DEVELOPMENT AND APPLICATION OF AN INDIVIDUALISED GENETIC APPROACH FOR CARDIOVASCULAR DISEASE RISK REDUCTION

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Abstract

The importance of assessing genetic risk factors to explain ethnic differences in risk of cardiovascular disease (CVD) has been clearly demonstrated in the genetically distinct populations of South Africa. However, the limitations of single-gene tests that focus on monogenic conditions such as familial hypercholesterolaemia (FH) have also been recognised. Single-gene defects affect a relatively small subset (5-10%) of patients at high risk of premature coronary heart disease, while multiple gene variations with minor effects contribute to CVD risk in the vast majority of individuals in the general population. Such multigenic effects depend predominantly on environmental influences, which imply that most premature cardiovascular deaths can be prevented if action is taken to avoid or modify external exposures that may cause a genetic predisposition to become clinically relevant. We have therefore developed a comprehensive cardiovascular genetic screening strategy that is applied in conjunction with nutrition and lifestyle assessments to identify individuals at increased risk of CVD for targeted intervention. To provide the necessary support base to medical practitioners who refer patients for testing, genetic counselling clinics were established in different regions of South Africa and dieticians were trained countrywide to implement cardio-protective dietary and lifestyle interventions based partly on genetic test results. This initiative facilitated the incorporation of applied nutritional and cardiovascular genetics into the South African health care system. Most importantly, a more accurate distinction can now be made between South African patients with FH requiring life-long drug treatment and those at increased CVD risk as a consequence of less severe genetic alterations that could be targeted by dietary and lifestyle changes.

Introduction: The quest for a new approach towards cardiovascular health

Worldwide, cardiovascular disease (CVD) accounts for more than 12 million deaths each year (World Health Organisation 2002). In South Africa 1 in 3 men and 1 in 4 women suffer from coronary heart disease (CHD) before the age of 60 years. A family history is indicative of a genetic predisposition of CVD and represents the most important heart disease risk factor, especially when affected at a young age. Identification of subgroups of patients in whom the impact of environmental risk factors may be stronger due to a genetic predisposition is therefore expected to lead to more effective preventive and therapeutic strategies.

The strategy outlined in this article for identification and management of individuals at increased risk of CVD in South Africa is based on a review of the literature, as well as personal experience in cardiovascular research and service delivery over a period of nearly 20 years. It also draws on data presented in November 2003 at a symposium hosted by the Royal Society of Medicine in London, UK, which focussed on novel ways to identify patients at high risk of coronary heart disease. The effectiveness of current methods to identify and treat individuals at high risk of developing CVD was acknowledged, but the need to find better ways of disease prevention in those classified as intermediate or low risk was highlighted. The majority of CVD-related deaths occur in these groups where targeted lifestyle and dietary intervention would be most beneficial. To have the desired impact, the problem has to be tackled by a multi-disciplinary approach focussing on modifiable risk factors, including genetic and environmental factors evaluated within the context of medical and biochemical risk traits.

Genetic testing as a component of overall CVD risk assessment

It is important to distinguish between population-specific and global genetic testing applications, since genetic risk factors may be relevant only to the specific population/ethnic group and environment from which the information was gathered. In addition to genetic make-up, several other factors such as diet composition, body fat content, physical activity, and personal and social behaviour may modify the risk in a population-specific manner. When genetic testing is combined with conventional risk assessment methods it has the potential to translate into more effective disease prevention and treatment. Risk evaluation will remain incomplete until genetic risk factor determination becomes part of routine health and disease management.

Computer-based programs can be used for CVD risk assessment, which also takes into account the presence or absence of a family history of myocardial infarction (MI) (http://www. CHD-taskforce.com). It is an important step towards the addition of genetic risk factors as part of an overall strategy to evaluate and reduce CVD risk. We have now taken this further by the development of a comprehensive genetic screening strategy that considers medical history, genetic risk factors, biochemical parameters, and environmental risk in a single test procedure. Although an individual's plasma risk-trait levels reflecting both genotype and environmental exposure may be considered the best predictor of clinical outcome (Humphries et al. 2004), the identification of genetic risk factors allows these markers to be used as preventative or therapeutic targets. An important advantage of genotyping is that it is not subjected to short-term fluctuations and measurement errors as is the case for measurements of plasma and other quantitative phenotypic risk factors.

