality during the developmental phase as well as in adulthood. Upon necropsy, 45% of these animals were found to have neoplasms of various types suggesting that this region could have a pivotal role in the regulation of cell proliferation and senescence[49]. This region is also associated with other phenotypes, such as sporadic amyotrophic lateral sclerosis, cutaneous nevi development, and intracranial aneurism[50-52].

The analysis of phenotypes, such as CAD or MI, presents two main obstacles: (1) the complexity of phenotypes (e.g. differences between early and late age onset MI, ST elevation MI and non-ST elevation MI) that can lead to non-replications[53]; and (2) corrections that must be applied when analyzing multiple variants[54], which can lead to false negatives. It is possible that, in the years to come, with the refinement of samples and the development of new methods, data unravelling the complexity of CAD will be easier to obtain. To give an idea of how quickly information on complex diseases increases, 5 new loci associated with CAD were identified in 2009 alone. Gudbjartsson *et al*[55] found genome-wide significance for a non-synonymous SNP on *SH2B3* gene (at 12q24) in association with inflammation in endothelial cells, elevated eosinophil count, and acute MI in six populations[55]. The Myocardial Infarction Genetics Consortium, studying a sample of early onset acute MI, identified three new variants: 21q22 near *MRPS6-SLC5A3-KCNE* (encoding genes for mitochondrial ribosomal protein 28s, a sodium and myo-inositol transporter in response to hypertonic stress, and a potassium channel involved in the pathogenesis of arrhythmias), 6p24 in *PHACTR1* (encoding for an inhibitor of protein phosphatase 1 involved in serine and threonine dephosphorylation crucial for cell growth and differentiation), and 2q33 in *WDR1*, a member of the Pes1-Bop1 complex (required for ribosome biogenesis and, once again, crucial for cell proliferation). This consortium also replicated genome-wide significance for 6 previously identified variants (9p21, 1p13 near *CELSR2-PSRC1-SORT1*, 10q11 near *CXCL12*, 1q41 in *MIA3*, 19p13 near *LDLR* and 1p32 near *PCSK9*)[56]. Erdmann *et al*[57] identified a new susceptibility locus on 3q22.3

(*MRAS*, a RAS related protein encoding gene involved with cell growth and differentiation.).

Although much effort has been spent on identifying and interpreting the involvement of different genetic variants in the pathogenesis of atherosclerosis, we can consider the problem far from being solved.

COMMON DISEASES AND VARIANT HYPOTHESES

Mutations that have a deleterious effect are usually associated with disease and, hence, often remain rare, with the result that the related disease is also rare. Variants conferring an advantage are often the basis for evolutionary change and tend to rise rapidly to high frequency, a phenomenon known as genetic hitchhiking[58]. On the other hand, a polymorphism is defined as a frequent variant that is often neutral. Although the majority of neutral mutants are lost by chance, a minority of them eventually become fixed in the population[59]. There are two hypotheses on the genetic basis of common diseases. On the one hand, the common disease common variant (CDCV) hypothesis postulates that genetic variants have low penetrance but high frequency in the population and contribute to the genetic background of common diseases; and on the other hand, the common disease rare variant (CDRV) hypothesis proposes that rare variants with strong penetrance provide this attribute to common diseases. On the basis of the second hypothesis, new generation sequencing methods are being tested to identify the rare variations that have escaped in genome-wide association studies[60]. Over the last few years, these studies have identified a number of SNPs in the genome, resulting in recognition of about 150 common variants in robust association with over 30 common phenotypes. Given the very low penetrance and number of studies that have analyzed such variants, it is difficult to give an accurate predictive value for a complex disease state such as CAD, diabetes or hypertension.

UTILITY AND LIMITATIONS OF TESTING GENETIC VARIATIONS

Despite the discussions and concerns that have been described elsewhere[60-68], there is an immense need to validate and provide a qualification process for genomic biomarkers before use in clinical practice, from a practical point of view.

Further, it is important to interpret the results of genetic testing using a set of parameters that includes family history and a scoring system for the range of clinical manifestations associated with the disease. Genome-wide association studies have so far identified only a small fraction of the heritability of CAD, so the ability to make meaningful predictions is still quite limited. Nonetheless, direct-to-consumer marketing of genetic risk prediction for CAD is attracting early adopters.

