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Genetic screening for familial hypercholesterolaemia

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Executive Summary

Familial hypercholesterolaemia (FH) is a condition where affected individuals are characterised with chronically elevated serum cholesterol and low density lipoprotein-cholesterol (LDL-C) levels. This condition is caused by mutations within the LDL receptor (LDLR) and apolipoprotein B (apoB) gene, resulting in the presence of dysfunctional LDL receptors which are not capable of clearing lipoproteins efficiently from the bloodstream. Consequent to the high concentrations of LDL-C and cholesterol, individuals with FH are associated with early onset coronary heart disease.

FH is one of the most common genetic disorders, affecting 1 in 500 individuals worldwide. The actual prevalence rate in Australia and New Zealand is unknown but is likely to correspond to worldwide estimates. Despite the fact that FH can be diagnosed early, research has shown that only approximately 20% of FH affected individuals have been diagnosed and only 7% are adequately treated (Williams et al. 1996).

The most commonly utilised method of diagnosis (clinical diagnosis) involves the measurement of total serum cholesterol levels and LDL-C levels, identifying the presence of tendinous xanthomas and a study of family history. However, there are several inherent flaws that plague this method of diagnosis. To begin with, the range of serum cholesterol and LDL-C levels in FH patients overlaps with that of normal individuals. Early studies conducted on children have shown misdiagnosis rates ranging from 4.5% to18.9% when utilising total cholesterol or LDL-C cutoff points (Leonard et al. 1977, Kwiterovich et al. 1974) while other studies have reported that some FH-positive patients may not have sufficiently elevated levels of cholesterol for clinical diagnosis. In addition, studies have shown that tendinous xanthomas are not always prevalent in FH patients and are rarely present until the fourth decade of life. Therefore, the presence of tendinous xanthomas is not a reliable diagnostic criterion, especially in children (Austin et al. 2004a).

Genetic testing has been advocated as the only unequivocal diagnosis method for FH that is capable of addressing the limitations of clinical diagnosis. Genetic testing is not dependent on biochemical measurements or clinical symptoms; instead diagnosis of FH is achieved by the molecular identification of mutations within the LDLR or apoB gene. However, studies have shown that mutation detection rate can vary significantly, ranging from 20% to 80% in patients with clinically diagnosed FH (Sozen et al. 2004, Hadfield and Humphries 2005). To date, no molecular technique is capable of detecting a mutation in all clinically diagnosed FH patients. It remains unclear as to what extent the low mutation detection rates are caused by the inadequacies of molecular testing, incorrect clinical diagnosis or the existence of polygenic mutations that result in the FH phenotype.

Both the Netherlands and Norway have established national genetic cascade screening programs for FH and have reported significant success. In the Netherlands, 93% of patients genetically diagnosed with FH had visited a physician and were prescribed lipid-lowering medication at 1-year post-screening (Umans-Eckenhausen et al. 2001), while 86% of patients remained on lipid-lowering medication at 2-years post-screening (Umans-Eckenhausen et al. 2003). LDL-C levels decreased by 30.2% at 2-years post-screening, with 66% of patients achieving the treatment LDL-C target levels of 135 mg/dl (3.5mmol/l) (Umans-Eckenhausen et al. 2003). Meanwhile, the Norwegian screening program achieved 9.6% ($p<0.0001$) and 14.7% ($p<0.0001$) reduction in total serum cholesterol and LDL-C levels respectively at 6 months post-screening; while 24.1% of adult relatives had LDL-C levels of less than 135 mg/dl (3.5mmol/l) at 6-months (Leren et al. 2004).

Cost-effectiveness studies of cascade screening utilising genetic confirmation techniques have shown that the cost per life-year gained is comparable to cascade screening utilising conventional clinical diagnosis techniques (Marks et al. 2000, Marks et al. 2002, Marang-van de Mheen et al. 2002, Wonderling 2004). However, there is some controversy with regards to the cost per life-year gained for the Dutch screening program (Marang van de Mheen et al. 2002, Wonderling 2004). It is important to note that none of these models are entirely appropriate for the Australia/New Zealand context. A model is needed that takes account of the recently published clinical guidelines for the management FH (Sullivan 2007) and which utilises costs appropriate for Australia/New Zealand costs and recognising the cost reductions possible with generic statins.

In conclusion, genetic screening for FH appears to be effective in increasing the proportion of FH patients receiving adequate medical treatment; therefore resulting in significant reductions of LDL-C and cholesterol levels which should translate to lower incidences of coronary heart disease (Umans-Eckenhausen et al. 2001, Umans-Eckenhausen et al. 2003, Leren et al. 2004). However, the ability of current genetic testing techniques to identify mutations within the LDLR gene is somewhat of a concern, especially when utilised to identify index cases. It may be prudent to utilise clinical diagnosis to identify potential index cases; followed by genetic testing to identify the exact mutation causing the FH phenotype. When genetic testing is utilised for cascade screening of relatives, the results are highly encouraging (Umans-Eckenhausen et al. 2003, Leren et al. 2004). In the context of the Australian and New Zealand healthcare system, the fact that there is no national screening program for FH is disconcerting considering the fact that FH is relatively common and can be effectively treated when early diagnosis is made. Regardless of whether clinical diagnosis methods or molecular genetic methods are utilised, some form of national screening initiative would be beneficial in both countries as there is now a national guideline for management of the condition (Sullivan 2007).

HealthPACT Advisory

HealthPACT has considered screening for familial hypercholesterolemia following a horizon scanning report based on identification of index cases and using a cascade approach to selecting cases for screening. There is international evidence that screening for familial hypercholesterolemia can be effective.

The number of LDL receptor mutations (850) has made screening complex but with identification of index cases the direct sequencing approach appears to provide a practical solution to effective screening.

However, there remains a number of substantial issues for resolution of the effectiveness of a screening program within the Australian community and HealthPACT has requested MSAC to undertake a full health technology assessment on genetic screening for familial hypercholesterolemia.

Introduction

The Australian Safety and Efficacy Register of New Interventional Procedures – Surgical (ASERNIP-S), on behalf of the Medical Services Advisory Committee (MSAC), has undertaken a Horizon Scanning Report to provide advice to the Health Policy Advisory Committee on Technology (Health PACT) on the state of play of the introduction and use of genetic screening for familial hypercholesterolaemia.

Genetic testing to identify individuals with familial hypercholesterolaemia is not conducted in Australia or New Zealand. To date, clinical diagnosis based on cholesterol levels, low-density lipoprotein cholesterol levels, family history and other phenotypic factors is the main technique utilised for the diagnosis of this condition. There is no national screening program to identify individuals with familial hypercholesterolaemia in Australia or New Zealand; however the MEDPED-FH (Make Early Diagnosis Prevent Early Death –Familial Hypercholesterolaemia) program is active in both countries and aims to register as well as support patients who have been clinically diagnosed with familial hypercholesterolaemia.

This Horizon Scanning Report is intended for the use of health planners and policy makers. It provides an assessment of the current state of development of genetic screening for familial hypercholesterolaemia, its present use, the potential future application of the technology, and its likely impact on the Australian health care system.

This Horizon Scanning Report is a preliminary statement of the safety, effectiveness, cost-effectiveness and ethical considerations associated with genetic screening for familial hypercholesterolaemia.

Background

Description of the condition

Note: Please refer to Appendix D for a glossary of terms.

Familial hypercholesterolaemia (FH) is a condition caused by mutations in the low-density-lipoprotein receptor (LDLR) gene. It is an autosomal codominant genetic disorder and is known to result in elevated total cholesterol and LDL-cholesterol (LDL-C) levels due to the presence of dysfunctional LDL receptors or a lack of hepatic LDL receptors that are capable of clearing LDL from the bloodstream. Consequent to chronically high LDL-C and total serum cholesterol levels, patients with FH are predisposed to premature atherosclerotic cardiovascular disease and tendinous xanthomas. Early detection of this condition is possible, and almost certainly will be beneficial to the individual.

Conventionally, FH can be detected at any age from clinical/biochemical testing and inspection of family history if necessary.

In order to prevent early onset cardiovascular disease, individuals diagnosed with this condition require aggressive treatment to lower LDL and cholesterol levels. Most patients are prescribed lipid-lowering drugs such as hydroxy-methylglutaryl-coenzyme A reductase (statin), which induce upregulation of the normal LDL receptor allele in the liver in order to reduce the concentration of LDL in the bloodsteam[1]. Further LDL level reduction can be achieved by administering a combination of high-dose statins with ezetimibe (a cholesterol-absorption inhibitor). In some severe cases, the combination of statins with cholesterol-absorption inhibitors may be insufficient; therefore clinicians would include bile acid sequestrant and/or niacin to the patient's medication regimen. Failing this, patients would require LDL apheresis to exert control over their elevated LDL-C levels (Rader 2007). In patients with homozygous FH, severe deficiencies or sometimes complete loss of LDL receptors (receptor-negative) may occur; therefore leading to extremely high LDL-C levels within the bloodsteam. Patients with this rare homozygous form of FH will exhibit symptoms of cutaneous xanthomas during childhood and devastating complications such as accelerated atherosclerosis very early in life. Without aggressive treatment, homozygous receptor-negative FH patients rarely survive beyond the second decade; whereas, homozygous patients with defective receptors usually have better prognosis but would invariably develop clinical atherosclerotic vascular disease by age 30 (Rader 2007).

Genetic basis of Familial Hypercholesterolaemia

Research since the 1970s has identified that the FH phenotype is a result of mutations within the LDLR gene. This gene spans 45 kilobases, has 18 exons and maps to the short arm of chromosome 19 at 19p13.1-pl3.3. At present, over 850 LDLR mutation variants have been identified in individuals with FH (http://www.ucl.ac.uk/fh/). However, not all variants are known to be functional mutations. In addition, over 80 gene deletions and duplications have been identified in the LDLR gene; these major rearrangements account for approximately 5% of FH mutations in genetically heterogenous populations (Austin et al. 2004b).

In the 1980s, additional research revealed that the same clinical FH phenotype may be caused by mutations in the receptor binding region of apolipoprotein B-100 (apoB-100), the ligand for the LDL receptor. Mutations to the apoB gene would impair the binding of the ligand and therefore delay the clearance of LDL from the bloodstream (Rader 2007). The disorder resulting from the mutations in this gene has been termed 'familial defective apolipoprotein B-100 (FDB)'. The

[1] Statins inhibits the enzyme hydroxy-methylglutaryl-coenzyme A, causing intracellular cholesterol to fall. This results in upregulation of LDL receptors to normalise intracellular cholesterol levels, resulting in a decrease of extracellular cholesterol levels.

most common mutation resulting in FDB is a substitution of glutamine for arginine at position 3500 in apoB-100; however it should be noted that there are other mutations that have a similar effect on apoB binding to the LDL receptor (Rader 2007). Several variants of the apoB gene have been identified; such as the R3500Q, R3500W and R3531C variants that reduce binding of LDL *in vitro*. However, R3531C has not been consistently demonstrated to be associated with hypercholesterolaemia (Austin et al. 2004b).

In addition to LDLR and apoB mutations, scientists have discovered other genes that contribute to monogenic elevated plasma LDL cholesterol levels. Two new loci have been identified in recent years and shown to cause recessive forms of hypercholesterolaemia; this condition is known as “autosomal recessive hypercholesterolaemia”. Clinical studies have revealed that patients with *homozygous autosomal recessive hypercholesterolaemia* exhibit cholesterol levels that are usually intermediate between those of FH heterozygotes and FH homozygotes. Meanwhile, *autosomal recessive hypercholesterolaemia heterozygotes* have similar lipid levels to the general population; the long-term risks of coronary disease in this subgroup are not known (Austin et al. 2004a). However, autosomal recessive hypercholesterolaemia is beyond the scope of this report and will not be discussed in greater detail.

Founder Populations

In certain populations, a small number of LDLR or apoB variants account for the molecular diagnosis of the majority of patients with FH. For example, in the North Karelian region of Finland, over 80% of FH individuals are heterozygous for the same LDLR variant (Austin et al. 2004b); while in the province of Quebec, the frequency of heterozygous FH patients is 1 in 270 overall, which is approximately twice the frequency observed in most population samples, and over 76% is caused by 5 mutations in the LDLR gene (Davignon and Roy 1993). This high frequency is attributable to a “founder effect”, which is the establishment of a new population by a few original founders who carry only a small fraction of the total genetic variation of the parental population.

In addition to these populations, there is some evidence that Tunisians, Christian Lebanese and South African Indians have a small number of variants in the LDLR gene which are responsible for a substantial portion of FH cases (Austin et al. 2004b).

Description of the Technology

The genetic diagnosis of FH is dependent on the identification of a mutation in the LDLR or apoB gene. There are several molecular techniques available for rapid mutational screening that will be briefly described in the following sections. This list is by no means comprehensive, but aims to summarise some of the more commonly utilised techniques in the genetic diagnosis of FH as well as a few relatively new techniques.

Methods of molecular (DNA) screening

Most molecular screening methods utilised for the identification of LDLR and apoB mutations involve initial amplification with polymerase chain reaction (PCR) or reverse transcriptase PCR (RT-PCR) in order to produce an adequate sample size for analysis.

There are two main approaches to the molecular diagnosis of FH; screening of the gene for *any* possible mutations, or screening for *specific* reported mutations. The following is a brief description of molecular screening methods presented within the literature as a means to diagnose FH:

1) Single-strand conformation polymorphism (SCCP) analysis

The SSCP technique is the most widely used among all techniques in the identification of mutations within the LDLR and apoB gene (Marks et al. 2004). This method is capable of identifying most sequence variations in a single strand of DNA, typically between 150 and 250 nucleotides in length. The basic principle of SCCP is that under non-denaturing conditions, a single strand of DNA will adopt a certain conformation (presumably dependant on internal base-pairing between short segments by fold-back) that is completely unique depending on its sequence composition. If a single base has been altered, it is likely that the conformation will be different. Changes in conformation alter the size of the DNA sufficiently that even though the variant sequence has the same charge, the configuration-to-charge ratio is different enough to be detectable as a mobility difference during electrophoresis through a retarding matrix such as acrylamide gel (Humphries et al. 1997).