Development of a comprehensive cardiovascular genetic screening strategy

Before the potential benefits of applied cardiovascular genetics could become a reality, the development of cost-effective genetic assays enabling the simultaneous analysis of multiple CVD genetic risk factors is required. By applying the reverse-hybridisation technique for test development to identify South African patients at high risk of CVD (Kotze et al. 2003a), it has become possible to translate genetic testing from a research environment to routine clinical medicine (Kotze et al. 2003b). Two separate DNA-based strip assays were developed and are now applied routinely to identify genetic risk factors in patient referrals (Kotze and Thiart 2003). The first test option includes eight mutations in the low-density receptor (LDLR) gene, which accounts for familial hypercholesterolaemia (FH) in the majority of affected South Africans. The second test option performed in conjunction with lifestyle and dietary assessments, includes twelve mutations of relatively low expression in ten different genes involved in lipid metabolism, homocysteine/folate metabolism, haemostasis and iron overload.

The appropriateness of applying the population-specific FH assay or globally applicable CVD multi-gene assay is determined by the personal risk profile of the individual and his/her family history of CVD, taking into account age of onset and cause of death. The genetic test results are evaluated within the context of clinical, biochemical, lifestyle and dietary information provided at referral, in order to formulate intervention strategies based on all the risk factors identified.

Dyslipidaemia, diet and smoking

Identification and treatment of FH patients are a health priority in South Africa. In the high-risk Afrikaner, Jewish and Indian populations the prevalence of FH is between 1/70 and 1/100 due to an increased frequency of a limited number of gene defects (Vergotine et al. 2001), compared to approximately 1/500 in most other populations worldwide. The deleterious effects of an unhealthy diet, physical inactivity and smoking are exemplified significantly in patients with FH and needs to be targeted aggressively. More than 10 years ago Kotze et al. (1993a) described a FH family where the same LDLR gene

mutation was associated with variable clinical expression, ranging from occurrence of a heart attack at an early age (<50 years) to good health into advanced age (>80 years). To our knowledge, this was the first study indicating that the deleterious effects of a specific FH mutation might be neutralised by a healthy lifestyle (gene-environment interaction) and an apparent favourable genetic background (gene-gene interaction). This finding highlighted the significance of modifier genes and other risk factors that may impact on disease expression in monogenic conditions such as FH, while also increasing CVD risk in the general population.

The importance of a genetic approach for FH case finding is emphasised by the fact that less than 10% of the estimated 120 000 patients with heterozygous FH in South Africa have been diagnosed despite extensive awareness and publicising of hypercholesterolaemia as a CVD risk factor (Marais et al. 2004). The need for DNA testing was demonstrated by Vergotine et al. (2001), who demonstrated that determination of total cholesterol levels in FH families might fail to provide the correct diagnosis in 29.4% of individuals when the 95th percentile for age and gender is used, and in 11.8% of cases when the 80th percentile is used. When high total cholesterol levels exceeding 7.5 mmol/l in adults is reported together with a family history of early onset (<55 years) coronary heart disease, FH mutation screening known to be highly cost-effective (Marks et al. 2002) is recommended. Genetic testing is also useful to distinguish between hyperlipidaemic patients with FH and those with a less severe mutation in the apolipoprotein (Apo) B or E genes, especially when high plasma cholesterol levels are detected in the absence of xanthomas or a family history of early-onset CHD.

Mutations in the genes encoding Apo B and E, which act as ligands for the FH gene are known causes of dyslipidaemia in the South African population and were obvious candidates for inclusion in the CVD multi-gene assay. Although familial defective apolipoprotein B-100 (FDB) appears to be relatively rare (~1 in 500) in the South African population, genetic testing remains important to distinguish between FH and less severe Apo B gene defects (Rubinsztein et al. 1993, 1995). Detection of the Apo E polymorphism is considered a useful indicator that E4 allele carriers with raised plasma cholesterol levels are likely to be responsive to a diet low in saturated fat and cholesterol, especially since they appear to be less responsive to statin therapy than E2 allele carriers (Gerdes et al. 2000; Schaefer 2002). A recent meta-analysis performed by Song et al. (2004) indicated that carriers of the Apo E4 allele had a 42% higher risk for CHD compared with individuals with the neutral E33 genotype. The impact of theE4 allele on CHD risk however appears to be confined to current smokers, because smoking may exacerbate the deleterious effect of this allele on arterial wall thickening (Humphries et al. 2001). To achieve healthy aging, life-long low dietary fat intake appears to be especially important in carriers with the E4 allele (Petot et al. 2003).