To date, the only genomic biomarkers that have been

Table 2 List of genetic markers that have been approved by the US Food and Drug Administration and by the European Medicines Agency (source: http://www.fda.gov)

Biomarker	Representative label	Drug
HLA-B*5701 allele presence	Patients who carry the HLA-B*5701 allele are at high risk for experiencing a hypersensitivity reaction to abacavir	Abacavir
Her2/neu over-expression	Over-expression of Her2/neu necessary for selection of patients appropriate for drug therapy (breast cancer)	Trastuzumab (Herceptin®)
EGFR expression with alternate context	Epidermal growth factor receptor presence or absence (colorectal cancer)	Cetuximab (Erbitux®)
UGT1A1 variants	UGT1A1 mutation patients, exposure to drug and hence their susceptibility to toxicity (colon-rectum cancer)	Irinotecan (Camptosar®)
TPMT variants	Increased risk of myelotoxicity associated to thiopurine methyltransferase deficiency or lower activity	Azathioprine (Imuran®)
Protein C deficiencies (hereditary or acquired)	Hereditary or acquired deficiencies of protein C or its cofactor protein S	Warfarin (Coumandin®)
C-KIT expression	Gastrointestinal stromal tumour c-kit expression	Imatinib mesylate (Glivec®)
CYP2C19 variants	CYPC19 variants (poor metabolizers PM and extensive metabolizers EM) with genetic defect leads to change in drug exposure	Voriconazole (Vfend®)
CYP2C9 variants	CYP2C9 variants PM and EM genotypes and drug exposure	Celecoxib (Celebrex®)
CYP2D6 variants	CYP2D6 variants PM and EM genotypes and drug exposure	Atomoxetine (Strattera®)
CYP2D6 with alternate context	CYP2D6 PM and EM variants and drug exposure and risk	Fluoxetine HCl (Prozac®)
DPD deficiency	Severe toxicity (stromatitis, diarrhoea, neutropenia and neurotoxicity) associated to deficiency of dihydropyrimidine dehydrogenase	Capecitabine (Xeloda®)
EGFR expression	Epidermal growth factor receptor presence or absence (NSCLC, pancreas cancer)	Erlotinib (Tarceva®)
EGFR expression with alternate context	Epidermal growth factor receptor presence or absence (squamous cell carcinoma of head and neck)	Cetuximab (Erbitux®)
G6PD deficiency	G6PD deficiency and risk for haemolysis	Rasburicase (Elitek®)
G6PD deficiency with alternate context	G6PD deficiency (or NADH methemoglobin reductase deficiency) and risk for haemolytic reactions	Primaquine (Primaquine®)
KRAS mutation	Retrospective subset analyses of metastatic colorectal cancer trials have not shown a treatment benefit for Vectibix in patients whose tumors had KRAS mutations in codon 12 or 13. Use of Vectibix is not recommended for the treatment of colorectal cancer with these mutations	Panitumumab (Cetuximab®)
NAT variants	N-Acetyltransferase slow and fast acetylators and toxicity	Rifampin isoniazid (Rifater® and pyrazinamide)
Philadelphia chromosome deficiency	Philadelphia (Ph1) chromosome presence and efficacy-Busulfan is less effective in patients with CML lacking the Philadelphia chromosome	
UCD efficiency disorders	Valproate therapy and urea cycle disorders interaction	Valproic acid (Depakene®)
VKORC1 variants	Polymorphisms of vitamin K epoxide reductase complex subunit identify warfarin-sensitive patients who require a lower dose of the drug	Warfarin (Coumandin®)
PML/RAR α gene expression (retinoic acid receptor responders and non-responders)	PML/RAR (α) fusion gene presence	Tretinoin (Avita®, Renova®, Retin-A®)

approved and are recommended by the European Medicines Agency (EMA) and by the Food and Drug Administration (FDA) are localized in the ambit of pharmacogenomics (e.g. *VKORC1/CYP2C9* genotype for warfarin dosing in coagulation defects and HLA-B*5701 for the prevention of adverse reactions in antiretroviral therapy in HIV infected patients[68]; Table 2 from http://www.fda.gov).

LEVELS OF BIOLOGICAL VARIATION

DNA is a very stable molecule that is easy to extract and is less prone to degradation compared to RNA. DNA is, hence, easier to study. However, one must consider somatic cell mutations, tissue-specific epigenetic effects such as DNA methylation, histone modification and microRNA expression, which can significantly and constantly change expression in the cell. Such changes can alter the activities of the cell and cannot be neglected when studying the biology of a complex disease[69]. Figure 2 shows a
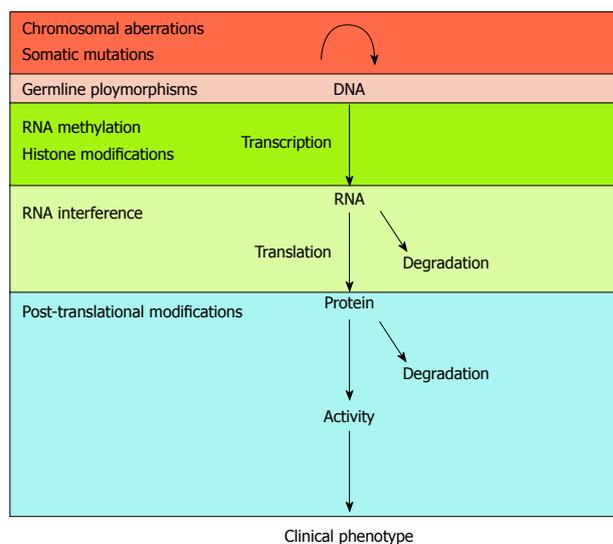
Figure 2 Biological variation at different levels. Modified from Brockmöller *et al*[69], 2008.