2) Denaturing gradient gel electrophresis (DGGE)

DGGE is a molecular fingerprinting method that separates PCR generated DNA products by subjecting the DNA to an electrophoresis gel that contains a denaturing agent. Certain denaturing gels are capable of inducing DNA to melt at various stages. During DGGE, the DNA encounters increasingly higher concentrations of the chemical denaturant as they migrate through the polyacrylamide gel. Upon reaching a threshold denaturant concentration, the weaker melting domains of the double-stranded DNA will begin to denature (partially or completely) therefore slowing its migration across the gel dramatically. Mutations to the DNA sequence are likely to result in differing denaturant thresholds and consequently result in visible differences in migration compared to normal wild type DNA. This distinctive characteristic of DNA when placed in denaturing gel allows researchers to discern differences in DNA

sequences or mutations of various genes when comparing one DNA sample to another (Nissen et al. 1998).

3) Denaturing high-performance liquid chromatography (DHPLC)

Prior to DHPLC, a 200-1000 base-pair DNA fragment is amplified by means of PCR from at least two chromosomes. Following this, the amplified fragments are denatured at 95ºC for 3 minutes before they are allowed to reanneal by gradually lowering the temperature to 65 ºC within 30 minutes. In the presence of a mutation in one of the chromosomes, the sense and anti-sense strands of either homoduplex will form two heteroduplices (in addition to the formation of the original homoduplex). Depending on the ‘Guanine -Cytosine’ content of the double-stranded DNA fragments, a column temperature of typically 50-70ºC is maintained to induce partial denaturation. The heteroduplices that are less thermally stable will denature more extensively and are less retained by the stationary phase. This generates a chromatograph pattern that is distinctive from the pattern that would be generated if the target DNA fragment and normal DNA fragments were identical (Bodamer et al. 2002).

4) Complementary DNA (cDNA) strand sequencing

Complete cDNA sequencing involves the generation of cDNA via RT-PCR from extracted RNA. This generates a set of overlapping fragments covering the whole coding region (LDLR or apoB) and all fragments are sequenced on both strands. Liguori et al. (2003) utilised a combination of four DNA sequence analysis programs; Phred, Phrap Consed, RefComp and PolyPhred to enable quantitative estimation of sequence quality, visual display of integrated sequence information and detection of mutations either in homo- or heterozygote. The process of sequencing is large automated and computer assisted; upon completion, the complete cDNA sequence is reassembled and compared with the normal wild type sequence to identify mutations (Liguori et al. 2003).

5) Melt-microplate array diagonal gel electrophoresis (Melt-MADGE)

The melt-MADGE technique essentially combines the principles of traditional MADGE technique with DGGE. The MADGE technique is essentially a high-throughput electrophoresis method that utilises a microplate-array (8 x 12 array). The same array is utilised in melt-MADGE with the key difference being the implementation of a DGGE-esque technique. Conventional DGGE involves the use of cumbersome apparatus and with inconveniences of vertical polyacrylamide gels and gradient-gel pouring, has an inherent low throughput, and is fundamentally incompatible with the spatial arrays of MADGE.

Melt-MADGE utilises the same principles as DGGE but exchanges the dimension of the gradient, so that it is a thermal ramp in time rather than a gradient in space. PCR products are loaded onto a homogenous gel and the temperature of the entire gel is raised during the course of electrophoresis. DNA fragments that begin to

denature at a lower temperature will display markedly reduced mobility at an earlier stage (heteroduplexes) and therefore will not migrate as far as the more thermostable homoduplexes. This reconfiguration of the denaturing gradient to have temperature as the dependent variable and time as the independent variable creates the option for high density arraying of wells, therefore enabling high-throughput of analyses (Day et al. 1998, National Genetics Reference Laboratory 2007).

6) Microelectronic array technology

Microelectronic array technology was recently utilised in Greece for the analysis of LDLR mutations in FH patients (Laios and Drogari 2006). This technique can only be utilised to identify *specific* known mutations within the population and is not capable of identifying new mutations. Primer pairs are used to amplify the region encompassing each single-nucleotide polymorphisms (SNP); and utilising the NanoChip® Molecular Biology Workstation[2], target SNPs are electronically deposited onto a proprietary cartridge composed of a 10 x 10 array of microelectrodes. A thin hydrogel permeation layer containing streptavidin coats the chip surface, therefore allowing the binding of the biotinylated PCR amplicon. Allele specific fluorescently-labelled oligonucleotide reporters (Cy5- and Cy3-labeled) were designed and used for detection of wild-type and SNP sequences. The wild-type reporter is Cy-3 labelled while the mutant reporter is Cy5-labelled. Following this, the microarray chip is washed and imaged. Fluorescence signal ratios of the reporters will allow the identification of wild-type, homozygote and heterozygote samples (Laios and Drogari 2006, Santacroce et al. 2002).

This technique is particularly useful in populations of low genetic heterogeneity as it can take advantage of the ‘founder effect’.

Methods of FH screening utilising genetic testing

1) Universal screening

Universal screening basically implies that every person will be tested, regardless of their apparent risk of having FH. For example, a screening program can be set up to test all 15 year-olds within the population. The key advantage of this screening method is that practically every FH positive individual can be identified if the diagnostic method is accurate; however this method is evidently laborious and would require substantial resources to execute effectively (Marks et al. 2000).

2) Cascade screening

[2] The NanoChip® Molecular Biology workstation is an automated microelectronic array technology system consisting of a cartridge, a sample loader and a fluorescence-based reader with a computer-controlled interface and data-analysis software.

Cascade screening is a form of targeted screening where relatives of index cases are screened due to their high risk of FH. This method of screening is currently being utilised in the Netherlands where the DNA of individuals that have been clinically diagnosed with FH are analysed for the presence of an LDLR mutation. If a mutation is present, the subject is classified as an index case. With consent from the patient, first-degree relatives (who have a 50% chance of being FH positive) are contacted and genetically tested for the mutation; if carrier status is confirmed then the relatives of this newly diagnosed patient are contacted as well (Umans-Eckenhausen et al. 2001).

Intended purpose

Genetic screening utilising molecular diagnosis techniques is intended to provide unequivocal identification of individuals with FH and consequently enable the administration of lipid-lowering medication in order to prevent the onset of coronary heart disease. As there are well established and effective clinical management and pharmacological means to treat FH; it is highly advantageous to identify individuals with this condition before the onset of cardiovascular disease in order to achieve marked reduction in the morbidity and mortality associated with FH.

Besides being a diagnosis tool, genetic screening may predict a differential response to medical therapy, depending on the 'severity' of the mutation. For example, patients with 'mild' mutations where residual receptor activity is high may have a greater lipid-lowering response compared to those with 'severe' mutation where receptor activity is much lower. Heath et al. (1999) compared FH patients with 'severe'[3] LDLR mutations to those with 'mild' mutations and found a significantly lower proportion of 'severe' mutation patients achieved the target cholesterol levels (4.9 and 4.1 mmol/l) compared to 'mild' mutation patients at the maximum statin dose of 40 mg/day (p = 0.018). This therefore indicates that patients with a more severe mutation may require more aggressive lipid-lowering treatment to attain LDL-C levels recommended to reduce the risk of coronary heart disease (Heath et al. 1999).

Clinical need and burden of disease

FH is one of the most common genetic disorders with a prevalence of heterozygotes of approximately 1 in 500 people in most western countries, while the more severe homozygous form occurs in approximately 1 in 1,000,000 people (Leren 2004). It is estimated that more than 10,000,000 individuals are affected by FH worldwide, of which as many as 200,000 die of premature coronary heart disease each year (Leren 2004).

A study involving 14 western countries has indicated that approximately 20% of FH affected subjects have been diagnosed, only 16% have been prescribed lipid-

[3] 'Severe' mutations were mutations in repeat 5 in exon 4 and null mutations

lowering drugs, and only 7% were adequately treated (Williams et al. 1996). There is no Australian or New Zealand prevalence data on FH; however is it likely to correspond with worldwide estimates.

1) Peripheral arterial disease and stroke

In early studies, the prevalence of symptomatic peripheral arterial disease (indicated by intermittent claudication) was reported to be 8%-16% in clinical FH heterozygotes (Hutter et al. 2004). Later, the use of echo-Doppler, which allows the identification of presymptomatic arterial lesions and reduced blood flow, revealed that peripheral arterial disease was present in 30%-45% of FH patients (Postiglione et al. 1985, Kuo et al. 1987).

Individuals with FH have a fivefold increase in arterial lesions in iliac arteries and an increase of three to fourfold in the prevalence of reduced blood flow in leg arteries compared to controls (Hutter et al. 2004). Another study noted an approximate 10-fold increase in the prevalence of peripheral artery disease as measured by ankle/arm blood pressure ratios and femoral artery blood flow (31%) compared to matched controls (3.7%) (Kroon et al. 1995). In some cases, peripheral vascular disease was manifest in 30 year-old heterozygous FH patients (Kroon et al. 1995).

The association of clinical FH phenotype with stroke events has been documented in various studies of several geographic locations (Austin et al. 2004a). Despite the substantial number of studies conducted, results have shown large variations and are often in disagreement with each other; therefore it is unclear if FH is truly associated with increased stoke events (Huxley et al. 2003). These discrepancies may be a result of varying study populations; some studies examined FH patients who survived incident cases of ischaemic stroke, while others examined overall mortality and stroke incidence in a cohort of FH patients. Other factors which may be involved are small sample sizes and differences in study endpoints. In the largest study to date on the risk of stoke in *treated* FH patients, the results showed that the mortality rate from stroke was 0.39 per 1000 person-years and the standardised mortality ratio for fatal stroke was similar to that of the general population (Huxley et al. 2003). However, it should be noted that although this study strongly suggests that treated FH patients are not at an increased risk for stroke, it does not exclude the possibility that untreated individuals may be at increased risk. In addition, the risk of stroke may have been substantially underestimated in this study as patients may have died beforehand due to coronary artery disease, which typically has an earlier stage of onset.

2) Coronary heart disease

In a 1974 cross-sectional study (Stone et al. 1974), the prevalence of coronary artery disease was analysed in 1023 relatives of 116 FH index patients. According to estimates from this study, FH-affected male relatives had a cumulative probability of non-fatal or fatal coronary artery disease of 16% at 40-years old and 52% at 60-years old; significantly higher compared to unaffected male relatives at 60-years (12.5%). Similar estimates were obtained for females at 60-years old, where a cumulative probability of fatal or non-fatal coronary artery disease of 32.8% was noted in affected female relatives compared to 9.1% in non-affected relatives (Stone et al. 1974).

In one prospective cohort study (Simon Broome Register Group 1991) of 282 men and 244 women with heterozygous FH followed up for 2234 person years during 1980-1989; the investigators found that these patients had a standardised mortality ratio of approximately 3.9 times higher compared to the general population in England and Wales for coronary heart disease (Table 1). Surprisingly, the excess mortality from coronary heart disease was highest at age 20-39 (standardised mortality ratio: 9686) and decreased significantly with age (age 60-74; standardised mortality ratio: 44). This implies that only younger FH patients are associated with higher mortality; and the risk decreases significantly if they survive beyond middle-age (Simon Broome Register Group 1991).

Table 1: Simon Broome coronary heart disease mortality in FH patients (1980-1989)

Age (years)	Person-years	Standardised mortality ratio	Observed deaths (total mortality)	No. of observed CHD deaths
20-39	774	9686**	6	6
40-59	1110	519**	13	8
60-74	358	44	5	1
20-74	2234	386*	24	15

*p<0.01; **p<0.001

In another study by the Simon Broome Register group (Simon Broome Register Group 1999), the authors prospectively followed a cohort of 605 men and 580 women (aged 20 – 79 years) with heterozygous FH from 1980 to 1995 (8770 person years). The results obtained verify the findings of the earlier study (Simon Broome Register Group 1991) that young adults (aged 20-39 years) with FH had a nearly 100-fold increase in relative risk for fatal coronary heart disease; and that this risk decreased significantly if the patient survived though to middle age. However, this study also revealed a decline in relative risk for coronary mortality in patients aged 20-59 years, from being 8-fold before 1992 to only 3.7-fold post-1992. This substantial reduction in mortality is explained by the more widespread usage of statins post-1992 (Table 2).

Table 2: Simon Broome coronary heart disease mortality in FH patients (1980-1995)

Age (years)	1980-1991		1992-1995	
	Relative risk	No. of observed CHD deaths	Relative risk	No. of observed CHD deaths

0-19	-	0	-	0
20-39	84.3****	7	17.5	1
40-59	5.3****	12	3.3*	7
60-79	1.2	5	2.1*	14
0-79	3.6****	24	2.5**	22

*p<0.05; **p<0.01; ****p<0.0001

The Australian Centre for Genetics Education states that without treatment, 50% of heterozygous FH males will develop coronary artery disease before the age of 50 and 100% by the age of 70. In heterozygous FH women, 12% will suffer coronary artery disease by age 50, and this increases to 74% by age 70 (Centre for Genetics Education 2005).

Stage of development

Genetic screening programs for FH has been utilised quite extensively in the Netherlands and Norway (Umans-Eckenhausen et al. 2001, Leren et al. 2004). In fact, both the Dutch and Norwegian genetic screening programs are the most established worldwide. To date, Australia and New Zealand do not have a national screening program for detecting individuals with FH utilising clinical or molecular diagnostic techniques. However, mutation analysis has been conducted in an effort to identify the extent of genetic heterogeneity in New Zealand FH patients originating from the United Kingdom (Thiart et al. 2000).