Apo E genotyping may also be useful to assess the impact of alcohol consumption on healthy aging. Anttila et al. (2004) have shown that the risk of dementia increased with increasing alcohol consumption only in those individuals carrying the Apo E4 allele. This finding may relate to previous studies showing an increased risk of dementia in patients with elevated cholesterol levels and other CVD risk factors. When LDL-cholesterol levels are compared among Apo E subgroups on the basis of drinking status, these levels are significantly higher in male drinkers with the Apo E4 allele than in non-drinkers (Corella et al. 2001). In women, the expected effect of Apo E alleles on LDL-C levels was present in both drinkers and non-drinkers. In a model with adjustment for age, sex, body mass index, smoking, exercise, waist-hip ratio, TV viewing, and study site, the increase in HDL-cholesterol associated with alcohol was significantly higher in subjects without the Apo E4 allele than in those with this allele (Djousse et al. 2004). In a study performed by Marques-Vidal et al. (2003) it was furthermore shown that the effects of the Apo E4 allele

on lipids and insulin levels are partly dependent on environmental variables such as body mass index and alcohol intake. These findings suggest that subjects carrying the Apo E4 allele may not benefit from moderate alcohol drinking as part of the dietary measures to lower CHD risk. Furthermore, while weight control is important in all people, it seems to be especially important in men with the Apo E4 allele to modify potentially elevated fasting insulin and glucose levels (Elosua et al. 2003)

When the Apo E polymorphism was studied in the general South African population, significantly elevated plasma cholesterol levels was detected in Caucasians with the E4 allele (Kotze et al. 1993b). An additive effect of Apo E polymorphism could however not be demonstrated in South African FH patients who already have abnormal lipid levels due to the presence of a mutation in the LDLR gene (Kotze et al. 1993b). This finding highlighted the difficulty to demonstrate the potential deleterious effect of a single minor gene variation in the presence of a major genetic alteration such as an LDLR gene mutation causing FH. The current value of Apo E genotyping in the South African population therefore lies mainly in the ability to distinguish dyslipidaemia related to the Apo E4 allele from patients with FH in the absence of xanthomas or a family history of early-onset CHD, because the treatment strategy will differ. Detection of the Apo E2 allele is also important because the majority of South African patients with type III hyperlipoproteinaemia are homozygous for this allele (Blom et al. 2002).

Hyperhomocysteinaemia, folate deficiency and alcohol
High plasma homocysteine concentration is an established risk factor for atherosclerosis and atherothrombosis (reviewed by Handy and Loscalzo 2003). The adverse effects of homocysteine include endothelial dysfunction, impairment of fibrinolysis (Toffler et al. 2002) and altered fibrin clot formation and stability (Sauls et al. 2003). Co-existence of other risk factors may further increase the CVD risk-associated effect of high homocysteine levels, as evidenced by the 30-fold increased risk reported when high blood pressure is also present (Graham et al. 1997).

Reduced activity of the methylenetetrahydrofolate reductase (MTHFR) enzyme results in hyperhomocysteinemia only when dietary folate intake is low (De Bree et al. 2003), which implies that genetic influences would only be detectable in populations with low folate status. Reduced MTHFR activity is of special concern in individuals with high alcohol intake since this may lead to impaired folate status due to malabsorption, increased excretion, or abnormal folate metabolism (Halsted et al. 2002).The negative effects of low intakes of the methyl-related nutrients with high intakes of alcohol are additive, therefore changes in overall dietary patterns is recommended to ensure the consumption of a protective high methyl diet, which is essential not only to reduce CVD risk, but also the incidence of cancer (Bailey 2003), impaired cognitive function and neural tube defects in babies. If alcohol is consumed, this should be restricted to less than 15 g/day or less than one drink per day (of any kind) and avoided in pregnant women and those who plan a pregnancy.

