Role of genomics in cardiovascular medicine

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Abstract
As all branches of science grow and 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.

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

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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. 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-myocardial infarcts. Furthermore, they can also be related to excessive physical activity and illicit drug and alcohol abuse. In all, these cases are defined as “secondary” [26-37]. 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 [26-37]. 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 six 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 [41] (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 atherosclerosis [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), but the heritability of a parental chromosomal translocation, or may be due to de novo mutations [44]. 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
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 aetiology. 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), cardiac myosin-binding protein C (MYBPC3, 11p11.2), cardiac troponin T (TNNT2, 1q32.1), cardiac troponin I (TNNT3, 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[29].

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[40-43].

With the exception of disease causing mutations that...
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\textsuperscript{(40)}. Since this discovery, many studies, in particular large scale genome-wide association studies, identified several common variants in genes encoding for

| 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, TXB1, JAG1, NOTCH2 |                  |
| Tricuspid atresia                      | NKX2-5          |                  |
| Pulmonary atresia                      | PTPN11, JAG1, NOTCH2 |                  |
| Ebstein’s anomaly of the tricuspid valve | NKX2-5          |                  |
| Double outlet right ventricle          | NKX2-5, THRAP2  |                  |
| Persistent truncus arteriosus          | TXB1            |                  |
| Anomalous pulmonary venous connection |                 |                  |
| Left-sided obstruction defects         |                 |                  |
| Hypoplastic left heart syndrome        | NOTCH1          |                  |
| Mitral stenosis                        | NOTCH1, PTPN11  |                  |
| Aortic coarctation                     | NOTCH1, PTPN11  |                  |
| Septation defects                      |                 |                  |
| Interrupted aortic arch                | TXB1            |                  |
| Atrial septation defects               | NKX2-5, GATA4, TXB20, MYH6, TXB5 |                  |
| Ventricular septal defects             | NKX2-5, GATA4, TXB20, TXB1, TXB5 |                  |
| Atrioventricular septal defects        | PTPN11, KRAS, SOS1, RAF1, CRELD1 |                  |
| Other congenital heart defects         |                 |                  |
| Bicuspid aortic valve                  | NOTCH1          |                  |
| Patent ductus arteriosus               | TFAPII          |                  |
| Non ischemic cardiopathies             |                 |                  |
| Structural defects                     |                 |                  |
| CMH                                    | MYH7, TNN2, TPM1, MYBPC3, PRKAG2, TNN3, MYL3, TTN, ML2, ACTC1, CSR3P, LAMP2 |                  |
| Dilated cardiomyopathy                 | ACTC, DES, SCD, MYH7, TNN2, TPM1, TTN, VCL, MYBPC, MLP, ACTN2, PIN, ZASP, MYH6, ABC, TNN1C1, TCP, EYA4, LMAA, SCN5A, DMD, TAZ, TNN1 |                  |
| Arrhythmogenic right ventricular       |                 |                  |
| dysplasia/cardiomyopathy               |                 |                  |
| Long QT syndrome                       | SCN5A, SCN4B, KCNQ1, KCNH2, KNE1, KNE2, ANK2, CAV3 |                  |
| Brugada syndrome                       | SCN5A, SCN1B, GPDC1L, CACNA1C, CACNBb |                  |
| Sindrome di Lev-Leñègre                | SCN5A           |                  |
| Short QT syndrome                      | KCHN2, KCNQ1, KCN2 |                  |
| Sindrome di Wolff-Parkinson-White      | AMPK            |                  |
| Tachycardia ventricolare               |                 |                  |
| Tachycardia ventricolare polimorifica  |                 |                  |
| catatolaminergica                      |                 |                  |
| Atrial fibrillation                    |                 |                  |
| Coronary artery disease, myocardial    |                 |                  |
| infarction                             |                 |                  |
| Mendelian inheritance                  |                 |                  |
| Complex disease                        |                 |                  |