The MEDPED-FH project, coordinated by the University of Utah, is active within the Asia-pacific region and aims to register and support the treatment of people with FH. The project currently operates in more than 30 countries worldwide with over 25,000 registered patients. In Australia, MEDPED is supported by Pfizer and has registered about 700 patients with FH, which is approximately 2% of the estimated 33000 patients overall (Hamilton-Craig 2005). In each capital city, MEDPED physicians are collaborating with nurse practitioners to perform FH cascade screening utilising biochemical diagnosis techniques.

Treatment Alternatives

Existing comparators

Clinical criteria

The most widely utilised diagnosis technique (and the only comparator to molecular/genetic testing) for familial hypercholesterolaemia is the identification of clinical FH phenotype (elevated total cholesterol and LDL-C levels, presence of tendinous xanthomas, early onset cardiovascular disease). As the understanding and knowledge of FH improved, researchers have developed sets of clinical criteria to identify individuals with FH. Three of the most commonly utilised clinical diagnosis criteria are: the United States MEDPED criteria, the United Kingdom Simon Broome Register criteria and the Dutch Lipid Clinic Screening Network criteria.

1) MEDPED criteria

The MEDPED diagnostic criteria for FH take account of the prior probability of an LDL receptor mutation, which varies for the first, second, and third degree relatives and the general population. Based on these factors, different cutpoints for total cholesterol levels are designated for each of these groups for four age brackets (Table 3).

Table 3: United States MEDPED Program diagnostic criteria for familial hypercholesterolemia

	Total cholesterol cutpoints (mmol/liter)			
Age (years)	**First-degree relative with FH†**	**Second-degree relative with FH**	**Third-degree relative with FH**	**General population**
<20	5.7	5.9	6.2	7.0
20–29	6.2	6.5	6.7	7.5
30–39	7.0	7.2	7.5	8.8
≤40	7.5	7.8	8.0	9.3
Diagnosis				
FH is diagnosed if total cholesterol levels exceed the cutpoint				

†FH: Familial hypercholesterolaemia

Austin et al. (2004b)

2) Simon Broome Register criteria

The Simon Broome Register criteria in the United Kingdom (Table 4) supplements cholesterol measurements with clinical signs and family history. It takes into account the fact that total cholesterol and LDL levels are different for adults and children; as well as the evidence of dominant transmission and the age of onset of coronary disease in relatives. The presence of tendinous xanthoma is the key feature for the 'definite' diagnosis of FH according to the Simon Broome Register. In 1994, the criteria were revised to include DNA evidence for LDLR mutation or familial defective apoB-100 as sufficient for a 'definite' diagnosis.

Table 4: Simon Broome Familial Hypercholesterolemia Register diagnostic criteria for familial hypercholesterolemia

	Description
Criteria	
a	Total cholesterol concentration above 7.5 mmol/liter in adults or a total cholesterol concentration above 6.7 mmol/liter in children aged less than 16 years, or
	Low density lipoprotein cholesterol concentration above 4.9 mmol/liter in adults or above 4.0 mmol/liter in children
b	Tendinous xanthomata in the patient or a first-degree relative
c	DNA-based evidence of mutation in the *LDLR* or *APOB* gene
d	Family history of myocardial infarction before age 50 years in a second-degree relative or before age 60 years in a first-degree relative
e	Family history of raised total cholesterol concentration above 7.5 mmol/liter in a first- or

second-degree relative
Diagnosis
A "definite" FH† diagnosis requires either criteria *a* and *b* or criterion *c*
A "probable" FH diagnosis requires either criteria *a* and *d* or criteria *a* and *e*

†FH: Familial hypercholesterolaemia

Austin et al. (2004b)

3) Dutch Lipid Clinic Screening Network criteria

The Dutch Lipid Screening Network criteria is similar to the Simon Broome criteria but with the addition of a numeric score. A 'definite' FH diagnosis is achieved if the score is greater than 8, considered 'probable' FH if the score is between 6 to 8 points and considered 'possible' between 3 to 5 points. Diagnosis is not made if the total score is below 3.

Table 5: Dutch Lipid Clinic Network diagnostic criteria for familial hypercholesterolemia*

Criteria	Points
Family history	
First-degree relative with known premature (men: <55 years; women: <60 years) coronary and vascular disease, or	
First-degree relative with known LDLC† above the 95th percentile	1
First-degree relative with tendinous xanthomata and/or arcus cornealis, or	
Children aged less than 18 years with LDLC above the 95th percentile	2
Clinical history	
Patient with premature (men: <55 years; women: <60 years) coronary artery disease	2
Patient with premature (men: <55 years; women: <60 years) cerebral or peripheral vascular disease	1
Physical examination	
Tendinous xanthomata	6
Arcus cornealis prior to age 45 years	4
Cholesterol levels (mmol/liter)	
LDLC, $\geq$ 8.5	8
LDLC, 6.5–8.4	5
LDLC, 5.0–6.4	3
LDLC, 4.0–4.9	1
DNA analysis	
Functional mutation in the *LDLR* gene	8
Diagnosis (based on the total number of points obtained)	
A "definite" FH† diagnosis requires more than 8 points	
A "probable" FH diagnosis requires 6–8 points	
A "possible" FH diagnosis requires 3–5 points	

†LDLC: low density lipoprotein cholesterol

Clinical Outcomes

There is a large body of evidence on FH and genetic screening. Many studies have been conducted to determine the genetic basis of FH in a population, however these are of little interest due to the fact that the genetic basis of FH in those countries are likely to be markedly different to Australia and New Zealand. In addition, numerous studies have also been conducted to increase the sensitivity of genetic screening techniques; however, detailed comparisons of technical modifications to increase throughput and new molecular innovations are beyond the scope of this report (a brief description of various molecular techniques was provided earlier in this report). Studies selected for inclusion are predominantly conducted in the Netherlands and Norway as these countries have the most developed national genetic cascade screening program for FH. Relevant studies that may be of interest to the Australian and New Zealand healthcare system, such as those which ascertain the effectiveness of genetic screening, are included as well.

Safety

All included studies did not report on the safety outcomes associated with the molecular diagnosis of FH. In principle, the actual procedure of molecular diagnosis poses little to no danger to the subject as he/she is not subjected to any physical stress while molecular analysis is conducted.

However, this does not mean that there are no risks to genetic screening of FH. As with any other disease which can be screened genetically, there are social and psychological implications which will be discussed in greater detail later in this report.

Effectiveness

In this section, the efficacy of genetic diagnosis of FH will be evaluated in comparison to conventional clinical diagnosis. Later, the utility of genetic diagnosis in identifying index cases and FH-positive first-degree relatives will be discussed; followed by the results of the genetic cascade screening program in the Netherlands and Norway. Refer to Appendix B for profiles of studies included and the genetic testing method utilised.

Clinical vs. genetic diagnosis

Diagnosis of FH is of paramount importance in order to initiate pharmacological interventions and lifestyle modifications to improve the prognosis of affected individuals. At present, the majority of individuals with FH are undiagnosed or are only diagnosed after their first coronary event (Williams et al. 1996). As stated previously, elevated cholesterol and LDL-C levels are recognised as one of the main clinical symptoms of FH; therefore measurement of plasma cholesterol and LDL-C concentrations have been utilised as an important diagnostic method. The three commonly utilised diagnosis criteria; the Simon Broome Register, MEDPED and the Dutch Lipid Clinic Network, placed substantial emphasis on the biochemical determination of cholesterol and LDL-C levels to aid in the identification of FH subjects.

Despite the fact that elevated total cholesterol and LDL-C levels are indicative of FH, there are inherent flaws that limit the sensitivity and specificity of this method of diagnosis. Firstly, there is substantial variation when measuring blood cholesterol levels. Studies have shown that blood cholesterol levels vary approximately 6% over time; therefore in order to improve accuracy, two blood cholesterol measurements are often required (Marks et al. 2003). In addition to this, the range of blood cholesterol and LDL-C levels for individuals with FH overlaps with that of normal individuals. In an early study on children aged 1-19 years (with one parent having heterogenous FH), Kwiterovich et al. (1974) reported that 16.9% were 'misclassified' based on LDL-C levels alone (7.2% false-positives, 9.7% false negatives); while 27.4% were 'misclassified' when plasma cholesterol levels was utilised for diagnosis of FH (8.5% false positives, 18.9% false negatives) (Kwiterovich et al. 1974). The potential for misdiagnosis utilising serum cholesterol levels was echoed in another early case series study by Leonard et al. (1977) where it was shown that 4.5% of children within the study cohort were misdiagnosed. The authors concluded that when total serum cholesterol is in the range of 6.5-7.0mmol/l in children, the diagnosis of FH cannot be made or excluded with confidence (Leonard et al. 1977). Further compounding the disadvantages of utilising total cholesterol or LDL-C levels for diagnosis is the fact that in some FH cases, LDL-C levels are not sufficiently elevated; therefore resulting in false-negative outcomes (Marks et al. 2000).

The presence of tendinous xanthomas is one of the main factors in identifying 'definite' FH cases in the Simon Broome Register and the Dutch Lipid Clinic Network criteria. However, research has highlighted some doubts with regards to its reliability in identifying definite cases of FH (van Aalst-Cohen 2006, Graham et al. 1999). In addition, tendinous xanthomas are usually not present until the fourth decade of life and therefore have limited applicability in younger subjects (Austin et al. 2004a).

The retrospective, multi-centre, cohort study by van Aalst-Cohen et al. (2006) investigated if patients diagnosed with molecular techniques[4] differ significantly from patients diagnosed with conventional clinical criteria[5]. The authors noted that despite the use of stringent clinical criteria to define FH patients, two patient groups could be identified within the study population; those with LDLR mutation (52.3%) and those without (47.7%). Analysis of this cohort revealed significantly different clinical (hypertension) and laboratory profiles (total cholesterol, LDL-C, triglyceride, high-density lipoprotein cholesterol, and glucose levels) between patients with and without LDLR mutations. This phenotypic heterogeneity challenges the value of the current clinical criteria utilised for the diagnosis of FH (van Aalst-Cohen et al. 2006).

Koivisto et al. (1992) examined the concordance of clinical[6] and molecular diagnosis for FH in 65 patients[7]. Utilising molecular diagnosis as the reference, 15% (10/65) patients were misclassified or were designated to the 'possible FH' category when clinical diagnosis criteria were utilised. For adults (>18 years), 11.7% (7/60) were misclassified; however when the investigators utilised age-, population-, and sex-specific 95th percentile values for serum LDL-C instead of common reference limits for all adults; only 5% (1/20) were incorrectly classified. Meanwhile in children (<18 years), 3/5 were misdiagnosed clinically due to the fact that serum LDL levels were lower than the diagnostic limits adopted (Koivisto et al. 1992).

Overall, there are no clear definitions of what the clinical diagnosis criteria of FH, or what the most sensitive cholesterol or LDL-C cut-off levels should be for diagnosis based on cholesterol measurements and family history. A more definitive diagnosis technique for FH is therefore required. Genetic testing may be capable of overcoming the uncertainties of measuring serum cholesterol or LDL-C levels as it eliminates the confusion caused by the overlap of cholesterol levels between the general population and FH individuals.

Utility of genetic testing to identify index cases for cascade screening

As mentioned previously, over 850 LDLR mutations for FH have been identified; therefore screening for each and every one of these mutations is time consuming and labour intensive. Researchers are continually developing or modifying existing molecular testing techniques in order to produce rapid, high-throughput and automated processes to assist in the complexity of this task. In many cases, LDLR and apoB mutation negative samples are sent for more expensive (and

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[4] van Aalst-Cohen et al. (2006) screened for the 14 most prevalent Dutch LDLR mutations.
[5] The FH diagnostic criteria utilized by van Aalst-Cohen was based on the Simon Broome Register criteria, MEDPED criteria and the Dutch Lipid Clinic Network criteria.
[6] Koivisto et al. (1992) derived the clinical diagnosis criteria from the Policy Statement of the European Atherosclerosis Society: <5.0mmol/l "not FH"; 5.0-5.9mmol/l "possible FH"; ≥6.0mmol/l "FH".
[7] Molecular diagnosis has confirmed the presence of LDLR mutations within these 65 patients.

laborious) complete sequencing techniques. However, it should be noted that the utilisation of molecular diagnosis for FH to detect index cases would require the development of country- or region-specific test kits; being largely dependent on the genetic heterogeneity within the population.

One of the concerns of utilising molecular diagnosis techniques for FH is the fact that mutation detection rates can vary significantly. Damgaard et al. (2005) investigated the sensitivity and specificity of the three main clinical diagnostic criteria[8] to predict the results of routine molecular genetic analysis of the LDLR and apoB genes in 408 Danish index patients. FH-causing mutations were identified in 135/408 (33.1%) index patients (117 LDLR, 16 apoB R3500Q, 2 LDLR *and* apoB mutations). Table 6 provides a comparison of the various sets of clinical criteria to identify patients with mutations in the gene for LDLR or apoB:

Table 6: The ability of the Simon Broome Register Group, MEDPED and Dutch Lipid Clinic Network criteria to predict the results of the molecular genetic analysis of the LDL receptor gene and of the apoB R3500Q mutation.