Nutrition requirements differ according to the MTHFR genotype (Moriyama et al. 2002; Herrmann et al. 2003). Determination of homocysteine levels and/or genetic testing performed in conjunction with assessment of dietary folate intake may therefore serve as useful parameters for folic acid supplementation. However, before wide implementation of such a test concept could be promoted, consideration had to be given to the uncertainty of whether the association between elevated serum homocysteine levels and CVD is indeed causal. The meta-analysis performed by Wald et al. (2002) provided strong evidence for causality, because both genetic and prospective studies (that do not share the same potential sources of error) yielded highly significant results. In a meta-analysis performed by Klerk et al. (2002), individuals with two copies of the most extensively studied 677C→T mutation were found to have a 16% higher risk of CHD

compared with individuals homozygous for the common variant. The increased risk of stroke associated with the T-allele was furthermore confirmed in a recent meta-analysis including 14870 individuals (Cronin et al. 2005). Lowering of homocysteine concentrations by 3 mmol/l by increasing folic acid intake could reduce the risk of ischaemic heart disease by 16%, deep vein thrombosis by 25%, and stroke by 24% (Wald et al. 2002). The benefits of folic acid supplementation are explained largely by favourable endothelial function outcomes after lowering homocysteine levels, as assessed by flow-mediated vasodilation or haemostatic markers (Brown and Hu 2001).

Mutation 677C→T that leads to a thermolabile variant of MTHFR is the most common genetic variation in enzymes of the homocysteine metabolic pathway. A second common MTHFR mutation, 1298A→C, is associated with decreased enzyme activity both *in vitro* and *in vivo*, but is only associated with elevated homocysteine levels in the presence of mutation 677C→T (Weisberg et al. 1998). Analysis of these two polymorphisms in the South African population demonstrated a heterogeneous distribution across ethnic groups (Scholtz et al. 2002). Failure to replicate the significant association detected between variation in the MTHFR gene and coronary events in one of two FH subpopulations studied by Scholtz et al. (2002) may be related to lifestyle changes towards healthier eating habits in high-risk FH patients. Raal et al. (1999) indeed reported markedly higher folate levels in FH patients attending the Johannesburg lipid clinic (these patients showed no MTHFR allelic differences in relation to coronary events) compared with control individuals. The potential value of MTHFR genotyping in FH heterozygotes was demonstrated by Kawashiri et al. (2000), who recommended routine determination of plasma homocysteine levels and MTHFR genotype for improvement of clinical management in males at risk of early-onset coronary events.

While genetic differences in population structure and epidemiological factors may lead to conflicting research results when low-expression mutations are studied, food fortification and folic acid supplementation represents further confounding factors. In a study performed by Shmeleva et al. (2003) in North Western Russia where the population has limited access to multivitamins and no food fortification, homozygosity for the T-allele of the MTHFR 677C→T polymorphism was detected in all the patients with homocysteine levels above 30 μm/l. In carriers with the T allele, thrombotic risk increased 1.9-fold in patients with arterial thrombosis, 1.7-fold for venous thrombosis and 2.5 fold for both conditions. These findings are in accordance with the risk for recurrent venous thrombosis ascribed to the MTHFR genotype by Keijzer et al. (2002). These authors reported a relative risk of between 1.4 and 1.6 in the presence of the T-allele and a combined risk of 18.7 if detected in the presence of the factor V Leiden mutation, the most common genetic risk factor for venous thrombosis.

Haemostasis, physical activity and hormone therapy
The relation between atherosclerosis and thrombosis is increasingly ascribed to the direct effect of certain haemostatic factors on atherogenesis, in addition to their role in thrombus formation (Voetsch and Loscalzo 2004). Clinical conditions associated with the development of thrombosis include atherothrombotic disorders (myocardial infarction, ischemic cerebrovascular disease, and peripheral vascular disease) and venous thrombotic disorders (deep vein thrombosis and pulmonary embolus). Souto et al. (2000) provided evidence that levels of several coagulation factors, tissue plasminogen activator, homocysteine, and resistance to activated protein C, correlates with genetic risk. Multiple subtle genetic variations occurring relatively frequently in the general population have been implicated in these processes. None of the thrombosis-related gene variations selected for inclusion in the CVD multi-gene assay cause disease on their own, but depending on the environmental triggers they may increase the risk imposed by physical inactivity, smoking, obesity, and an unhealthy diet. Therefore the

potential impact of genetic variation relevant to haemostasis and thrombosis is evaluated and reported within the context of environmental factors in patients at risk of CVD or with existing disease.