simplified scheme of these variations. As an example, in the cardiovascular field, we have already mentioned the "mutation theory of atherosclerosis", which underlines the similarity between atherosclerotic and carcinogenic processes[41,42]. Furthermore, different microRNAs have been found to be involved in different phases of ischemic heart disease[70]. These levels of variation are much harder to analyze, and are not constant during the individual's lifetime as DNA variations, but are important in the pathology of all diseases, including cardiovascular diseases. The study of epigenetics, transcriptomics and proteomics is, therefore, another important issue in all disease studies and needs to be well integrated with studies of genome variability. These discussions and integration of these issues is beyond the scope of this mini-review.

REFERENCES

1   **Bruneau BG**. The developmental genetics of congenital heart disease. *Nature* 2008; **451**: 943-948

2   **Garg V**, Kathiriya IS, Barnes R, Schluterman MK, King IN, Butler CA, Rothrock CR, Eapen RS, Hirayama-Yamada K, Joo K, Matsuoka R, Cohen JC, Srivastava D. GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. *Nature* 2003; **424**: 443-447

3   **Sarkozy A**, Conti E, Neri C, D'Agostino R, Digilio MC, Esposito G, Toscano A, Marino B, Pizzuti A, Dallapiccola B. Spectrum of atrial septal defects associated with mutations of NKX2.5 and GATA4 transcription factors. *J Med Genet* 2005; **42**: e16

4   **Brassington AM**, Sung SS, Toydemir RM, Le T, Roeder AD, Rutherford AE, Whitby FG, Jorde LB, Bamshad MJ. Expressivity of Holt-Oram syndrome is not predicted by TBX5 genotype. *Am J Hum Genet* 2003; **73**: 74-85

5   **Ching YH**, Ghosh TK, Cross SJ, Packham EA, Honeyman L, Loughna S, Robinson TE, Dearlove AM, Ribas G, Bonser AJ, Thomas NR, Scotter AJ, Caves LS, Tyrrell GP, Newbury-Ecob RA, Munnich A, Bonnet D, Brook JD. Mutation in myosin heavy chain 6 causes atrial septal defect. *Nat Genet* 2005; **37**: 423-428

6   **Ruiz-Perez VL**, Ide SE, Strom TM, Lorenz B, Wilson D, Woods K, King L, Francomano C, Freisinger P, Spranger S, Marino B, Dallapiccola B, Wright M, Meitinger T, Polymeropoulos MH, Goodship J. Mutations in a new gene in Ellis-van Creveld syndrome and Weyers acrodental dysostosis. *Nat Genet* 2000; **24**: 283-286

7   **Ruiz-Perez VL**, Tompson SW, Blair HJ, Espinoza-Valdez C, Lapunzina P, Silva EO, Hamel B, Gibbs JL, Young ID, Wright MJ, Goodship JA. Mutations in two nonhomologous genes in a head-to-head configuration cause Ellis-van Creveld syndrome. *Am J Hum Genet* 2003; **72**: 728-732

8   **Goldmuntz E**, Bamford R, Karkera JD, dela Cruz J, Roessler E, Muenke M. CFC1 mutations in patients with transposition of the great arteries and double-outlet right ventricle. *Am J Hum Genet* 2002; **70**: 776-780

9   **Mégarbané A**, Salem N, Stephan E, Ashoush R, Lenoir D, Delague V, Kassab R, Loiselet J, Bouvagnet P. X-linked transposition of the great arteries and incomplete penetrance among males with a nonsense mutation in ZIC3. *Eur J Hum Genet* 2000; **8**: 704-708

10 **McElhinney DB**, Geiger E, Blinder J, Benson DW, Goldmuntz E. NKX2.5 mutations in patients with congenital heart disease. *J Am Coll Cardiol* 2003; **42**: 1650-1655