CMH: Hypertrophic cardiomyopathy; HOS: Holt-Oram syndrome.
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\[47,49\] 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\[50\] found genome-wide significance for a non-synonymous SNP on 3H2B3 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 Pse1-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-PARC1-SORT1, 10q11 near CXCL12, 1q41 in ML43, 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 mutations 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
Polymorphisms of vitamin K epoxide reductase complex subunit identify Tretinoin (Avita UGT1A1 mutation patients, exposure to drug and hence their susceptibility Philadelphia (Ph1) chromosome presence and efficacy—Busulfan is less Atomoxetine (Strattera Imatinib mesylate (Glivec G6PD deficiency (or NADH methemoglobin reductase deficiency) and risk CYP2D6 PM and EM variants and drug exposure and risk G6PD deficiency and risk for haemolysis CYP2D6 variants PM and EM genotypes and drug exposure CYP2D6 variants PM and EM genotypes and drug exposure DPD deficiency Severe toxicity (stomatitis, diarrhoea, neutropenia and neurotoxicity) associated to deficiency of dihydropyrimidine dehydrogenase EGFR expression Epidermal growth factor receptor presence or absence (NSCLC, pancreas cancer) CYP2C9 variants CYP2C19 variants (poor metabolizers PM and extensive metabolizers EM) with genetic defect leads to change in drug exposure CYP2D6 variants PM and EM genotypes and drug exposure CYP2D6 variants PM and EM genotypes and drug exposure DPD deficiency with alternate context Severe toxicity (stomatitis, diarrhoea, neutropenia and neurotoxicity) CYP2D6 with alternate context and drug exposure and risk Protein C deficiencies (hereditary or acquired) Hereditary or acquired deficiencies of protein C or its cofactor protein S C/KIT expression Gastrointestinal stromal tumour c-kit expression with alternate context CYP2C9 variants G6PD deficiency G6PD deficiency with alternate context KRAS mutation KRAS mutation G6PD deficiency (or NADH methemoglobin reductase deficiency) and risk for haemolytic reactions KRAS mutation for the treatment of colorectal cancer with these mutations NAT variants N-Acetyltransferase slow and fast acetylators and toxicity Philadelphia chromosome deficiency Philadelphia chromosome deficiency UCD efficiency disorders UCD efficiency disorders with alternate context VKORC1 variants VKORC1 variants with alternate context PML/RAR α gene expression (retinoic acid receptor responders and non-responders) Patients who carry the HLA-B*5701 allele are at high risk for experiencing a hypersensitivity reaction to abacavir Over-expression of Her2/neu necessary for selection of patients appropriate for drug therapy (breast cancer) UGT1A1 mutation patients, exposure to drug and hence their susceptibility to toxicity (colon-rectum cancer) Increased risk of myelotoxicity associated to thiopurine methyltransferase deficiency or lower activity Hereditary or acquired deficiencies of protein C or its cofactor protein S Epidermal growth factor receptor presence or absence (squamous cell carcinoma of head and neck) G6PD deficiency and risk for haemolysis G6PD deficiency (or NADH methemoglobin reductase deficiency) and risk for haemolytic reactions 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 N-Acetyltransferase slow and fast acetylators and toxicity Philadelphia (Ph1) chromosome presence and efficacy—Busulfan is less effective in patients with CML lacking the Philadelphia chromosome Valproic acid (Depakene) and pyrazinamide Rifampin (Rifater) and pyrazinamide Valproic acid (Depakene) Warfarin (Coumadin®) Tretinoin (Avita®, Renova®, Retin-A®) 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         | Abacavir                 |
|                            | hypersensitivity reaction to abacavir                                                |                          |
| Her2/neu over-expression   | Over-expression of Her2/neu necessary for selection of patients appropriate for      | Trastuzumab (Herceptin®) |
|                            | drug therapy (breast cancer)                                                         |                          |
| 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        | Irinotecan (Camptosar®)  |
|                            | toxicity (colon-rectum cancer)                                                       |                          |
| TPMT variants              | Increased risk of myelotoxicity associated to thiopurine methyltransferase           | Azathioprine (Imuran®)   |
|                            | deficiency or lower activity                                                          |                          |
| Protein C deficiencies     | Hereditary or acquired deficiencies of protein C or its cofactor protein S           | Warfarin (Coumadin®)     |
| (hereditary or acquired)   |                                                                                      |                          |
| C/KIT expression           | Gastrointestinal stromal tumour c-kit expression                                      | Imatinib mesylate (Glivec®) |
| CYP2C9 variants            | CYP2C19 variants (poor metabolizers PM and extensive metabolizers EM) with genetic   | Verucanozole (Vlend®)    |
|                            | defect leads to change in drug exposure                                              |                          |
| CYP2D6 variants            | CYP2C9 variants PM and EM genotypes and drug exposure                                | Celecoxib (Celebrex®)    |
|                            | CYP2D6 variants PM and EM genotypes and drug exposure                                | Atomoxetine (Strattera®) |
|                            | CYP2D6 variants with alternate context CYP2D6 PM and EM variants and drug exposure   | Fluoxetine HCl (Prozac®) |
|                            | Severe toxicity (stomatitis, diarrhoea, neutropenia and neurotoxicity) associated to  | Cepacitabine (Xeloda®)   |
|                            | deficiency of dihydropyrimidine dehydrogenase                                        |                          |
| 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 (Elitrek®)   |
| G6PD deficiency with alternate context | G6PD deficiency (or NADH methemoglobin reductase deficiency) and risk for              | Primaquine (Primaquine®) |
|                            | haemolytic reactions                                                                 |                          |
| KRAS mutation              | Retrospective subset analyses of metastatic colorectal cancer trials have not        | Panitumumab (Cetuximab®) |
|                            | 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                                               |                          |
| NAT variants               | N-Acetyltransferase slow and fast acetylators and toxicity                            | Rifampin ironiazid (Rifater® and pyrazinamide) |
| Philadelphia chromosome    | Philadelphia (Ph1) chromosome presence and efficacy—Busulfan is less effective in   |                          |
| deficiency                  | 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-       | Warfarin (Coumadin®)     |
|                            | sensitive patients who require a lower dose of the drug                                |                          |
| PML/RAR α gene expression  | (retinoic acid) 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[69]; 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 micro-RNA 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 Brockmüller et al[69], 2008.](http://www.wjgnet.com)
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.\(^6\,^12\) Furthermore, different microRNAs have been found to be involved in different phases of ischemic heart disease.\(^3\) 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.

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