Knowledge of gene-gene and gene-environment interactions as determinants for understanding the role of genetic polymorphisms on thrombosis has been clearly demonstrated for the factor V Leiden mutation (1691G→A). This mutation is the most common risk factor for the inherited form of hypercoagulability and deep vein thrombosis. Although a single copy of the factor V Leiden mutation occurs in approximately 5% of the Caucasian population, the prevalence of venous thrombosis is only 1 in 1000 individuals in the general population. In heterozygotes the risk of thrombosis is increased 5-10 fold and in homozygotes the risk increases 50-100 fold. It has been estimated that approximately 80% of persons with two copies (homozygous) of the factor V Leiden mutation and 10% of heterozygotes will have thrombosis at some point in their lifetime. The risk of venous thrombosis is increased more than 40-fold when this mutation occurs in combination with the prothrombin mutation (20210G→A), which confers a 3-5 fold higher risk of thrombosis on its own. The importance of environmental triggers for venous thromboembolism associated with the factor V Leiden and prothrombin mutations are particularly evident during pregnancy, with use of oral contraceptives, or hormone replacement therapy (Gerhardt et al. 2000; Rosendaal et al. 2001). Although the presence of factor V Leiden alone increases the risk of venous thrombosis by a factor of about 7 and use of oral contraceptives increases the risk of by a factor of about 4, their joint effect results in a more than 30-fold increased risk.

Association of the factor II (prothrombin 20210G→A), factor V (Leiden,1691G→A), and factor XIII (Val34Leu) mutations with myocardial infarction was convincingly demonstrated in the study of Butt et al. (2003), which included 500 MI patients and 500 control subjects from the genetically isolated Newfoundland population. A 12-fold higher risk resulting in more than 90% disease penetrance was reported in combined carriers of the factor II and XIII mutations. The importance of studying combined genotypic effects is highlighted by the fact that various previous studies have ascribed a protective role to the V34L polymorphism when studied in isolation. Although the valine to leucine amino acid substitution of factor XIII enhances the rate of activation of thrombin is not in itself sufficient to impart a significant effect on the development of CVD (Ariens et al. 2002; Butt et al. 2003). Therefore analysis of V34L in the context of increased CVD risk should always be performed together with factor II 20210G→A genotyping to test for a possible synergistic effect.

In addition to the factor II, V and XIII mutations discussed above, three extensively studied mutations in the β-fibrinogen (β-fib, 455G→A), plasminogen activator inhibitor 1 (PAI-1, -675 4G/5G), and glycoprotein IIIa (GPIIIa, 1565T→C) genes are also included in the CVD multi-gene assay. Although contradictory results have been reported for these three polymorphisms, their functional effects correlate consistently with biochemical abnormalities implicated in CVD risk. The effects of the gene variations are clearly context-dependant and therefore modifying factors has to be taken into account during studies and interpretation of test results. Testing for these polymorphisms discussed in more detail below is of relevance in individuals who may be at increased risk of thrombosis due to risk factors such as cigarette smoking, high body mass, physical inactivity and a diet low in omega-3 fatty acids.

Among the components of the coagulation system, elevated plasma fibrinogen levels have been most consistently associated with atherothrombotic disorders. Fibrinogen may contribute to atherosclerosis and thrombosis by increasing blood coagulability, plasma viscosity, platelet aggregability and by promoting fibrin deposition in the vessel wall. Lysis