Clinical criteria		**Sensitivity (%)**	**Mutation detection rate (%)**	**Specificity (%)[a]**	**1-Specificity (%) = False positive rate [a]**
Simon Broome Register	Definite FH	34.1 (26.1-42.7) *35.9*	61.3 (49.4-72.4) *59.2*	89.4 (85.1-92.8)	10.6 (7.2-14.9)
	Definite or possible FH	90.4 (84.1-94.8) *93.2*	38.5 (33.1-44.1) *35.4*	28.6 (23.3-34.3)	71.4 (65.7-76.7)
MEDPED	Total cholesterol	63.4 (54.5-71.6) *69.0*	53.5 (45.4-61.6) *52.0*	73.4 (67.8-78.6)	26.6 (21.4-32.2)
	LDL-Cholesterol	70.3 (61.2-78.4) *75.5*	51.6 (43.6-59.5) *49.7*	69.8 (63.8-75.3)	30.2 (24.7-36.2)
Dutch Lipid Clinic Network	Definite FH	41.5 (33.1-50.3) *42.7*	62.9 (52.0-72.9) *60.2*	87.9 (83.4-91.5)	12.1 (8.5-16.6)
	Definite or probable FH	66.7 (58.0-74.5) *68.3*	48.1(40.8-55.5) *45.2*	64.5 (58.5-70.1)	35.5 (29.9-41.5)
	Definite, probable or possible FH	99.3 (95.9-100.0) *99.1*	34.3 (29.6-39.2) *31.1*	5.9 (3.4-9.3)	94.1 (90.7-96.6)

95% confidence intervals are given in parentheses. Data concerning only LDLR mutation carriers are given in *italics*.
Sensitivity – ability of clinical criterion to identify individuals with mutations; **Mutation detection rate** – proportion of patients who fulfilled the criteria [*(1)* total cholesterol >8mmol/L, LDL-C >6mmol/l and triglycerides <2.5mmol/l; *(2)* tendon xanthoma; *(3)* history of coronary artery disease before the age of 60 years in the patient and/or in a first degree relative and/or hypercholesterolaemia in a first degree relative]; **Specificity** – proportion of patients without a mutation who did not fulfil the clinical criterion of FH; **1-Specificity** – proportion of patients without mutations who did fulfil the clinical criterion of FH.
[a] Specificity and 1-Specificity are the same for LDLR mutations only

Damgaard et al. (2005)

[8] Simon Broome Register, MEDPED and Dutch Lipid Clinic Network criteria

The results show a relatively low *sensitivity* (34%-42%) for 'definite' FH; however values of over 90% was achieved for 'possible' or 'probable' FH. Conversely, the *specificity* for the presence of a detectable mutation was approaching 90% for 'definite' FH (both Simon Broome and Dutch Lipid Clinic Network), however it was much lower for Simon Broome 'possible' (29%) and Dutch Lipid Clinic 'probable' (6%) FH. The mutation detection rates were approximately 63% for both Simon Broome and Dutch 'definite' subjects, which decreased substantially to 34%-38% when 'possible' FH subjects were included. The investigators pointed out that this low mutation detection rate may be due to the inadequacy of the molecular screening strategy employed on this study; indicating that the SSCP sequencing technique utilised could not detect all mutations (e.g. intronic LDLR mutations, apoB mutations other than R3500Q etc.). Alternatively, the definite clinical FH phenotype may have been caused by polygenic mutations located outside the LDLR and apoB genes. Damgaard and colleagues (2005) conclude that while molecular diagnosis is useful, it leaves out a group of patients that have polygenic mutations; therefore clinical diagnosis would be helpful in these cases (Damgaard et al. 2005).

Other studies have reported varying percentages of mutation detection rates; a Dutch study stated that approximately 20% of clinically diagnosed FH patients did not have detectable LDLR and apoB mutations (Fouchier et al. 2001). Concurrent with the statement by Damgaard et al. (2005), the investigators attributed this to the weakness of the molecular diagnosis technique utilised, in this study it was DGGE, which was not able to detect all single base-pair changes and the fact that other genes may be responsible for the FH phenotype. Meanwhile, a study on Southeast Asians reported that the majority of the cohort (73%) did not have detectable mutations in the LDLR and apoB gene although these patients had been clinically diagnosed with FH (Khoo et al. 2000). The investigators found that patients without detectable LDLR/apoB mutations had significantly lower rates of coronary heart disease, LDL levels and tendinous xanthomas. This significantly milder phenotype infers that Southeast Asians may have a different mutation which leads to the manifestation of this mild FH phenotype (Khoo et al. 2000).

Historically, the reported percentage of clinically diagnosed FH patients in which a mutation is detected varies from 20% in 'possible' or 'probably' FH to 60%-80% in 'definite' FH patients (Sozen et al. 2004, Hadfield and Humphries 2005). At the time of writing, no study has identified a mutation in 100% of clinically diagnosed patients. Even with very strict clinical criteria (>9.5mmol/l LDL-C), DGGE detected only 81% of mutations in one small sample of 32 patients from Holland, a country where genetic heterogeneity is low (Lombardi et al. 1995). It remains unclear as to what extent the low mutation detection rates are due to the inadequacies of molecular testing, incorrect clinical diagnosis or existence of polygenic mutations causing the FH phenotype. Further work is required to compare the analytical and clinical specificity and sensitivity of the different

molecular techniques currently available and the difference of clinical diagnostic criteria in use. Therefore, it would be prudent to utilise *both* clinical and molecular approaches in identifying proband/index FH individuals, unless the genetic basis of FH within the population of interest has been well documented and can be detected with existing molecular techniques.

Utility of genetic testing to diagnose FH in relatives of index cases

Damgaard et al. (2005) examined samples from 385 relatives of clinically diagnosed FH subjects and found considerable variation in biochemical phenotype for relatives of patients with an LDLR or apoB mutation. This confirms that LDL-C percentiles were poor predictors of mutation detection in relatives, as 23% of mutation carriers had an LDL-C level below the 90th percentile, and 14.7% of non-carriers had an LDL-C above the 90th percentile (Damgaard et al. 2005).

Cost-analysis studies have revealed that the best strategy to diagnose FH is screening of first-degree relatives of patients already diagnosed with FH (detailed under cost analysis section), otherwise known as cascade genetic screening. Cascade genetic screening for FH is being conducted in several countries worldwide, with the Netherlands having the most extensive program (The Dutch National Foundation for Identification of Familial Hypercholesterolaemia) (Umans-Eckenhausen et al. 2001). In this program, index patients were identified as having FH only if they had a known genetic mutation. This case-finding program is structured to identify close relatives of index patients with FH utilising molecular screening techniques.

Between January 1994 to January 1999, family screening was initiated in 237 Dutch families of which 5442 members participated in the screening program (Umans-Eckenhausen et al. 2001). Of these, 945 men and 1094 women had an LDLR gene mutation. At screening, 51% (875/2039 patients) of adults reported previous knowledge of their condition, while 39% (667/2039 patients) have received statin treatment. At 1-year post-screening, 93% of patients identified with FH via molecular screening had visited a physician and started on lipid-lowering medication. The investigators reported that the best available cutoff point to diagnose FH in relatives by total cholesterol concentration is the age-specific and sex-specific 90th percentile. Approximately 18% of FH-positive patients had total cholesterol concentration below these percentiles (false-negatives) and would have been missed if only cholesterol concentrations were measured. On the other hand, 18% of patients would be above the 90th percentile cutoff point (false-positives) (Umans-Eckenhausen et al. 2001).

Considering the fact that achieving low plasma LDL levels in FH patients requires lifelong treatment with lipid-lowering drugs, it is important to appraise the compliance of patients diagnosed with genetic screening to provide a more accurate assessment of the effectiveness of the program. Umans-Eckenhausen et al. (2003) sent a questionnaire to 747 patients, with follow-up to determine long-

term compliance with lipid-lowering medication after genetic screening. It revealed that the overall percentage of treated patients rose from 37.6% at screening to 92.5% 1-year later.

Table 7: Lipoprotein levels at screening and 2 years later in patients with FH with and without cholesterol-lowering medication at the time of diagnosis. Dutch FH genetic screening program.

Receiving cholesterol - lowering medication at screening	No. of patients	Lipoprotein	Mean (SD) level, mg/dl		% change
			At screening	2 years after screening	
No	183	Total cholesterol	298(58)	230 (48)	-22.8
		Tryglycerides	165 (88)	150 (79)	-9.1
		HDL cholesterol	44 (11)	47 (12)	+6.8
		LDL cholesterol	219 (58)	153 (58)	-30.1
Yes	118	Total cholesterol	274 (64)	249 (63)	-9.1
		Tryglycerides	169 (136)	157 (117)	-7.1
		HDL cholesterol	45 (12)	46 (11)	+2.2
		LDL cholesterol	195 (60)	175 (56)	-10.3

*p-value (between levels at screening and after 2 years) was calculated by means of a paired *t* test. P<0.001 for all comparisons

Umans-Eckenhausen et al. (2003)

Between the 1-year and 2-year follow-up, 14 FH carriers died and another 47 patients who were taking medication or started medication after screening discontinued their medication because of disinterest and own choice (29.8%, 14/47 patients), adverse effects (14.9%, 7/47 patients), physician's advice (34.0%, 16 patients), to become pregnant or breastfeeding (17%, 8 patients) and unknown reasons (4.3%, 2 patients). As a result of this, the proportion of patients on lipid-lowering medication decreased to 86.0% (630/733 patients still living). In a subgroup of patients where total cholesterol and LDL-C levels were recorded (Table 7), the investigators showed that mean total cholesterol, LDL-C and triglycerides decreased by an additional 9.1%, 10.3% and 7.1%, respectively; while HDL cholesterol levels increased by 2.2% (p<0.01 for all). The investigators reported that mean baseline LDL-C level was 219 mg/dl (5.7 mmol/l) but decreased to 153 mg/dl (4.0 mmol/l) after 2 years, therefore representing a 30.2% reduction in LDL-C level. The therapeutic goal in these patients includes a reduction of LDL-C concentrations to less than 135 mg/dl (3.5 mmol/l) for primary prevention according to European guidelines. Overall, 66% managed to achieve treatment target levels for LDL-C of 135mg/dl (3.5 mmol/l) or lower. Considering the fact that previous studies have shown that a 1% reduction in LDL-C level will lead to a decrease in cardiovascular disease

incidence of 1.0% to 1.7%; it is reasonable to assume that the changes achieved from this Dutch genetic screening program will translate to a substantial decrease in cardiovascular disease incidence (Umans-Eckenhausen et al. 2003).

Molecular genetic testing for FH has been utilised routinely in Norway since 1998. The program is centralised at the Medical Genetics Laboratory, Rikshopitalet. A total of 851 relatives of 188 index patients have undergone genetic testing, of which 401 (47.9%) have tested positive and 444 (52.1%) have tested negative. These results are concurrent with the strategy of primarily testing first-degree relatives; who have a 50% risk of inheriting the FH mutation. Investigators have reported that only 41.5% of the FH-positive relatives were on lipid-lowering medication; in adults (>18 years old) the proportion receiving lipid-lowering medication was 52.5%. At the time of genetic screening, only 6.1% (24/407) of the affected relatives had a value for total serum cholesterol below 193 mmol/l (5.0 mmol/l) which was considered the target total serum cholesterol level in these FH patients; while as many as 31.7% (129/407) had total serum cholesterol above 309 mg/dl (8.0 mmol/l). As 41.5% of these affected individuals were on lipid-lowering medication, these data demonstrate that the majority of patients receiving lipid-lowering medication are not adequately treated. Only a small portion (7.5%) of the affected relatives had tendinous xanthomas, and only 4.7% had xanthelasma; illustrating again that xanthomas are not a sensitive criterion for identifying individuals with hypercholesterolaemia due to mutation within the LDLR gene (Leren et al. 2004).

To determine if genetic screening results in more appropriate therapeutic measures to lower total serum cholesterol and LDL-C, Leren et al. (2004) conducted a survey among affected relatives aged 10 years and older at least 6 months after the genetic test was performed. A total of 61.9% (146/236) of the affected relatives contacted responded to the survey. At 6 months after screening, 31.3% of the 146 affected relatives reported changes in their diet and 53.4% reported changes in medical therapy. At the time of screening, 41.5% of affected relatives were on lipid-lowering medication; this increased to 81.0% at 6-months follow-up. In adults (>18 years old), the corresponding increase was from 52.5% to 88.5% (Leren et al. 2004).

The changes in lipoprotein levels (total cholesterol, HDL cholesterol, triglycerides, LDL-C) at least 6 months after screening are presented in Table 8. It should be noted that only 86.3% of subjects were in the fasting state[9] at the time of genetic screening and that only 79.l% were fasting at the 6-month follow-up. These results on lipoprotein levels should therefore be interpreted with caution. For all 146 patients, total serum cholesterol and LDL-C decreased by 9.6% (p<0.0001) and 14.7% (p<0.0001), respectively. In relatives that were already on lipid-lowering medication (n=80), the corresponding reductions were smaller and not statistically significant. Conversely, relatives not on lipid-lowering medication

[9] The fasting state allows for more accurate measurements of lipoprotein levels, eating prior to testing would result in elevated levels of total cholesterol and LDL-C.

at the time of screening reported significant overall reductions in total serum cholesterol (18.8%) and LDL-C (22.0%) at least 6 months after. In adults (>18 years), the reductions in total serum cholesterol and LDL-C were 21.2% and 30.0%, respectively (p<0.0001 for both). The investigators reported that among affected adult relatives, 24.1% achieved LDL-C levels of less than 135mg/dl (3.5mmol/l); which is substantially lower than the 66% achieved in the Netherlands (Umans-Eckenhausen et al. 2003). However the Dutch study had a substantially longer follow-up (2 years) compared to the Norwegian study (at least 6 months).

Table 8: Lipoprotein levels at screening and at least 6 months later in patients with FH. Norwegian FH screening program.