11 **Prescott K**, Woodfine K, Stubbs P, Super M, Kerr B, Palmer R, Carter NP, Scambler P. A novel 5q11.2 deletion detected by microarray comparative genomic hybridisation in a child referred as a case of suspected 22q11 deletion syndrome. *Hum Genet* 2005; **116**: 83-90

12 **Cai CL**, Liang X, Shi Y, Chu PH, Pfaff SL, Chen J, Evans S. Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. *Dev Cell* 2003; **5**: 877-889

13 **Yagi H**, Furutani Y, Hamada H, Sasaki T, Asakawa S, Minoshima S, Ichida F, Joo K, Kimura M, Imamura S, Kamatani N, Momma K, Takao A, Nakazawa M, Shimizu N, Matsuoka R. Role of TBX1 in human del22q11.2 syndrome. *Lancet* 2003; **362**: 1366-1373

14 **Stoller JZ**, Epstein JA. Identification of a novel nuclear localization signal in Tbx1 that is deleted in DiGeorge syndrome patients harboring the 1223delC mutation. *Hum Mol Genet* 2005; **14**: 885-892

15 **Li L**, Krantz ID, Deng Y, Genin A, Banta AB, Collins CC, Qi M, Trask BJ, Kuo WL, Cochran J, Costa T, Pierpont ME, Rand EB, Piccoli DA, Hood L, Spinner NB. Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. *Nat Genet* 1997; **16**: 243-251

16 **Tartaglia M**, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, van der Burgt I, Crosby AH, Ion A, Jeffery S, Kalidas K, Patton MA, Kucherlapati RS, Gelb BD. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. *Nat Genet* 2001; **29**: 465-468

17 **Tartaglia M**, Cordeddu V, Chang H, Shaw A, Kalidas K, Crosby A, Patton MA, Sorcini M, van der Burgt I, Jeffery S, Gelb BD. Paternal germline origin and sex-ratio distortion in transmission of PTPN11 mutations in Noonan syndrome. *Am J Hum Genet* 2004; **75**: 492-497

18 **Tartaglia M**, Martinelli S, Stella L, Bocchinfuso G, Flex E, Cordeddu V, Zampino G, Burgt I, Palleschi A, Petrucci TC, Sorcini M, Schoch C, Foa R, Emanuel PD, Gelb BD. Diversity and functional consequences of germline and somatic PTPN11 mutations in human disease. *Am J Hum Genet* 2006; **78**: 279-290

19 **Schubbert S**, Zenker M, Rowe SL, Böll S, Klein C, Bollag G, van der Burgt I, Musante L, Kalscheuer V, Wehner LE, Nguyen H, West B, Zhang KY, Sistermans E, Rauch A, Niemeyer CM, Shannon K, Kratz CP. Germline KRAS mutations cause Noonan syndrome. *Nat Genet* 2006; **38**: 331-336

20 **Carta C**, Pantaleoni F, Bocchinfuso G, Stella L, Vasta I, Sarkozy A, Digilio C, Palleschi A, Pizzuti A, Grammatico P, Zampino G, Dallapiccola B, Gelb BD, Tartaglia M. Germline missense mutations affecting KRAS Isoform B are associated with a severe Noonan syndrome phenotype. *Am J Hum Genet* 2006; **79**: 129-135

21 **Tartaglia M**, Pennacchio LA, Zhao C, Yadav KK, Fodale V, Sarkozy A, Pandit B, Oishi K, Martinelli S, Schackwitz W, Ustaszewska A, Martin J, Bristow J, Carta C, Lepri F, Neri C, Vasta I, Gibson K, Curry CJ, Siguero JP, Digilio MC, Zampino G, Dallapiccola B, Bar-Sagi D, Gelb BD. Gain-of-function SOS1 mutations cause a distinctive form of Noonan syndrome. *Nat Genet* 2007; **39**: 75-79

22 **Pandit B**, Sarkozy A, Pennacchio LA, Carta C, Oishi K, Martinelli S, Pogna EA, Schackwitz W, Ustaszewska A, Landstrom A, Bos JM, Ommen SR, Esposito G, Lepri F, Faul C, Mundel P, López Siguero JP, Tenconi R, Selicorni A, Rossi C, Mazzanti L, Torrente I, Marino B, Digilio MC, Zampino G, Ackerman MJ, Dallapiccola B, Tartaglia M, Gelb BD. Gain-of-function RAF1 mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy. *Nat Genet* 2007; **39**: 1007-1012

23 **Digilio MC**, Conti E, Sarkozy A, Mingarelli R, Dottorini T, Marino B, Pizzuti A, Dallapiccola B. Grouping of multiple-lentigines/LEOPARD and Noonan syndromes on the PTPN11 gene. *Am J Hum Genet* 2002; **71**: 389-394