by thrombin produces soluble fibrin fragments that are then stabilised in a clot by factor XIII. Aside from the role of fibrinogen in thrombus formation after plaque rupture, it plays an important role in the inflammatory process underlying the development of atherosclerosis. It remains uncertain whether elevated fibrinogen levels exert an increased risk of CVD or is a consequence of the disease, but the finding of significantly elevated fibrinogen levels in asymptomatic relatives of vascular disease patients supports a causal role (Mills et al. 2002; Lansbury et al. 2002). Presence of the A-allele of the β-fib polymorphism 455G→A appears to promote a strong acute-phase response in fibrinogen, forming the pathogenetic basis for progression of coronary atherosclerosis in patients with this allele (de Maat et al. 1998). Presence of the A-allele of the 455G→A polymorphism would suggest implementation of intensive risk reduction therapy, since statin treatment was shown to offset the deleterious effect of this allele. Individuals homozygous for the A-allele have fibrinogen levels that are 7-10% higher than in individuals with the G-allele, which correlates with the increased rate of basal transcription exerted by the mutated allele (Brown and Fuller 1998; van 't Hooft et al. 1999). An increase in 1 g/l of fibrinogen is associated with a relative risk of 1.8 for CHD. Genetic variation interacts with smoking, physical activity, gender, use of drugs and infection in determining an increase in fibrinogen levels (Vischetti et al.2002). The risk of CVD increases further when hypercholesterolaemia or diabetes is also present (Carter et al. 1996a).

Elevated levels of PAI-1 are associated with increased risk of arterial vascular disease, thrombosis, obesity, metabolic syndrome, insulin resistance, type II diabetes, and correlates with smoking and hypertriglyceridaemia (Huber et al. 2001, Festa et al. 2002). Emotional or psychosocial stress and inflammation also cause elevated expression of PAI-1. The 4G/5G insertion-deletion polymorphism at nucleotide position –675 upstream of the PAI-1 gene affects gene transcription and concentrations are highest when two copies of the 4G allele are present (Panahloo et al. 1995). This allele is therefore considered to be a CVD risk marker, especially in obese individuals (Hoffstedt et al. 2002). Since the -675 4G/5G polymorphism increases the risk for obesity 2-3 fold, detection of the 4G allele emphasises the importance of high physical activity and a healthy diet to maintain a healthy body weight or to promote weight loss. Analysis of the 4G/5G PAI-1 polymorphism in patients with different types of thrombosis and unrelated healthy controls, showed that both the 4G/4G and 4G/5G genotypes are associated with a higher risk of thrombosis development in patients with internal organ thrombosis (Balta et al. 2002). This association was strongest in a subgroup of patients with portal vein thrombosis (PVT) and the 4G/4G and 4G/5G genotypes conferred more than 10- and 6-fold increases in the risk of developing PVT, respectively. However, several studies showed a protective effect (Roest et al. 2000) or failed to demonstrate 4G as a genetic risk factor for CVD. Since these findings are in contrast with elevation of PAI-1 levels in homozygous 4G individuals, confounding factors not taken into account, could explain these contradictory results.

Recent studies have confirmed the important role of increased platelet activation in development of vascular disease (Cassar et al. 2003; Cherian et al. 2003). Glycoprotein IIb/IIIa is the primary platelet surface receptor for fibrinogen and within this complex, the common 1565T→C polymorphism in exon 2 of the GPIIIa gene represents the clinically most relevant genetic alteration in the gene. This Leu33Pro amino acid substitution results in a conformational change in the amino-terminal disulfide loop involved in fibrinogen binding (Honda et al. 1995). Weiss et al. (1996) first reported the association between the 33Pro isoform, also known as PI[A2] or HPA-1b, and the risk for myocardial infarction. In the total patient group studied, PI[A2] was associated with a 2.8-fold increase in risk for coronary artery disease, whereas in a small subgroup (21 patients) under the age of 60 years an even higher risk with an odds ratio of 6.2 was reported. By using

several meta-analytical methods, Burr et al. (2003) confirmed the role of the GPIIIa Leu33Pro polymorphism in coronary artery disease. When combined with smoking, the effect of the PL^{A2} allele becomes very strong and the strength of the association appears to increase with early onset CVD (Carter et al 1996b; Ardissino et al. 1999). It became evident that information of a patient's GPIIIa genotype is particularly informative for smokers and those with a family history of early onset cardiovascular disease, since approximately 50% of early onset myocardial infarction could be attributed to interaction of cigarette smoking with the PL^{A2} allele. It was estimated that smokers with this allele are 13 times more likely to suffer premature myocardial infarction than non-smoking non-carriers. The PL^{A2} allele represents an inherited risk factor that may promote the thromboembolic complications of coronary heart disease.