	At genetic screening (Mean ± SD)	6 months after genetic screening (Mean ± SD)	% change	p-value*
All patients (n=146)				
Total serum cholesterol	7.10 (±1.61)	6.42 (±1.51)	-9.6	<0.0001
HDL cholesterol	1.37 (±0.33)	1.47 (±0.38)	+7.3	<0.0001
Triglycerides	0.95 (±0.54)	0.97 (±0.64)	+2.1	n.s.
LDL cholesterol	5.29 (±1.60)	4.51 (±1.47)	-14.7	<0.0001
Patients on lipid-lowering therapy at the time of genetic testing (n=80)				
Total serum cholesterol	6.50 (±1.26)	6.29 (±1.27)	-3.2	n.s.
HDL cholesterol	1.40 (±0.34)	1.50 (±0.39)	+7.1	<0.0001
Triglycerides	0.97 (±0.47)	0.94 (±0.63)	+3.1	n.s.
LDL cholesterol	4.66 (±1.18)	4.36 (±1.23)	-7.1	n.s.
Patients not on lipid-lowering therapy at the time of genetic testing (n=47)				
Total serum cholesterol	7.97 (±1.84)	6.47 (±1.83)	-18.8	<0.0001
HDL cholesterol	1.38 (±0.33)	1.48 (±0.40)	+7.2	<0.05
Triglycerides	0.87 (±6.19)	0.99 (±0.71)	+13.8	n.s.
LDL cholesterol	6.19 (±1.85)	4.54 (±1.81)	-22.0	<0.0001
Patients aged 18 and above not on lipid-lowering therapy at the time of genetic testing (n=39)				
Total serum cholesterol	7.98 (±1.96)	6.29 (±1.85)	-21.2	<0.0001
HDL cholesterol	1.40 (±0.329)	1.55 (±0.39)	+10.7	<0.01
Triglycerides	0.92 (±0.69)	0.94 (±0.64)	+2.2	n.s.
LDL cholesterol	6.16 (±1.96)	4.31 (±1.77)	-30.0	<0.0001

n.s.: not significant
*p-values (between levels at screening and at least 6 months after screening) were calculated by means of paired *t* test.

Leren et al. (2004)

From the results presented, it is evident that both the Dutch and Norwegian genetic screening programs have achieved significant success. Both genetic screening programs have succeeded in reducing LDL-C level by at least 20% (Umans-Eckenhausen et al. 2003, Leren et al. 2004); and the Dutch program has reported that 66% of affected relatives have achieved treatment target LDL-C levels at 2-years follow-up (Umans-Eckenhausen et al. 2003). In addition, both genetic screening programs have significantly increased the proportion of FH-positive subjects receiving medical treatment for their condition.

Psychological and social effects of screening

The psychological and social consequences associated with genetic screening have been widely discussed in the literature (Marks et al. 2000). Concerns regarding the psychological well-being of the index patient's relatives when they are first contacted may hinder the implementation of a cascade screening program in a population. There is a possibility that the relatives may become anxious and upset when they are informed of their high risk of having FH; and some may even be offended that such information has been revealed to clinicians. Other concerns include the sense of fatalism in patients with genetically confirmed FH, which could potentially lead to depression and hopelessness as they feel 'destined' to suffer early coronary heart disease.

However, although a great deal has been written and published; most of these are opinion papers which are unsupported by any data. The health technology assessment by Marks et al. (2000) highlighted the lack of qualitative work on the psychological and social effects on screening of any disease. In the case of FH, only a handful of studies have been conducted and most are Dutch or Norwegian based.

The randomised controlled trial by Marteau et al. (2004) randomly assigned 469 patients (341 probands, 128 adult relatives) to: a) routine clinical diagnosis or b) routine clinical diagnosis plus genetic testing, to test the hypothesis that finding a genetic mutation reduces the patient's perceptions of control over the disease and adherence to risk-reducing lifestyle modifications/medication. The investigators found no support for the hypothesis; mutation detection had no effect on the perceived control or adherence to risk-reducing behaviour (no increase in perceived fatalism, control over FH, control over cholesterol or control over heart disease). However, patients with identified genetic mutations were less convinced that dietary changes were effective in reducing their cholesterol level ($p = 0.02$ at 6 months) compared to those without mutations. Instead, patients with identified mutations tended to believe more strongly that lipid-lowering medications were more effective ($p = 0.06$ at 6 months) (Marteau et al. 2004).

van Maarle et al. (2001) utilised questionnaires to assess participants' views on, and the psychological impact of the cascade genetic screening program in the Netherlands. Overall, the investigators found that only 2% of the people approached for screening did not participate due to various reasons (not interested, already diagnosed clinically, afraid of insurance consequences). The majority of participants (88%) would recommend the screening program to someone of similar circumstance; and only 2 (1%) would not recommend the screening program. Less than 5% of respondents were unhappy with the approach taken to contact them, but a substantial proportion of patients (20%) felt social pressure to participate in the screening program. Overall, van Maarle et al. (2001) concluded that screening for FH was highly acceptable within the Dutch population.

In another study, van Maarle et al. (2003) examined the transient and long-term quality of life (QoL) effects of genetic screening for FH in the Netherlands in a consecutive cohort of 677 participants. On the depression scale and the overall score of the Hospital Anxiety and Depression Scale (HADS), older participants and those with chronic diseases scored worse. Participants who felt more or less forced to take part in the screening program scored worse on the depression scale. However, none of these factors were significantly associated with the change over time. The more a participant experienced a feeling of social pressure, the higher the perceived risk of heart attack later in life and their QoL is lower; however these factors were small and practically negligible relative to the scale. The investigators concluded that QoL in FH screening participants remained essentially unchanged during FH genetic screening and there were no differences in QoL between FH positive and FH negative subjects (van Maarle et al. 2003).

To date, no quantitative evidence has been presented that supports the occurrence of significant negative psychological effects due genetic screening for FH or in any other diseases that can be screened genetically. A population based survey by Tonstad et al. (1995) revealed that 77% of respondents would voluntarily enter a cascade genetic screening program for FH if they had a family member with the condition; confirming the high acceptance of the screening program. Bhatnagar et al. (2000) stated that 99% of index patients gave consent that first-degree relatives should be contacted for cascade genetic screening, illustrating that patients are willing to pass on information to their family and are less concerned about the confidentiality of this information than previously thought.

In the case of children, Leren (2004) reported that most Dutch parents (87%) wanted their children aged below 16 to undergo cascade genetic screening. Studies on the psychological state have so far concluded that children who are aware they have FH do not differ to those without FH. However, it should be noted that at the time of writing, it is still unclear if the benefit of coronary heart disease risk reduction is outweighed by the potential long-term risk of taking lipid-lowering medication in children (Marks et al. 2000).

Cost Analysis

Four cost-effectiveness analyses studies were retrieved, including one British health technology assessment report, on genetic screening for FH.

In the UK health technology assessment report by Marks et al. (2000), a comprehensive model to investigate the cost effectiveness of various forms of population screening (universal and opportunistic) and cascade screening was constructed. The strategies examined involved a two-stage process; the first step is identifying individuals with cholesterol levels sufficiently elevated to be compatible with a diagnosis of FH and then either making the diagnosis based on clinical signs and a family history of coronary disease or carrying out genetic tests. Marks et al. (2000) found that case finding/cascade screening is the most effective strategy to identify FH individuals based on the fact the numbers needed to be screened to identify one case of identifiable mutation was significantly lower compared to universal population screening (2.6 people screened vs. 2729 people screened). However, genetic confirmation in a heterogenous population not considered at high risk for FH is less effective in finding cases compared to clinical confirmation. This is due to the fact that the probability of identifying a genetic mutation in subjects where a mutation has not been identified in the family is only 50% (in the United Kingdom); hence double the number of people will have to be screened if genetic testing is utilised instead of clinical criteria.

The cost per new patient detected with FH using the *clinical criteria* ranged from £133 (cascade screening) to £9754 (universal screening); meanwhile cost per case for *genetic confirmation* ranged from £1873 (cascade screening) to £72140 (universal screening), considerably higher compared to detection with clinical criteria.

Table 9: Comparison of the overall cost-effectiveness of clinical and genetic strategies

Strategy	Cost per life years gained (clinical) (£)	Cost per life years gained (genetic) (£)
Universal (16)	2777	14842
Universal	13029	78060
Oppurtunistic (GP)	11310	70009
Oppurtunistic (MI)	9281	21106
Case finding	3097	3300 (relatives **only**: proband with known mutation) 4914 (cost of testing probands included)

Explanation of screening strategies
Universal (16): Screening of all school leavers at the age of 16 years
Universal: Screening at the ages of 16-55 years

Oppurtunistic (GP): Screening of people aged 16-55 years who visit their GP for other medical reasons
Oppurtunistic (MI): Screening of people who have been admitted to hospital with an early myocardial infarction (aged 16-55 years)
Case finding: Screening of family members of and 'index' patient (proband) who has been identified with FH and is attending a lipid clinic; also known as cascade screening.

Marks et al. (2000)

Marks et al. (2000) concluded that generally cascade screening was the most cost-effective strategy while universal screening is the least cost-effective. Where genetic mutation is found for an index case, there is little difference in cost-effectiveness of cascade screening, regardless of whether the diagnostis was clinically or genetically based. Even in the case where the genetic status has not been determined in a family, genetic diagnosis for women of all ages and men up to the age of 34 years is as cost-effective as clinical diagnosis (Marks et al. 2000). With the use of genetic confirmation, an unequivocal diagnosis is made (assuming a genetic mutation is identified) and therefore younger family members can be informed of their predisposition to FH without the confusion caused by overlapping cholesterol and LDL-C levels with the normal population; which has been proven to be most problematic in children (Koivisto et al. 1992, Leonard et al. 1977, Kwiterovich et al. 1974). The paper published in 2002 by Marks et al. (2002) reiterates the findings of the health technology assessment report and reinforced the conclusion that cascade screening is the most cost-effective screening method.

Marang-van de Mheen et al. (2002) estimated the cost-effectiveness of the current genetic screening program (cascade screening) on FH in relatives of index patients in the Netherlands utilising data from 2229 screened FH-relatives (age, sex, risk factor status and screening outcome) with the Framingham risk function and national disease-specific cost data. A mathematical model was constructed to compare survival and cost, with or without the genetic screening program. The total screening cost was approximately €640,000 for the 2229 relatives; which equates to €288 per individual screened. Assuming that all FH-positive patients (n=759) will be treated, the follow-up cost was €26,000,000; resulting in cost per individual treated of almost €35,000. The model assumes that with the additional life-years gained from FH treatment; these individuals will survive and develop other diseases as they age.

Table 10: Difference in cost-effectiveness between treatment strategies (genetic screening)

Treatment strategy	N treated	Total costs(€)	Years of life gained	Costs per year of life gained(€)
All FH+	759	27 048 563	865	31 260
FH+ with elevated cholesterol level	461	18 247 529	610	29 918
FH+ as in cholesterol consensus	265	9 251 537	361	25 613
Untreated FH+	430	16 704 039	519	32 164
Untreated FH+ with elevated	303	12 554 834	407	30 843

cholesterol level				
Untreated FH+ as in cholesterol consensus	133	5 637 424	204	27 700

Explanations of treatment strategies
All FH+: All FH+ relatives from genetic screening are treated
FH+ with elevated cholesterol levels: only FH+ relatives with high cholesterol levels are treated;
FH+ as in cholesterol consensus: only FH+ relatives with cholesterol levels that fulfil the treatment criteria in the national Dutch Institute of Healthcare Improvement consensus guideline on hypercholesterolaemia are treated
Untreated FH+: only FH+ relatives who were not on lipid-lowering medication at screening are treated **Untreated FH+ with elevated cholesterol level:** only FH+ relatives with high cholesterol levels who were not on lipid-lowering medication at screening are treated
Untreated FH+ as in cholesterol consensus: only untreated FH+ relatives with cholesterol levels that fulfil the treatment criteria in the national Dutch Institute of Healthcare Improvement consensus guideline on hypercholesterolaemia are treated

Marang-van de Mheen et al. (2002)

Table 11: Difference in cost-effectiveness between treatment strategies in the case of alternative screening (lipid profile screening)

Treatment strategy	N treated	Total costs(€)	Years of life gained	Costs per year of life gained(€)
All with elevated cholesterol level	658	25 030 932	836	29 957
All as in cholesterol consensus	406	12 368 034	507	24 376
Untreated with elevated cholesterol level	489	19 038 280	623	30 558
Untreated as in cholesterol consensus	263	8 555 188	337	25 360

Explanations of treatment strategies
All with elevated cholesterol level: All individuals with elevated cholesterol levels above the 95th percentile of the general Dutch population
All as in cholesterol consensus: All individuals who fulfil the treatment criteria in the national Dutch Institute of Healthcare Improvement consensus guideline
Untreated with elevated cholesterol level: See “All with elevated cholesterol level”, but only if untreated at screening
Untreated as in cholesterol consensus: See “All as in cholesterol consensus”, but only if untreated at screening

Marang-van de Mheen et al. (2002)

As summarised in Table 9 and Table 10, the cost effectiveness of genetic screening in the Netherlands (€25,500 to €32,000; depending on treatment strategy) is similar to that of lipid profile screening (€24,000 to €31,000; depending on treatment strategy). It is important to note that the screening costs did not contribute much to the total cost in both screening scenarios (genetic vs. alternative); instead it was the cost of follow-up, or more specifically the cost of cholesterol-lowering drugs, that accounted for 80% of total cost. Marang-van de Mheen and colleagues concluded that the cost effectiveness of family based screening for FH exceeds the cut-off point of €18,151 per life year gained as set by the Dutch cholesterol consensus guideline and therefore warrants substantial political consideration (Marang-van de Mheen et al. 2002).