24 **Sarkozy A**, Conti E, Seripa D, Digilio MC, Grifone N, Tandoi C, Fazio VM, Di Ciommo V, Marino B, Pizzuti A, Dallapiccola B. Correlation between PTPN11 gene mutations and congenital heart defects in Noonan and LEOPARD syndromes. *J Med Genet* 2003; **40**: 704-708

25   **Sarkozy A**, Obregon MG, Conti E, Esposito G, Mingarelli R, Pizzuti A, Dallapiccola B. A novel PTPN11 gene mutation bridges Noonan syndrome, multiple lentigines/LEOPARD syndrome and Noonan-like/multiple giant cell lesion syndrome. *Eur J Hum Genet* 2004; **12**: 1069-1072

26   **Ingles J**, Semsarian C. Sudden cardiac death in the young: a clinical genetic approach. *Intern Med J* 2007; **37**: 32-37

27   **Zheng ZJ**, Croft JB, Giles WH, Mensah GA. Sudden cardiac death in the United States, 1989 to 1998. *Circulation* 2001; **104**: 2158-2163

28   **Morita H**, Rehm HL, Menesses A, McDonough B, Roberts AE, Kucherlapati R, Towbin JA, Seidman JG, Seidman CE. Shared genetic causes of cardiac hypertrophy in children and adults. *N Engl J Med* 2008; **358**: 1899-1908

29   **Keren A**, Syrris P, McKenna WJ. Hypertrophic cardiomyopathy: the genetic determinants of clinical disease expression. *Nat Clin Pract Cardiovasc Med* 2008; **5**: 158-168

30   **Maisch B**, Richter A, Sandmöller A, Portig I, Pankuweit S. Inflammatory dilated cardiomyopathy (DCMI). *Herz* 2005; **30**: 535-544

31   **Jefferies JL**, Towbin JA. Dilated cardiomyopathy. *Lancet* 2010; **375**: 752-762

32   **Awad MM**, Calkins H, Judge DP. Mechanisms of disease: molecular genetics of arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Nat Clin Pract Cardiovasc Med* 2008; **5**: 258-267

33   **Basso C**, Corrado D, Marcus FI, Nava A, Thiene G. Arrhythmogenic right ventricular cardiomyopathy. *Lancet* 2009; **373**: 1289-1300

34   **Marbán E**. Cardiac channelopathies. *Nature* 2002; **415**: 213-218

35   **Brugada J**, Brugada R, Brugada P. Channelopathies: a new category of diseases causing sudden death. *Herz* 2007; **32**: 185-191

36   **Roberts R**. Genomics and cardiac arrhythmias. *J Am Coll Cardiol* 2006; **47**: 9-21

37   **Antzelevitch C**, Pollevick GD, Cordeiro JM, Casis O, Sanguinetti MC, Aizawa Y, Guerchicoff A, Pfeiffer R, Oliva A, Wollnik B, Gelber P, Bonaros EP Jr, Burashnikov E, Wu Y, Sargent JD, Schickel S, Oberheiden R, Bhatia A, Hsu LF, Haïssaguerre M, Schimpf R, Borggrefe M, Wolpert C. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. *Circulation* 2007; **115**: 442-449

38   **Stambader JD**, Dorn L, Mikuz G, Sergi C. Genetic polymorphisms in dilated cardiomyopathy. *Front Biosci* (Schol Ed) 2010; **2**: 653-676

39   **Campuzano O**, Brugada R, Iglesias A. Genetics of Brugada syndrome. *Curr Opin Cardiol* 2010; Epub ahead of print

40   **Watkins H**, Farrall M. Genetic susceptibility to coronary artery disease: from promise to progress. *Nat Rev Genet* 2006; **7**: 163-173

41   **Andreassi MG**, Botto N. DNA damage as a new emerging risk factor in atherosclerosis. *Trends Cardiovasc Med* 2003; **13**: 270-275

42   **Federici C**, Botto N, Manfredi S, Rizza A, Del Fiandra M, Andreassi MG. Relation of increased chromosomal damage to future adverse cardiac events in patients with known coronary artery disease. *Am J Cardiol* 2008; **102**: 1296-1300

43   **Salpea KD**, Humphries SE. Telomere length in atherosclerosis and diabetes. *Atherosclerosis* 2010; **209**: 35-38

44   **Detweiler DK**. Genetic aspects of cardiovascular diseases in animals. *Circulation* 1964; **30**: 114-127

45   **Yusuf S**, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* 2004; **364**: 937-952

46   **Wang TJ**, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, Jacques PF, Rifai N, Selhub J, Robins SJ, Benjamin EJ, D'Agostino RB, Vasan RS. Multiple biomarkers for the prediction of first major cardiovascular events and death. *N Engl*