Haemochromatosis, red meat and alcohol
The cardiomyopathy of idiopathic or acquired haemochromatosis suggests that the heart may be especially sensitive to toxic effects of excess iron (Sullivan 1990). The features of inherited iron overload include cardiac problems (arrhythmias and heart failure), and/or cirrhosis of the liver, diabetes, arthritis and skin pigmentation. Since haemochromatosis can be easily treated by phlebotomy once diagnosed, this condition is considered a preventable form of heart disease amongst other equally important health risks. Analysis of hereditary haemochromatosis (HH) in the South African population has shown that the carrier frequency of the most common iron overload mutation (C282Y) is 1 in 6 among white Afrikaners, implying that approximately 1 in 115 individuals are homozygous for this mutation (de Villiers et al. 1999).

Not only homozygosity for the HFE C282Y mutation, but also the heterozygous state is associated with increased serum iron parameters (Rossi et al. 2001). Individuals with serum transferrin saturation levels above 55% carry an increased all-cause mortality risk especially when combined with high red meat intake (Mainous et al. 2004a,b). These results are in accordance with earlier findings of Ascherio et al. (1994), who reported an increased risk of fatal myocardial infarction over a 4-year period with intake of red meat. Intakes of highly bioavailable forms of iron (supplemental iron and red meat) and of fruit (a dietary source of vitamin C) promoted high iron stores, whereas foods containing phytate (whole grains) decreased these stores (Fleming et al. 2002).The significant increase in ferritin concentration reported with increasing frequency of red meat consumption above a baseline of 1-2 times per week and alcohol intakes greater than 10 g/day, emphasised the important health implication of iron overload in relation to gene-diet interaction (Heath and Fairweather-Tait 2003). The important role that dietary intervention could play to reduce CVD risk was further highlighted by the finding that risk of fatal coronary disease increases with 60% among men who consumed meat 6 times a week compared with men who consumed meat less than once a week.

Heart disease rates rise sharply from age 20 onwards in males, who begin accumulating excess iron from late adolescence. Healthy women, on the other hand, do not accumulate excess iron in their tissues until after menopause, when they rapidly develop the same risk of CVD as men of the same age. Detection of an association between heterozygosity for mutation C282Y and increased risk of acute myocardial infarction in men (Tuomainen et al. 1999), as well as with cardiovascular death in postmenopausal women (Roest et al. 1999), support the iron-heart link. The risk of cardiovascular death in postmenopausal women with at least one copy of the C282Y mutation appeared to be stronger in women who were hypertensive or current smokers, with a nearly 20-fold increased risk when both risk factors are present compared with nonsmokers, nonhypertensives, and noncarriers. This finding again emphasised the importance of multiple risk factor assessment when low-expression mutations are analysed. The potential combined effects of elevated body iron stores and hypercholesterolaemia is of particular concern, seeing that two independent studies demonstrated worse clinical

outcomes under such circumstances (Salonen et al. 1992; Wells et al. 2004). Persons with both elevated transferrin saturation and elevated LDL showed a significantly greater hazards ratio for all-cause and CVD mortality than persons when both parameters normal or elevated LDL without elevated transferrin saturation.

Defining multigenic effects for targeted intervention
Various well-designed studies link the individual genotypes discussed above and/or their phenotypic effect to CVD. As indicated in table 1, the relevant biological processes are targeted for intervention if appropriate, based on genetic, biochemical and environmental risk factors identified. This approach is very powerful since it links the genotype with the overall risk profile and choice of intervention strategy. Selection of the mutations included in the CVD multi-gene assay was based on their phenotypic effect (clinical manifestation), allele frequencies and availability of appropriate intervention or treatment options that may be required. The genotypes being tested for (1) affect the function or level of the gene products, (2) affect biological processes involved in CVD or related co-morbidities, and (3) have apparent metabolic/clinical implications, either alone or in combination with other genetic or environmental risk factors.

Table 1. Intervention strategy based on the CVD risk profile compiled for each individual relating to lipid metabolism, homocysteine/folate metabolism, haemostasis and iron homeostasis.