A more recent Dutch study by Wonderling (2004) disagrees with the findings of Marang-van de Mheen et al. (2002). The model that Wonderling utilised shows that there are no health benefits from treating patients beyond the age of 60 years, while Marang-van de Mheen et al. (2002) assumed that treatment costs and effects would continue up to the age of 85 years. Wonderling (2004) assumed more conservative conditions in the calculations, in relation to mortality rates in FH patients, including discounting conventions, inclusion of children in the cohort, reduced drug compliance, and prescription data from clinical practice. Wonderling (2004) stated that the number of life years gained on average (male and female) for new untreated FH cases was 3.3 years; or 0.9 years when discounted at 4%. The incremental cost of the genetic screening program includes the cost of screening plus the cost of drugs that would not have been prescribed in the absence of the program minus the cost saving associated with having fewer coronary events to treat. The calculations revealed that screening cost per new untreated case diagnosed was US$1,768; small compared to the expected drug costs of US$6790.

The cost per life-year gained was US$2,200 (life expectancy undiscounted) or US$8,800 per life-year gained (discounting life expectancy at 4% per year). It should be noted that this estimate of cost-effectiveness assumes that those individuals newly diagnosed with FH but already on medication would not have any health gain as a result of the genetic screening program (also assuming they would bot have any additional drug costs). However, evidence from the screening program (Umans-Eckenhausen et al. 2001) revealed that most of these patients are undertreated. Therefore assuming that these patients had 50% of the total health gain and 50% of the incremental drug costs; the cost per life-year gained would be reduced to US$2,000 (life expectancy undiscounted) or to US$8,000 when discounting at 4%. Therefore in contrast to Marang-van de Mheen et al. (2002), Wonderling (2004) concluded that the results obtained represent good value for money and therefore genetic screening for FH in the Netherlands is highly cost-effective.

Overall, the cost-effectiveness of cascade screening utilising genetic confirmation techniques appear to be at least comparable to cascade screening utilising conventional clinical criteria (Marks et al. 2000, Marks et al. 2002, Marang-van de Mheen et al. 2002, Wonderling 2004). However, there is some contention as to whether cascade genetic screening is truly good value for money, at least in the Netherlands (Marang-van de Mheen et al. 2002, Wonderling et al. 2004). Further analyses would have to be conducted to address this contention. In addition, studies in the Australian and New Zealand context are required for an informed conclusion as to whether this screening strategy utilising genetic diagnosis is feasible in this region. In addition, several generic versions of statins are now available and should substantially improve cost effectiveness.

Ethical Considerations

Genetic testing raises numerous ethical issues which are normally beyond the scope of a horizon scanning report. Several key ethical issues relating to genetic screening for FH will be discussed briefly in this section.

Informed Consent

Although there is no national genetic screening program for FH in Australia and New Zealand, there is some effort in identifying FH individuals via clinical diagnosis and family history via the MEDPED program. The extent of the application of genetic diagnosis for FH in both Australia and New Zealand is unknown. Nevertheless, suspected FH patients must be informed by clinicians that genetic screening for FH is entirely voluntary. Clinicians should provide adequate information to patients and highlight the fact that FH has been extensively studies and there are effective treatment options that can significantly reduce the risk of coronary heart disease. However, it is also important to counsel FH negative patients that a negative genetic diagnosis does not preclude them from developing coronary heart disease in the future from non LDLR/apoB genetic mutation causes. In addition to this, clinicians should highlight that genetic testing is currently not capable of detecting mutations in 100% of clinically diagnosed FH subjects. Therefore, a LDLR/apoB mutation negative diagnosis does not necessarily mean that the individual will not developed symptoms of FH due to polygenic mutations.

Privacy and discrimination issues

As with other medical conditions, the diagnosis of FH may lead to difficulties in obtaining life insurance due to the higher risk of fatal coronary heart disease. In the UK, discrimination as a result of genetic tests has been ruled out for policies under £100,000. In one UK study, the investigators utilised a fictional FH patient to determine insurance premiums before and after effective lipid-lowering treatment and found that insurance companies increased premiums based on the phenotype and not the genotype. No insurance company refused cover, but premiums were 150% higher; when the condition was managed effectively premiums decreased to and average of 56% higher (Neil et al. 2004).

Access Issues

If a national genetic screening program is initiated in Australia or New Zealand, suspected FH subjects from rural areas would have blood samples taken by their local general practitioners/nurses and sent to an accredited laboratory for genetic analysis. Both the Dutch and Norwegian screening programs are centralised, this ensures that there is no geographic variation in the genetic testing service and that all family information is registered centrally. It is likely that a similar approach will be adopted by Australia and New Zealand (if a national program is initiated) as it is a proven working model.

Training and Accreditation

Training

The genetic diagnosis of FH requires trained molecular pathologists who are familiar with the techniques utilised to identify mutations within the LDLR and apoB genes; such as SSCP, DHPLC, cDNA sequencing, PCR, RT-PCR and others.

Clinical Guidelines

Guidelines for the diagnosis and management of FH are available from the Cardiac Society of Australia and New Zealand. These guidelines have been developed and co-ordinated by members of the Cardiovascular Genetics Diseases Working Group and are endorsed by the National Heart Foundation of Australia (Sullivan 2007).

Diagnosis

The diagnosis of an index case is dependent on recognition of the relevant clinical features. However, clinicians must be aware of the fact that approximately 15% of patients are misdiagnosed if elevated LDL-C levels is utilised as the main method of diagnosis. The guidelines recommended the use of the Dutch Lipid Clinic Network criteria and the modified Simon Broome criteria to assist in the diagnosis of FH. Although the clinical diagnosis of FH is likely to be straightforward in most cases, the guidelines acknowledges that genetic testing can provide certainty of diagnosis in cases with confounding factors such as borderline cholesterol levels, inconclusive family histories or tendon injuries. The process of detecting a genetic abnormality within the FH disease genes may be considered expensive and time-consuming. However, new techniques are improving the yield and FH appears to be one of the first conditions where a diagnostic chip may be developed (Sullivan 2007).

Treatment

The guideline recommends regular review of clinical symptoms and signs of cardiovascular disease. Exercise stress testing is recommended in adults and other forms of non-invasive testing to assist the early identification of clinically relevant atherosclerosis may become accepted practice. Patients must be made aware that proper diet, exercise and avoidance of smoking are mandatory and all cardiovascular risk factors should be evaluated and treated. In addition, clinicians should consider general measures to protect against vascular events, including the use of aspirin.

Cholesterol-lowering treatments such as statins provide good control of inherited high cholesterol levels. Statins reduces the production of cholesterol by cells, therefore stimulating the expression of the LDLR gene. The receptors produced by the normal gene will reduce LDL-C levels in the bloodstream. The effect of

statins can be enhanced with the use of bile acid sequestrants or cholesterol absorption inhibitors such as plant sterols or ezetimibe.

Statin treatment of FH males is one of the most cost-effective medical interventions currently available and research has shown major improvements in cardiovascular disease event rates and total mortality of FH patients. However, the management of young FH patients is still developing; diet and abstinence to smoking are safe and effective measures while statins are only considered in children of the most severe FH families (Sullivan 2007).

Asymptomatic family members

The guidelines reiterate that with the high rate of morbidity and mortality associated with the onset of coronary heart disease, it is inappropriate to wait for the onset of clinical symptoms before treated asymptomatic family members who may have inherited the condition. Although family follow-up (cascade testing) has been shown to be cost-effective for case detection, it is often overlooked for a variety of reasons. Even in the absence of legal and privacy issues, the resources required to identify and inform family members are beyond the scope of the primary care physician. The MEDPED program can assist in contacting and supporting family members of index cases and may be accessed at http://www.athero.org.au/MEDPED/index.htm.

Limitations of the Assessment

Methodological issues and the relevance or currency of information provided over time are paramount in any assessment carried out in the early life of a technology.

Horizon Scanning forms an integral component of Health Technology Assessment. However, it is a specialised and quite distinct activity conducted for an entirely different purpose. The rapid evolution of technological advances can in some cases overtake the speed at which trials or other reviews are conducted. In many cases, by the time a study or review has been completed, the technology may have evolved to a higher level leaving the technology under investigation obsolete and replaced.

A Horizon Scanning Report maintains a predictive or speculative focus, often based on low level evidence, and is aimed at informing policy and decision makers. It is not a definitive assessment of the safety, effectiveness, ethical considerations and cost effectiveness of a technology.

In the context of a rapidly evolving technology, an Horizon Scanning Report is a ‘state of play’ assessment that presents a trade-off between the value of early, uncertain information, versus the value of certain, but late information that may be of limited relevance to policy and decision makers.

This report provides an assessment of the current state of development of genetic screening for familial hypercholesterolaemia, its present and potential use in the Australian public health system, and future implications for the use of this technology.

Search Strategy used for the Report

The sources utilised in this assessment are listed in Table11. The medical literature was searched with the search terms outlined in Table 12 to identify relevant studies up to April 2007 in English only. In addition to this, major international health technology assessment databases and clinical trial registers were searched.

Table 12: Literature sources utilised in assessment

Source	**Location**
Electronic databases	
AustHealth	University of Adelaide library
Australian Medical Index	University of Adelaide library
CINAHL	University of Adelaide library
Cochrane Library – including Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects, the Cochrane Central Register of Controlled Trials (CENTRAL), the Health Technology Assessment Databese, the NHS Economic Evaluation Database	University of Adelaide library
Current Contents	University of Adelaide library
Embase	Personal subscription
Pre-Medline and Medline	University of Adelaide library
PyscINFO	Personal subscription
RACS electronic library	Personal subscription
Internet	
Blue Cross and Blue Shield Association's Technology Evaluation Center	http://www.bcbs.com/tec/
Canadian Agency for Drugs and Technologies in Health	http://www.cadth.ca
Current Controlled Trials metaRegister	http://www.controlled-trials.com/

EuroScan	http://www.euroscan.bham.ac.uk/
Health Technology Assessment International	http://www.htai.org/
International Network for agencies for Health Technology Assessment	http://www.inahta.org
Medicines and Healthcare products Regulatory Agency (UK)	http://www.mhra.gov.uk/
US Food and Drug Administration, Center for Devices and Radiological Health	http://www.fda.gov/cdrh/index.html
US Food and Drug Administration, Manufacturer and User Facility Device Experience Database	http://www.fda.gov/cdrh/maude.html
UK National Research Register	http://www.nrr.nhs.uk/
Websites of specialty organisations	Dependent on technology assessed

Table 13: Search terms utilised

Search terms
MeSH Genetic Screening*; Hypercholesterolemia/blood; Hypercholesterolemia/diagnosis*; Hypercholesterolemia/genetics*; Receptors, LDL/genetics*; Genetic Screening/economics*; Mutation. **Text words** Familial hypercholesterolemia; genetic screen*; cascade screen*; DNA test*; molecular diagnos*; clinical diagnos* **Limits** English, human

Availability and Level of Evidence

One randomised controlled trial (Level II screening evidence), five comparative studies (four level III-2 diagnosis evidence, one level III-3 screening evidence) and ten peer reviewed case series studies (six level IV screening evidence, four level IV diagnosis evidence) reported outcomes on the use of genetic screening for familial hypercholesterolaemia. In addition, three cost-effectiveness studies were retrieved for assessment in this report. The profiles of the included case series studies are summarised in Appendix B.

The medical literature (Table 12) was searched utilising the search terms outlined in Table 13 to identify relevant studies and reviews, until April 2007. In addition, major international health assessment databases were searched.

Sources of Further Information

The following is a list of studies relevant to genetic screening of FH which are currently underway (UK National Research Register). Note that this list is not comprehensive and studies on molecular diagnosis methods for FH as well as identification of new mutations causing FH are not included in this list.

Communicating genetic information within families

This study intends to examine how genetic test information received in the clinic is communicated through the family. A total of 60 adults with genetically diagnosed FH will be recruited from the Lipid Clinic at the Royal Free and University College Medical School. The projected end date is 28 Feb 2009. (Publication ID: N0652174800).

DNA testing from Familial Hypercholesterolaemia (FH): clinical and psychological outcomes

To compare the number of affected relatives found by cascade testing from index patients where specific DNA mutations were identified to relatives of FH patients without detectable DNA mutation. The projected completion date is 28 Feb 2009. (Publication ID: N0256183897).

The monitoring of familial hypercholesteroaemia patients attending lipid clinics, and their families, including putting their details and results onto a computerised database

This study aims to determine how many relatives are contracted for proband (by the proband) in the year following entry onto the database, and how many of these relative are mutation carriers with increased cholesterol levels. Expected completion date: 1 October 2008 (Publication ID: N0263132906).

Using genotype to predict coronary heart disease: assessing impact upon medication adherence

The study objective is to describe the impact upon adherence to lipid lowering medication of using genotype to assess risk of heart disease in relatives of patients with FH. Expected completion date: 31 December 2008 (Publication ID: N0201182082).

A comparison of DNA testing for Familial Hypercolesterolemia (FH) with traditional diagnostic methods; implications for cascade testing

This study aims to compare the number of affected relatives found by cascade testing from index patients where a specific DNA mutation has been found and where no mutation has been found. Expected completion date: 31 July 2008 (Publication ID: N0012165136).

Conclusions

FH is a condition where affected individuals are characterised with chronic elevated serum cholesterol and LDL-C levels. It is one of the most common genetic disorders, affecting 1 in 500 people worldwide. The actual prevalence rate in Australia and New Zealand is unknown but is likely to correspond to worldwide estimates. Consequent to the high concentrations of LDL-C and cholesterol within the bloodstream of affected individuals, FH is associated with early onset coronary heart disease. It is therefore important to diagnose patients before their first coronary event. Despite the fact that FH can be diagnosed early, research has shown that only approximately 20% of FH affected individuals have been diagnosed and only 7% are adequately treated (Williams et al. 1996). These findings are extremely worrying considering the fact that 50% of heterozygous FH males will develop coronary artery disease before the age of 50, and this increases to 100% by the age of 70. Meanwhile, 12% of untreated female FH patients will suffer from coronary heart disease by the age of 50, and this subsequently increases to 74% by age 70 (Centre for Genetics Education 2005).