J Med 2006; **355**: 2631-2639

47   **Helgadottir A**, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, Jonasdottir A, Jonasdottir A, Sigurdsson A, Baker A, Palsson A, Masson G, Gudbjartsson DF, Magnusson KP, Andersen K, Levey AI, Backman VM, Matthiasdottir S, Jonsdottir T, Palsson S, Einarsdottir H, Gunnarsdottir S, Gylfason A, Vaccarino V, Hooper WC, Reilly MP, Granger CB, Austin H, Rader DJ, Shah SH, Quyyumi AA, Gulcher JR, Thorgeirsson G, Thorsteinsdottir U, Kong A, Stefansson K. A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science* 2007; **316**: 1491-1493

48   **Palomaki GE**, Melillo S, Bradley LA. Association between 9p21 genomic markers and heart disease: a meta-analysis. *JAMA* 2010; **303**: 648-656

49   **Visel A**, Zhu Y, May D, Afzal V, Gong E, Attanasio C, Blow MJ, Cohen JC, Rubin EM, Pennacchio LA. Targeted deletion of the 9p21 non-coding coronary artery disease risk interval in mice. *Nature* 2010; **464**: 409-412

50   **van Es MA**, Veldink JH, Saris CG, Blauw HM, van Vught PW, Birve A, Lemmens R, Schelhaas HJ, Groen EJ, Huisman MH, van der Kooi AJ, de Visser M, Dahlberg C, Estrada K, Rivadeneira F, Hofman A, Zwarts MJ, van Doormaal PT, Rujescu D, Strengman E, Giegling I, Muglia P, Tomik B, Slowik A, Uitterlinden AG, Hendrich C, Waibel S, Meyer T, Ludolph AC, Glass JD, Purcell S, Cichon S, Nöthen MM, Wichmann HE, Schreiber S, Vermeulen SH, Kiemeney LA, Wokke JH, Cronin S, McLaughlin RL, Hardiman O, Fumoto K, Pasterkamp RJ, Meininger V, Melki J, Leigh PN, Shaw CE, Landers JE, Al-Chalabi A, Brown RH Jr, Robberecht W, Andersen PM, Ophoff RA, van den Berg LH. Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. *Nat Genet* 2009; **41**: 1083-1087

51   **Falchi M**, Bataille V, Hayward NK, Duffy DL, Bishop JA, Pastinen T, Cervino A, Zhao ZZ, Deloukas P, Soranzo N, Elder DE, Barrett JH, Martin NG, Bishop DT, Montgomery GW, Spector TD. Genome-wide association study identifies variants at 9p21 and 22q13 associated with development of cutaneous nevi. *Nat Genet* 2009; **41**: 915-919

52   **Yasuno K**, Bilguvar K, Bijlenga P, Low SK, Krischek B, Auburger G, Simon M, Krex D, Arlier Z, Nayak N, Ruigrok YM, Niemelä M, Tajima A, von und zu Fraunberg M, Dóczi T, Wirjatijasa F, Hata A, Blasco J, Oszvald A, Kasuya H, Zilani G, Schoch B, Singh P, Stüer C, Risselada R, Beck J, Sola T, Ricciardi F, Aromaa A, Illig T, Schreiber S, van Duijn CM, van den Berg LH, Perret C, Proust C, Roder C, Ozturk AK, Gaál E, Berg D, Geisen C, Friedrich CM, Summers P, Frangi AF, State MW, Wichmann HE, Breteler MM, Wijmenga C, Mane S, Peltonen L, Elio V, Sturkenboom MC, Lawford P, Byrne J, Macho J, Sandalcioglu EI, Meyer B, Raabe A, Steinmetz H, Rüfenacht D, Jääskeläinen JE, Hernesniemi J, Rinkel GJ, Zembutsu H, Inoue I, Palotie A, Cambien F, Nakamura Y, Lifton RP, Günel M. Genome-wide association study of intracranial aneurysm identifies three new risk loci. *Nat Genet* 2010; **42**: 420-425

53   **Moonesinghe R**, Khoury MJ, Liu T, Ioannidis JP. Required sample size and nonreplicability thresholds for heterogeneous genetic associations. *Proc Natl Acad Sci USA* 2008; **105**: 617-622

54   **Dudbridge F**, Gusnanto A. Estimation of significance thresholds for genomewide association scans. *Genet Epidemiol* 2008; **32**: 227-234