CVD RISK PROFILE (conside rmedical and family history, lifestyle and nutrition assessment scores, biochemical parameters, genetic test results)	*INTERVENTION BASED ON OVERALL RISK PROFILE*	
	Biochemical, Nutrition and Lifestyle Assessment*	**Genetic assessment (individualised approach in the presence of a genetic alteration)[#]**
Lipid metabolism Dyslipidaemia may result from unhealthy lifestyle and/or genetic risk factors, which may include mutations in the following genes LDLR Apo B Apo E	Restrict saturated fat, trans fatty acids, refined carbohydrates and cholesterol intake, avoid smoking, encourage regular exercise and healthy body weight.	FH or FDB diagnosis justifies life-long drug treatment to reduce serum cholesterol and aggressive reduction of all other CVD risk factors. Dietary intervention and lifestyle changes are the treatment of choice in dyslipidaemic Apo E2 or E4 carriers without existing CVD.
Folate metabolism High homocysteine levels may result from nutrition deficiencies and/or genetic risk factors, which may include MTHFR 677TT 677CT/1298AC	Ensure that dietary intake meets the recommended dietary allowance (RDA) for folate and other B vitamins.	Presence of two copies of MTHFR mutation 677C_T, or single copy in combination with mutation 1298A_C, requires increased folate intake above RDA (800 ug per day). When daily intake of folate is increased >400 ug, the intake of vitamins B6, B12 and riboflavin should be increased accordingly.

Haemostasis
Thrombosis results from acquired and/or genetic risk factors, which may include mutations in the following genes
    Factor V (Leiden)
    Prothrombin (FII)
    FII/Factor XIII
    β-Fibrinogen
    PAI-1/HPA-1
	Encourage high physical inactivity, avoid smoking, achieve or maintain a healthy body weight.	Avoid relevant environmental risk factors such as smoking, inactivity, obesity and encourage regular intake of omega-3 fatty acids.
		Detection of the factor V Leiden and/or prothrombin mutations highlights importance of preventative measures during long-distance travel or pregnancy, and careful consideration of OCP and HRT use.

Iron homeostasis
Abnormal iron status may be caused by environmental factors and/or risk genotypes, which may include
    HFE C282Y/C282Y
    HFE C282Y/H63D
    HFE C282Y/S65C
	Dietary restriction based on serum ferritin and transferrin saturation levels, reduced intake of red meat and alcohol may be advisable.	Identification of gene defect(s) in the presence of abnormal iron profile confirms HH diagnosis, requires regular phlebotomies to prevent organ damage when iron stores are high. The frequency of blood donation can be reduced by dietary intervention to limit iron bioavailability.

*In the absence of the gene variations included for each of these areas, population-based health recommendations apply based on the CVD risk profile. #The relevant risk implications (gene-environment interactions) discussed in the test are highlighted in the presence of any gene variation detected to develop an individualised risk reduction program.

Conclusions

Genetic testing forms an important component of continuous efforts to optimise disease diagnosis and lifestyle intervention for CVD risk reduction in the general South African population and in patients at risk of recurrent coronary events. Identification of FH patients forms the basis of the CVD genetic test since mutation screening in genetically characterised families allows accurate diagnosis and preventative treatment in about half of close relatives, the majority of whom usually are unaware of their FH status. In order to prevent misinterpretation of genetic data when performing the multi-gene CVD assay, information on non-genetic risk factors, biochemical parameters, family history, and known gene-gene or gene-environment interactions are taken into account when the genetic results are reported. The formulation of individualised risk reduction strategies and health monitoring programs implemented by referring health professionals are based on conventional risk factors integrated with the genetic test results (Kotze and Badehorst 2005; Avenant 2005). The test results are used to determine whether high- or low-expression mutations and/or diet and lifestyle factors are most likely to cause the disease or contribute to clinical outcome. In the genetic test report it is clearly stated that the high-frequency, low-expression gene variations included in the cardiovascular genetic test are not deterministic by itself in the development of CVD, but may increase the risk imposed by environmental factors such as smoking, physical inactivity or unhealthy food choices.

The key objective of nutritional genetics is to identify genetic and lifestyle risk factors that may interact to increase the risk of chronic diseases and to intervene effectively. Knowledge that the modest effect of a mutation in a low-risk environment may translate into a major effect in a high-risk environment empowers patients to take responsibility for

their health. By linking functional genetic alterations to clinical outcome and treatment, medical care may over time move from primarily disease management to health management.

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