Conventional clinical diagnosis of FH involves the measurement of total serum cholesterol levels and LDL-C levels, determining the presence of tendinous xanthomas and study of family history. However, studies have shown that although the presence of elevated total cholesterol and LDL-C levels is usually indicative of FH, biochemical measurement of lipids is not a reliable diagnosis technique as there are several inherent flaws that plague this method. One pertinent flaw with this technique is that the range of blood cholesterol levels and LDL-C levels in FH patients overlaps with that of normal individuals, therefore there is potential for substantial misdiagnosis. Early studies conducted on children have shown misdiagnosis rates ranging from 4.5% to18.9% when utilising total cholesterol or LDL-C cutoff points (Leonard et al. 1977, Kwiterovich et al. 1974) while other studies have reported that some FH-positive patients may not have sufficiently elevated levels of cholesterol for clinical diagnosis. In addition, tendinous xanthomas are not always prevalent in FH patients therefore limiting its applicability as a diagnostic criterion. Compounded by the fact that tendinous xanthomas are rarely present until the fourth decade of life, it is not helpful in diagnosing children (Austin et al. 2004a). Overall, there are no clear definitions as to what the best clinical diagnosis criteria are and what cholesterol/LDL-C cutoff points should be utilised to ensure accurate diagnosis of FH.

Genetic testing has been advocated as the only unequivocal diagnosis method for FH that is capable of addressing all the limitations of clinical diagnosis. This is due to the fact that it is not dependent on biochemical measurements and clinical symptoms, but is instead dependant on the molecular identification of mutations within the LDLR or apoB gene. However, considering the fact that there are over 850 LDLR mutations for FH (University College London 2007), screening for each and every one of these mutations to identify an index case (for cascade screening) may be considered time consuming and expensive. Therefore it is

common to scan for several mutations that account for the majority of FH mutations within a population, complete sequencing techniques are only utilised when mutations were not detected from initial genetic testing. However, one of the concerns of utilising molecular genetic testing is that the mutation detection rate can vary significantly. Studies have reported that the percentage of clinically diagnosed FH patients in which a mutation is detected varies from 20% in 'possible' and 'probable' FH to 60% to 80% in 'definite' FH patients (Sozen et al. 2004, Hadfield and Humphries 2005). To date, no molecular technique has been able to detect a mutation in 100% of clinically diagnosed FH patients. It remains unclear as to what extent the low mutation detection rates are caused by the inadequacies of molecular testing, incorrect clinical diagnosis or the existence of polygenic mutations that result in the FH phenotype. So it appears to be advisable that the identification of index cases should involve clinical diagnosis followed by genetic testing to determine the presence of a mutation.

Both the Netherlands and Norway have established national genetic cascade screening programs for FH and have reported significant success. Umans-Eckenhausen et al. (2001) reported that at 1-year post-screening (Netherlands), 93% of patients identified with FH via genetic screening had visited a physician and were placed on lipid-lowering medication, and 86% of patients remained on lipid-lowering medication at 2-years post-screening (Umans-Eckenhausen et al. 2003). In a subgroup of patients with pre-screening cholesterol measurements, the investigators highlighted that a mean 30.2% reduction in LDL-C level was achieved at 2-years post-screening, with 66% of patients achieving the treatment target levels of 135 mg/dl (3.5mmol/l). Meanwhile, Leren et al. (2004) reported similar outcomes for the Norwegian screening program; total serum cholesterol and LDL-C decreased by 9.6% ($p<0.0001$) and 14.7% ($p<0.0001$) respectively at 6 months post-screening. Overall, 24.1% of adult relatives in the Norwegian program achieved LDL-C levels of less than 135 mg/dl (3.5mmol/l) at 6-months.

Although various papers have been published on the negative psychological and social effect on genetic screening, no quantitative evidence has been presented to support this notion for FH screening. The randomised trial by Marteau et al. (2004) did not find any indication that patients genetically diagnosed with FH have a higher incidence of fatalism or a feeling of decreased control over their condition compared to patients clinically diagnosed with FH. Dutch studies have revealed that genetic screening for FH was highly acceptable within the Dutch population (only 2% did not choose to participate and only 2% would not recommend the screening program to others) (van Maarle et al. 2001) and there appears to be no significantly negative effects towards the patient's quality of life (van Marle et al. 2003). Meanwhile, children who were found to be genetically positive for FH did not exhibit any decline in their psychological state compared to those without FH (Leren 2004).

Cost-effectiveness studies of cascade screening utilising genetic confirmation techniques have shown that the cost per life-year gained is comparable to cascade

screening utilising conventional clinical diagnosis techniques (Marks et al. 2000, Marks et al. 2002, Marang-van de Mheen et al. 2002, Wonderling 2004). However, Marang van de Mheen et al. (2002) stated that the cost per life-year gained for cascade genetic screening (€25,500 to €32,000) exceeds the cutoff point of €18,151 per life year gained as set by the Dutch cholesterol consensus guideline. In contrast, Wonderling (2004) utilised a different model and stated that the cost per life-year gained for genetic cascade screening in the Netherlands was US$8,800; substantially lower than the estimate provided by Marang-van de Mheen et al. (2002). However, none of these models are entirely appropriate for the Australia/New Zealand context. A model is needed that takes account of the recently published clinical guidelines for the management FH and which uses the costs appropriate for Australia/New Zealand and recognising the cost reductions possible with generic statins.

In conclusion, genetic screening for FH appears to be effective in increasing the proportion of FH patients receiving adequate medical treatment; therefore resulting in significant reductions of LDL-C and cholesterol levels which should translate to lower incidences of coronary heart disease (Umans-Eckenhausen et al. 2001, Umans-Eckenhausen et al. 2003, Leren et al. 2004). However, the ability of current genetic testing techniques to identify mutations within the LDLR gene is somewhat of a concern (20% to 80% mutation detection rate in clinically diagnosed FH patients), especially if they are utilised to identify index cases. Perhaps clinical diagnosis should be utilised first to identify potential index cases; followed by genetic testing to identify the exact mutation causing the FH phenotype. When genetic testing is utilised for cascade screening of relatives, the results are highly encouraging (Umans-Eckenhausen et al. 2003, Leren et al. 2004) and the health benefits are indisputable (~20% reduction of LDL-C levels). Nevertheless, the issue of whether genetically positive individuals with *low* cholesterol levels should be treated with statins remains unclear. In the context of the Australian and New Zealand healthcare system, the fact that there is no national screening program for FH is disconcerting considering the fact that FH is relatively common and can be effectively treated when early diagnosis is made. Regardless of whether clinical diagnosis methods or molecular genetic methods are utilised, some form of national screening initiative would be beneficial in both countries as there is now a national guideline for management of the condition (Sullivan 2007).

Appendix A: Levels of Evidence

Designation of levels of evidence according to type of research question

Level	Intervention [§]	Diagnosis [**]	Prognosis	Aetiology [†††]	Screening
I [*]	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies
II	A randomised controlled trial	A study of test accuracy with: an independent, blinded comparison with a valid reference standard, [§§] among consecutive patients with a defined clinical presentation [††]	A prospective cohort study [***]	A prospective cohort study	A randomised controlled trial
III-1	A pseudorandomised controlled trial (i.e. alternate allocation or some other method)	A study of test accuracy with: an independent, blinded comparison with a valid reference standard, [§§] among non-consecutive patients with a defined clinical presentation[††]	All or none [§§§]	All or none [§§§]	A pseudorandomised controlled trial (i.e. alternate allocation or some other method)
III-2	A comparative study with concurrent controls: Non-randomised, experimental trial [†] Cohort study Case-control study Interrupted time series with a control group	A comparison with reference standard that does not meet the criteria required for Level II and III-1 evidence	Analysis of prognostic factors amongst untreated control patients in a randomised controlled trial	A retrospective cohort study	A comparative study with concurrent controls: Non-randomised, experimental trial Cohort study Case-control study
III-3	A comparative study without concurrent controls: Historical control study Two or more single arm study [‡] Interrupted time series without a parallel control group	Diagnostic case-control study [††]	A retrospective cohort study	A case-control study	A comparative study without concurrent controls: Historical control study Two or more single arm study
IV	Case series with either post-test or pre-test/post-test outcomes	Study of diagnostic yield (no reference standard) [‡‡]	Case series, or cohort study of patients at different stages of disease	A cross-sectional study	Case series

Tablenotes

[*] A systematic review will only be assigned a level of evidence as high as the studies it contains, excepting where those studies are of level II evidence.

[§] Definitions of these study designs are provided on pages 7-8 *How to use the evidence: assessment and application of scientific evidence* (NHMRC 2000b).

[†] This also includes controlled before-and-after (pre-test/post-test) studies, as well as indirect comparisons (ie. utilise A vs B and B vs C, to determine A vs C).

[‡] Comparing single arm studies ie. case series from two studies.

[**] The dimensions of evidence apply only to studies of diagnostic accuracy. To assess the effectiveness of a diagnostic test there also needs to be a consideration of the impact of the test on patient management and health outcomes. See *MSAC (2004) Guidelines for the assessment of diagnostic technologies*. Available at: www.msac.gov.au .

[§§] The validity of the reference standard should be determined in the context of the disease under review. Criteria for determining the validity of the reference standard should be pre-specified. This can include the choice of the reference standard(s) and its timing in relation to the index test. The validity of the reference standard can be determined through quality appraisal of the study. See Whiting P, Rutjes AWS, Reitsma JB, Bossuyt PMM, Kleijnen J. The development of QADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Medical Research Methodology*, 2003, 3: 25.

[††] Well-designed population based case-control studies (eg population based screening studies where test accuracy is assessed on all cases, with a random sample of controls) do capture a population with a representative spectrum of disease and thus fulfil the requirements for a valid assembly of patients. These types of studies should be considered as Level II evidence. However, in some cases the population assembled is not representative of the use of the test in practice. In diagnostic case-control studies a selected sample of patients already known to have the disease are compared with a separate group of normal/healthy people known to be free of the disease. In this situation patients with borderline or mild expressions of the disease, and conditions mimicking the disease are excluded, which can lead to exaggeration of both sensitivity and specificity. This is called spectrum bias because the spectrum of study participants will not be representative of patients seen in practice.

[‡‡] Studies of diagnostic yield provide the yield of diseased patients, as determined by an index test, without confirmation of accuracy by a reference standard. These may be the only alternative when there is no reliable reference standard.

[***] At study inception the cohort is either non-diseased or all at the same stage of the disease.

[§§§] All or none of the people with the risk factor(s) experience the outcome. For example, no smallpox develops in the absence of the specific virus; and clear proof of the causal link has come from the disappearance of small pox after large-scale vaccination.

[†††] If it is possible and/or ethical to determine a causal relationship using experimental evidence, then the 'Intervention' hierarchy of evidence should be utilised. If it is only possible and/or ethical to determine a causal relationship using observational evidence (ie. cannot allocate groups to a potential harmful exposure, such as nuclear radiation), then the 'Aetiology' hierarchy of evidence should be utilised.

Note 1: Assessment of comparative harms/safety should occur according to the hierarchy presented for each of the research questions, with the proviso that this assessment occurs within the context of the topic being assessed. Some harms are rare and cannot feasibly be captured within randomised controlled trials; physical harms and psychological harms may need to be addressed by different study designs; harms from diagnostic testing include the likelihood of false positive and false negative results; harms from screening include the likelihood of false alarm and false reassurance results.

Note 2: When a level of evidence is attributed in the text of a document, it should also be framed according to its corresponding research question eg. level II intervention evidence; level IV diagnostic evidence; level III-2 prognostic evidence etc.

Hierarchies adapted and modified from: NHMRC 1999; Lijmer et al 1999; Phillips et al 2001; Bandolier editorial 1999)

Study	Location	Study design	Study population	Study details	Outcomes assessed
Damgaard D, Larsen ML, Nissen PH, Jensen JM, Jensen HK, Soerensen VR, Jensen LG, Faergeman O. (2005)	Denmark	Level III-2 diagnosis evidence	408 patients referred to the Lipid Clinic	Clinical diagnosis: using MEDPED criteria, Simon Broome Register criteria, Dutch Lipid Clinic Network criteria. Genetic diagnosis: Initial screening for 3 most common mutations followed by SCCP analysis.	Ability of the three clinical diagnosis criteria to predict the results of genetic diagnosis (mutation detection rates).
Fouchier SW, Defesche JC, Umans-Eckenhausen MAW, Kastelein JJP. (2001)	Amsterdam, The Netherlands	Level IV screening evidence	1641 clinically diagnosed FH patients	Genetic analysis: DGGE, sequencing of PCR fragments, detection of large deletions or insertions (through long-segment PCR and Southern Blotting) for mutation detected to characterise LDLR gene defects within the population.	Various LDLR mutation frequencies in specific parts of the Netherlands. Proportion of patients without detectable mutations.
Graham CA, McClean E, Ward AJM, Beattie ED, Martin S, O'Kaine M, Young IS, Nicholls DP. (1999)	Belfast, United Kingdom	Level III-2 diagnosis evidence	158 families with clinical definitions of possible (120) or definite (38) FH.	Genetic diagnosis: DGGE and fluorescent sequencing for mutation detection. Clinical diagnosis: Cholesterol and triglyceride levels measured. Presence of tendinous xanthomas.	Accurate diagnosis of FH with genetic/clinical diagnosis. Genotype and phenotype correlation.
Khoo KL, van Acker P, Defesche JC, Tan H, van de Kerkhof L, Heijnen-van Eijk SJ, Kastelein JJP, Deslypere JP. (2000)	Amsterdam, The Netherlands.	Level IV diagnosis evidence	86 unrelated patients of South East Asian origin clinically diagnosed with FH	Genetic diagnosis: DGGE and gene sequencing (LDLR and apolipoprotein B) Clinical diagnosis: Total cholesterol, HDL-cholesterol, and triglyceride levels.	Types of mutations within LDLR and apoB genes. Correlation between type of mutation and lipoprotein levels and clinical signs or atherosclerosis.
Koivisto PVI, Koivisto UM, Miettinen TA,	Helsinki, Finland	Level III-2 diagnosis evidence	65 subjects (10 propositi and 55 first-degree	Clinical diagnosis: serum LDL cholesterol concentration,	Correlation between clinical and molecular genetic diagnosis.