55   **Gudbjartsson DF**, Bjornsdottir US, Halapi E, Helgadottir A, Sulem P, Jonsdottir GM, Thorleifsson G, Helgadottir H, Steinthorsdottir V, Stefansson H, Williams C, Hui J, Beilby J, Warrington NM, James A, Palmer LJ, Koppelman GH, Heinzmann A, Krueger M, Boezen HM, Wheatley A, Altmuller J, Shin HD, Uh ST, Cheong HS, Jonsdottir B, Gislason D, Park CS, Rasmussen LM, Porsbjerg C, Hansen JW, Backer V, Werge T, Janson C, Jönsson UB, Ng MC, Chan J, So WY,

Ma R, Shah SH, Granger CB, Quyyumi AA, Levey AI, Vaccarino V, Reilly MP, Rader DJ, Williams MJ, van Rij AM, Jones GT, Trabetti E, Malerba G, Pignatti PF, Boner A, Pescollderungg L, Girelli D, Olivieri O, Martinelli N, Ludviksson BR, Ludviksdottir D, Eyjolfsson GI, Arnar D, Thorgeirsson G, Deichmann K, Thompson PJ, Wjst M, Hall IP, Postma DS, Gislason T, Gulcher J, Kong A, Jonsdottir I, Thorsteinsdottir U, Stefansson K. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet* 2009; **41**: 342-347

56   **Kathiresan S**, Voight BF, Purcell S, Musunuru K, Ardissino D, Mannucci PM, Anand S, Engert JC, Samani NJ, Schunkert H, Erdmann J, Reilly MP, Rader DJ, Morgan T, Spertus JA, Stoll M, Girelli D, McKeown PP, Patterson CC, Siscovick DS, O'Donnell CJ, Elosua R, Peltonen L, Salomaa V, Schwartz SM, Melander O, Altshuler D, Ardissino D, Merlini PA, Berzuini C, Bernardinelli L, Peyvandi F, Tubaro M, Celli P, Ferrario M, Fetiveau R, Marziliano N, Casari G, Galli M, Ribichini F, Rossi M, Bernardi F, Zonzin P, Piazza A, Mannucci PM, Schwartz SM, Siscovick DS, Yee J, Friedlander Y, Elosua R, Marrugat J, Lucas G, Subirana I, Sala J, Ramos R, Kathiresan S, Meigs JB, Williams G, Nathan DM, MacRae CA, O'Donnell CJ, Salomaa V, Havulinna AS, Peltonen L, Melander O, Berglund G, Voight BF, Kathiresan S, Hirschhorn JN, Asselta R, Duga S, Spreafico M, Musunuru K, Daly MJ, Purcell S, Voight BF, Purcell S, Nemesh J, Korn JM, McCarroll SA, Schwartz SM, Yee J, Kathiresan S, Lucas G, Subirana I, Elosua R, Surti A, Guiducci C, Gianniny L, Mirel D, Parkin M, Burtt N, Gabriel SB, Samani NJ, Thompson JR, Braund PS, Wright BJ, Balmforth AJ, Ball SG, Hall AS, Schunkert H, Erdmann J, Linsel-Nitschke P, Lieb W, Ziegler A, König I, Hengstenberg C, Fischer M, Stark K, Grosshennig A, Preuss M, Wichmann HE, Schreiber S, Schunkert H, Samani NJ, Erdmann J, Ouwehand W, Hengstenberg C, Deloukas P, Scholz M, Cambien F, Reilly MP, Li M, Chen Z, Wilensky R, Matthai W, Qasim A, Hakonarson HH, Devaney J, Burnett MS, Pichard AD, Kent KM, Satler L, Lindsay JM, Waksman R, Knouff CW, Waterworth DM, Walker MC, Mooser V, Epstein SE, Rader DJ, Scheffold T, Berger K, Stoll M, Huge A, Girelli D, Martinelli N, Olivieri O, Corrocher R, Morgan T, Spertus JA, McKeown P, Patterson CC, Schunkert H, Erdmann E, Linsel-Nitschke P, Lieb W, Ziegler A, König IR, Hengstenberg C, Fischer M, Stark K, Grosshennig A, Preuss M, Wichmann HE, Schreiber S, Hólm H, Thorleifsson G, Thorsteinsdottir U, Stefansson K, Engert JC, Do R, Xie C, Anand S, Kathiresan S, Ardissino D, Mannucci PM, Siscovick D, O'Donnell CJ, Samani NJ, Melander O, Elosua R, Peltonen L, Salomaa V, Schwartz SM, Altshuler D. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. *Nat Genet* 2009; **41**: 334-341

57   **Erdmann J**, Grosshennig A, Braund PS, König IR, Hengstenberg C, Hall AS, Linsel-Nitschke P, Kathiresan S, Wright B, Trégouët DA, Cambien F, Bruse P, Aherrahrou Z, Wagner AK, Stark K, Schwartz SM, Salomaa V, Elosua R, Melander O, Voight BF, O'Donnell CJ, Peltonen L, Siscovick DS, Altshuler D, Merlini PA, Peyvandi F, Bernardinelli L, Ardissino D, Schillert A, Blankenberg S, Zeller T, Wild P, Schwarz DF, Tiret L, Perret C, Schreiber S, El Mokhtari NE, Schäfer A, März W, Renner W, Bugert P, Klüter H, Schrezenmeir J,

Rubin D, Ball SG, Balmforth AJ, Wichmann HE, Meitinger T, Fischer M, Meisinger C, Baumert J, Peters A, Ouwehand WH, Deloukas P, Thompson JR, Ziegler A, Samani NJ, Schunkert H. New susceptibility locus for coronary artery disease on chromosome 3q22.3. *Nat Genet* 2009; **41**: 280-282

58   **Smith JM**, Haigh J. The hitch-hiking effect of a favourable gene. *Genet Res* 1974; **23**: 23-35

59   **Kimura M**. The neutral theory of molecular evolution. New York: Cambridge University Press, 1983

60   **Schork NJ**, Murray SS, Frazer KA, Topol EJ. Common vs. rare allele hypotheses for complex diseases. *Curr Opin Genet Dev* 2009; **19**: 212-219

61   **Mihaescu R**, van Hoek M, Sijbrands EJ, Uitterlinden AG, Witteman JC, Hofman A, van Duijn CM, Janssens AC. Evaluation of risk prediction updates from commercial genome-wide scans. *Genet Med* 2009; **11**: 588-594

62   **Khoury MJ**, McBride CM, Schully SD, Ioannidis JP, Feero WG, Janssens AC, Gwinn M, Simons-Morton DG, Bernhardt JM, Cargill M, Chanock SJ, Church GM, Coates RJ, Collins FS, Croyle RT, Davis BR, Downing GJ, Duross A, Friedman S, Gail MH, Ginsburg GS, Green RC, Greene MH, Greenland P, Gulcher JR, Hsu A, Hudson KL, Kardia SL, Kimmel PL, Lauer MS, Miller AM, Offit K, Ransohoff DF, Roberts JS, Rasooly RS, Stefansson K, Terry SF, Teutsch SM, Trepanier A, Wanke KL, Witte JS, Xu J. The Scientific Foundation for personal genomics: recommendations from a National Institutes of Health-Centers for Disease Control and Prevention multidisciplinary workshop. *Genet Med* 2009; **11**: 559-567

63   **Gulcher J**, Stefansson K. Genetic risk information for common diseases may indeed be already useful for prevention and early detection. *Eur J Clin Invest* 2010; **40**: 56-63

64   **Talmud PJ**, Cooper JA, Palmen J, Lovering R, Drenos F, Hingorani AD, Humphries SE. Chromosome 9p21.3 coronary heart disease locus genotype and prospective risk of CHD in healthy middle-aged men. *Clin Chem* 2008; **54**: 467-474

65   **Brautbar A**, Ballantyne CM, Lawson K, Nambi V, Chambless L, Folsom AR, Willerson JT, Boerwinkle E. Impact of adding a single allele in the 9p21 locus to traditional risk factors on reclassification of coronary heart disease risk and implications for lipid-modifying therapy in the Atherosclerosis Risk in Communities study. *Circ Cardiovasc Genet* 2009; **2**: 279-285

66   **Green RC**, Roberts JS, Cupples LA, Relkin NR, Whitehouse PJ, Brown T, Eckert SL, Butson M, Sadovnick AD, Quaid KA, Chen C, Cook-Deegan R, Farrer LA. Disclosure of APOE genotype for risk of Alzheimer's disease. *N Engl J Med* 2009; **361**: 245-254

67   **Kane RA**, Kane RL. Effect of genetic testing for risk of Alzheimer's disease. *N Engl J Med* 2009; **361**: 298-299

68   **Rothstein MA**. Currents in contemporary ethics. GINA, the ADA, and genetic discrimination in employment. *J Law Med Ethics* 2008; **36**: 837-840

69   **Brockmöller J**, Tzvetkov MV. Pharmacogenetics: data, concepts and tools to improve drug discovery and drug treatment. *Eur J Clin Pharmacol* 2008; **64**: 133-157

70   **Silvestri P**, Di Russo C, Rigattieri S, Fedele S, Todaro D, Ferraiuolo G, Altamura G, Loschiavo P. MicroRNAs and ischemic heart disease: towards a better comprehension of pathogenesis, new diagnostic tools and new therapeutic targets. *Recent Pat Cardiovasc Drug Discov* 2009; **4**: 109-118