Kontula K. (1992)			relatives) from 10 families with FH.	presence of xanthomas. Genetic diagnosis: presence of FH-Helsinki mutation (of LDLR gene), apolipoprotein B mutation determined using DGGE.	Impact of genetic variation of apolipoproteins B and E on LDL cholesterol levels. Relationship or any discrepancy between clinical and molecular genetic diagnosis.
Kwiterovich PO, Fredrickson DS, Levy RI. (1974)	Maryland, United States	Level IV diagnosis evidence	134 children with one first-degree relative with FH	Clinical diagnosis: cholesterol and LDL-C levels	Distributions of cholesterol and LDL-C levels
Leonard JV, Whitelaw AGL, Wolff OH, Lloyd JK, Slack J. (1977)	London, United Kingdom	Level IV diagnostic evidence	134 children aged 1 – 16 years with at least one first degree relative with presumed FH	Blood samples collected from children after an overnight fast whenever possible. Serum cholesterol measured by semi-automated ferric chloride technique.	Serum cholesterol concentration.
Leren TP, Manshaus T, Skovholt U, Skodje T, Nossen IE, Teie C, Sorensen S, Bakken KS. (2004)	Oslo, Norway	Level IV screening evidence	851 first degree relatives of index cases from 188 unrelated families	Genetic screening: testing for 12 most common mutations, testing for 19 most common mutations, DNA sequencing of one strand, DNA sequencing of both strands.	Proportion of patients on lipid-lowering medication at 6-months. Total cholesterol and LDL-C levels at 6-months. Percentage of patients achieving target treatment LDL-C level.
Marks D, Wonderling D, Thorogood M, Lambert H, Humphries S, Neil HAW. (2000)	London, United Kingdom	Level III-3 screening evidence	Review of published studies on genetic screening for FH	Systematic review and cost effectiveness analysis of population screening and cascade screening.	Suitability of FH screening Cost effectiveness of different screening systems. psychological effects
Marteau T, Senior V, Humphries SE, Bobrow M, Cranston T, Crook MA, Day L, Fernandez M, Horne R, Iversen A, Jackson Z, Lynas J, Middleton-Price H, Savine R, Sikorski J, Watson M,	London, United Kingdom	Level II screening evidence	341 families (341 index cases, 128 relatives)	Patients randomised to (a) clinical diagnosis or (b) clinical diagnosis + genetic testing.	Perception of control over hypercholesterolemia. Adherence to cholesterol-lowering medication, diet, physical activity, and smoking.

Weinman J, Weirzbicki AS, Wray R. (2004)					
Sozen M, Whittall R, Humphries SE. (2004)	London, United Kingdom	Level IV diagnosis evidence	Patients with FH	Clinical diagnosis: Simon Broome designation 96 well capillary machine was used to develop a single strand confirmation polymorphism and heteroduplex method for mutation detection. Technique was applied to 101 known mutations of LDLR gene.	Mutation detection in the LDLR gene
Umans-Eckenhausen MAW, Defesche JC, Sijbrands EJG, Scheerder RLJM, Kastelein JJP. (2001)	Amsterdam, The Netherlands	Level IV screening evidence	5442 relatives of 237 FH index cases	Analysis of patient with clinical FH for LDLR mutations (index case identification). Genetic diagnosis of first-degree relatives.	Proportion of patients on lipid lowering medication after 1 year. Mean reduction of cholesterol level after 1 year.
Umans-Eckenhausen MAW, Defesche JC, van Dam MJ, Kastelein JJP. (2003)	Amsterdam, The Netherlands	Level IV screening evidence	747 patients with genetically diagnosed FH	Questionnaire sent to patients 2-years post-screening.	Extent of lipid lowering medication compliance following diagnosis. Mean reduction in LDL-C levels. Proportion of patients achieving treatment target LDL-C levels.
van Aalst Cohen ES, Jansen ACM, Tanck MWT, Defesche JC, Trip MD, Lansberg PJ, Stalenhoef AFH, Kastelein JJP. (2006)	Amsterdam, The Netherlands.	Level III-2 diagnosis study.	4000 hypercholesterolemic patients from the Dutch Lipid Clinic network database.	Comparison of clinical characteristics of patients with and without detectable LDLR mutations Clinical diagnosis: using MEDPED criteria, Simon Broome Register criteria, Dutch Lipid Clinic Network criteria. Genetic diagnosis: PCR analysis, DGGE, long range PCR, Southern Blotting and DNA sequencing for 14 common LDLR mutations in the	Laboratory parameters (including total cholesterol, LDL-C, HDL-C, triglycerides and glucose) of patients with and without LDLR mutation.

				Netherlands.	
van Maarle, Stouthard MEA, Bonsel GJ. (2003)	Amsterdam, The Netherlands.	Level IV screening study.	677 patients who participated in genetic screening.	Quality of life questionnaire to determine any differences between FH negative or FH positive subjects.	Quality of life scores (SF-36, EuroQol, HADS).
van Maarle, Stouthard MEA, Marang-van de Mheen, Klazinga NS, Bonsel GJ. (2001)	Amsterdam, The Netherlands.	Level IV screening study.	1434 relatives of index patients.	Questionnaire to assess psychological impact of screening.	Qualitative judgement on screening program. Quality of life scores (HADS, EuroQol).

Appendix C: HTA Internet Sites

AUSTRALIA

- Centre for Clinical Effectiveness, Monash University
  http://www.med.monash.edu.au/healthservices/cce/evidence/

- Health Economics Unit, Monash University
  http://chpe.buseco.monash.edu.au

AUSTRIA

- Institute of Technology Assessment / HTA unit
  http://www.oeaw.ac.at/ita/welcome.htm

CANADA

- Agence d'Evaluation des Technologies et des Modes d'Intervention en Santé (AETMIS) http://www.aetmis.gouv.qc.ca/en/

- Alberta Heritage Foundation for Medical Research (AHFMR)
  http://www.ahfmr.ab.ca/publications.html

- Canadian Coordinating Office for Health Technology Assessment (CCOHTA)
  http://www.cadth.ca/index.php/en/

- Canadian Health Economics Research Association (CHERA/ACRES) – Cabot database http://www.mycabot.ca

- Centre for Health Economics and Policy Analysis (CHEPA), McMaster University http://www.chepa.org

- Centre for Health Services and Policy Research (CHSPR), University of British Columbia http://www.chspr.ubc.ca

- Health Utilities Index (HUI) http://www.fhs.mcmaster.ca/hug/index.htm

- Institute for Clinical and Evaluative Studies (ICES) http://www.ices.on.ca

DENMARK

- Danish Institute for Health Technology Assessment (DIHTA) http://www.dihta.dk/publikationer/index_uk.asp

- Danish Institute for Health Services Research (DSI) http://www.dsi.dk/engelsk.html

FINLAND

- Finnish Office for Health Technology Assessment (FINOHTA) http://finohta.stakes.fi/FI/index.htm

FRANCE

- L'Agence Nationale d'Accréditation et d'Evaluation en Santé (ANAES)

  http://www.anaes.fr/

GERMANY

- German Institute for Medical Documentation and Information (DIMDI) / HTA

  http://www.dimdi.de/dynamic/en/

THE NETHERLANDS

- Health Council of the Netherlands Gezondheidsraad
  http://www.gr.nl/adviezen.php

NEW ZEALAND

- New Zealand Health Technology Assessment (NZHTA)
  http://nzhta.chmeds.ac.nz/

NORWAY

- Norwegian Centre for Health Technology Assessment (SMM)

  http://www.kunnskapssenteret.no/

SPAIN

- Agencia de Evaluación de Tecnologias Sanitarias, Instituto de Salud "Carlos III" / Health Technology Assessment Agency (AETS)

  http://www.isciii.es/htdocs/investigacion/Agencia_quees.jsp

- Catalan Agency for Health Technology Assessment (CAHTA)
  http://www.aatrm.net/html/en/dir394/index.html

SWEDEN

- Swedish Council on Technology Assessment in Health Care (SBU)

  http://www.sbu.se/www/index.asp

- Center for Medical Health Technology Assessment

  http://www.cmt.liu.se/

SWITZERLAND

- Swiss Network on Health Technology Assessment (SNHTA)

  http://www.snhta.ch/

UNITED KINGDOM

- NHS Quality Improvement Scotland

  http://www.nhshealthquality.org

- National Health Service Health Technology Assessment (UK) / National Coordinating Centre for health Technology Assessment (NCCHTA)

  http://www.hta.nhsweb.nhs.uk/

- University of York NHS Centre for Reviews and Dissemination (NHS CRD)

  http://www.your.ac.uk/inst/crd/

- National Institute for Clinical Excellence (NICE)

  http://www.nice.org.uk/

UNITED STATES

- Agency for Healthcare Research and Quality (AHRQ)

  http://www.ahrq.gov/clinic/techix.htm

- Harvard School of Public Health – Cost-Utility Analysis Registry

  http://www.tufts-nemc.org/cearegistry/index.html

- U.S. Blue Cross / Blue Shield Association Technology Evaluation Center (TEC)

  http://www.bcbs.com/tec/index.html

Appendix D: Glossary

Allele: One of the variant forms of a gene at a particular locus, or location, on a chromosome. Different alleles produce variation in inherited characteristics such as hair colour or blood type. In an individual, one form of the allele (the dominant one) may be expressed more than another form (the recessive one).

Autosome: A chromosome not involved in sex determination. The diploid human genome consists of 46 chromosomes, 22 pairs of autosomes, and 1 pair of sex chromosomes (the X and Y chromosomes).

Codominant: A situation where both alleles affect the phenotype of a heterozygous genotype, and neither is dominant over the other.

Cytosine: A DNA nucleotide that which is one member of the base pair in DNA consisting of guanine and cytosine.

Exon: The region of a gene that contains the code for producing the gene's protein. Each exon codes for a specific portion of the complete protein. In some species (including humans), a gene's exons are separated by long regions of DNA (called introns or sometimes "junk DNA") that have no apparent function.

Genetic polymorphism: The occurrence together in the same population of more than one allele or genetic marker at the same locus with the least frequent allele or marker occurring more frequently than can be accounted for by mutation alone.

Genotype: The specific allelic composition of a cell, either of the entire cell or more commonly for a certain gene or a set of genes. The genes that an organism possesses.

Guanine: One of the four bases in DNA/RNA, Guanine always pairs with cytosine to form the base pair G-C (guanine-cytosine).

Heteroduplex: A duplex molecule of nucleic acid whose strands are derived from different sources, such as from different homologous chromosomes or even from different organisms.

Heterozygote: Possessing two different forms of a particular gene, one inherited from each parent.

Homozygote: Possessing two identical forms of a particular gene, one inherited from each parent.

Homoduplex: DNA composed of two strands which are complementary in sequence.

Index case: See proband.

Kilobases: Unit of length of DNA equal to 1000 nucleotide bases.

Ligand: A molecule that binds to another; often a soluble molecule such as a hormone or neurotransmitter that binds to a receptor.

Locus: The place on a chromosome where a specific gene is located, a kind of address for the gene. The plural is "loci".

PCR: Polymerase chain reaction (PCR): A method for amplifying a DNA base sequence using a heat- stable polymerase and two 20- base primers, one complementary to the (+)- strand at one end of the sequence to be amplified and the other complementary to the (-)- strand at the other end. Because the newly synthesized DNA strands can subsequently serve as additional templates for the same primer sequences, successive rounds of primer annealing, strand elongation, and dissociation produce rapid and highly specific amplification of the desired sequence. PCR also can be used to detect the existence of the defined sequence in a DNA sample.

Phenotype:
(1) The form taken by some character (or group of characters) in a specific individual.
(2) The detectable outward manifestations of a specific genotype.
(3) The observable attributes of an organism.

Polymorphism: The occurrence in a population (or among populations) of several phenotypic forms associated with alleles of one gene or homologs of one chromosome. See genetic polymorphism.

Primer: A nucleic acid strand (or related molecule) that serves as a starting point for DNA replication. A primer is required because most DNA polymerases (enzymes that catalyse the replication of DNA) cannot begin synthesizing a new DNA strand from scratch, but can only add to an existing strand of nucleotides.

Proband: The family member through whom a family's medical history comes to light. The proband may also be called the index case, propositus (if male), or proposita (if female).

RNA: Ribonucleic acid, a chemical similar to a single strand of DNA. In RNA, the letter U, which stands for uracil, is substituted for T (thymine) in the genetic code. RNA delivers DNA's genetic message to the cytoplasm of a cell where proteins are made.

RT-PCR: RT-PCR (Reverse transcriptase-polymerase chain reaction) is a highly sensitive technique for the detection and quantitation of mRNA (messenger

RNA). The technique consists of two parts: 1) The synthesis of cDNA (complementary DNA) from RNA by reverse transcription (RT) and 2) The amplification of a specific cDNA by the polymerase chain reaction (PCR).

Single nucleotide polymorphisms (SNPs): is a DNA sequence variation occurring when a single nucleotide - A, T, C, or G - in the genome differs between members of a species (or between paired chromosomes in an individual). For example, two sequenced DNA fragments from different individuals, AAGC**C**TA to AAGC**T**TA, contain a difference in a single nucleotide. In this case we say that there are two alleles: C and T.

Wild-type: Refers to 'normal' DNA, not affected by mutations.

Sources for definitions:
http://www.genome.gov/glossary.cfm
http://www.medterms.com
http://linkage.rockefeller.edu/wli/glossary/genetics.html

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