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Horizon Scanning Technology Prioritising Summary Update

Lipochip® for the genetic diagnosis of familial hypercholesterolaemia

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AHTA

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PRIORITISING SUMMARY: UPDATE 2010

REGISTER ID: **000419**

NAME OF TECHNOLOGY: **LIPOCHIP®**

PURPOSE AND TARGET GROUP: **GENETIC DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLAEMIA**

2010 SAFETY AND EFFECTIVENESS ISSUES:

A single arm study (n=808) analysed DNA samples of Spanish patients with clinically diagnosed familial hypercholesterolaemia (FH) using the LIPOchip® genetic diagnostic platform (level III-1 diagnostic evidence). The main aims of this study were to assess effectiveness, sensitivity, specificity and costs of identifying patients with FH using the LIPOchip® three-tiered system.[1] Further testing by DNA sequencing and MLPA[2] analysis at two separate laboratories confirmed the primary results. Diagnosis of FH is usually made on the basis of clinical symptoms. However, these physical signs typically develop late in life, making detection in younger patients difficult without genetic tools such as LIPOchip®.[3] The 808 subjects recruited for testing were unrelated FH cases diagnosed by Dutch Lipid Clinic Network criteria.[4] Using these criteria, all patients scored at least six points, making FH diagnosis probable or certain. After three-tiered analysis was complete, mutations were found in 537 patients (66%), of which 521 were mutations in the low-density lipoprotein receptor (LDLR) gene and 16 were mutations in the apolipoprotein B (ApoB) gene. DNA microarray detected mutations in 419 patients (52% of total patients and 78% of mutation carriers), representing 403 in the LDLR gene and 16 in the ApoB gene. Large deletions or insertions were found in 77 of the remaining 389 negative cases (10% of total cases and 15% of all mutation carriers). Complete sequencing of LDLR was performed for the remaining 312 cases, with 41 found to have one of 18 mutations newly detected by resequencing (5% of all patients analysed and 8% of all mutation carriers (Figure 1)[5].

[1] DNA microarray, large genetic rearrangement analysis (deletions and insertions), and automated resequencing of the LDLR or ApoB genes. The DNA-array is capable of detecting 191 mutations in LDLR and four in ApoB.
[2] Multiplex ligation-dependent probe amplification.
[3] Early detection is desirable since treatment with cholesterol-lowering statins provides best outcomes for patients who commence therapy before progression to symptomatic disease.
[4] Physical symptoms including xanthomata, and family history, cardiovascular disease and LDL-cholesterol.
[5] QMFSP = quantitative multiplex PCR methodology, used in the large genetic rearrangement analysis.

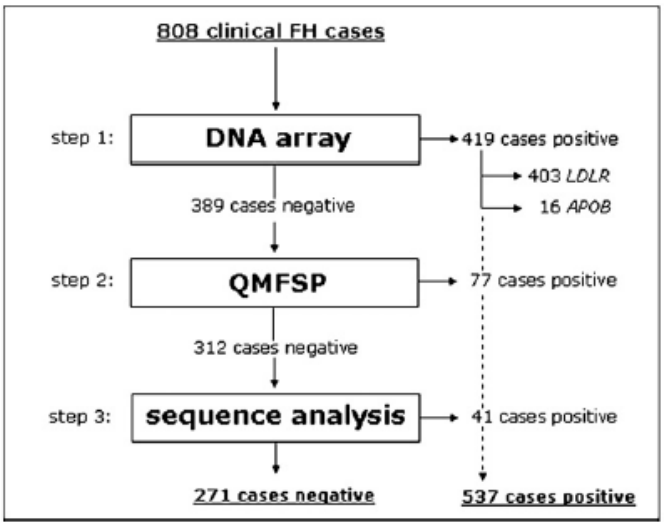

Figure 1 Summary of results from the three step LIPOchip® platform (Alonso et al 2009).

Average time to complete the LIPOchip® platform was 68 days (range = 10–93 days). DNA microarray with extraction and analysis took an average of 15 days, large rearrangement detection extended the process seven days, and sequencing added another 45 days. However, most mutations were detected in 15 to 22 days after commencement of analysis. Sensitivity and specificity of the LIPOchip® was 99.8 and 99.5 per cent, respectively, with only one false negative and one false positive reported. Other genetic techniques developed over the past two decades, such as single-strand conformation polymorphism analysis, have a sensitivity of only 75 to 85 per cent by comparison. All results were substantiated independently in two separate laboratories with blind analysis of Dutch and Spanish samples, again with only one false positive and one false negative outcome. The diagnostic evidence in this study suggests LIPOchip® is an appropriate system given the low incidence of errors associated with testing. Sensitivity and specificity rates approaching 100 per cent indicate LIPOchip® can certify a previous clinical diagnosis of FH. Unequivocal determination of FH in a patient may provide a basis for cascade screening which targets first degree relatives of known FH sufferers (Leren et al 2008). Such a strategy has been found to be cost-effective in the United Kingdom and the Netherlands (Wonderling et al 2004; Marks et al 2003; Marks et al 2000). Total cost of FH diagnosis using LIPOchip® was reported to be US $350 per person (Alonso et al 2009).

Diagnostic ability of MLPA analysis[6] was investigated in a British study of 611 patients referred from lipid clinics with suspected FH. MLPA and QMFSP,[7] both multiplex amplification techniques used for large genetic rearrangement analysis, are generic methods analogous to the second tier in the LIPOchip® platform. The 611 patients were all tested using an ARMS[8] assay, an approach which targets the most

[6] The method of analysis used for independent and blinded verification of results in Alonso et al 2009.
[7] Used in the original LIPOchip® protocol by Alonso et al 2009.
[8] Amplification refractory mutation system.

common point mutations in the screening population, but has limited utility for populations in which significant genetic heterogeneity exists. (Laurie & George 2009) ARMS cannot detect large gene rearrangements (deletions or duplications), but these are thought to comprise only five per cent of LDLR mutations in the United Kingdom, making ARMS a more appropriate initial test than MLPA. ARMS detected point mutations in 234 (38.3%) patients. Of the 377 patients in whom no mutations were found using ARMS, subsequent analysis with MLPA identified 19 patients with large LDLR rearrangements, equating to 7.5 per cent of the 253 mutations detected in total. All patients were also categorised as definite FH or possible FH status on the basis of standardised clinical criteria. Prevalence of detectable rearrangements among patients with definite and possible FH was 7.5 and 4.5 per cent, respectively, but this difference was not significant (p=0.32). The authors reported that the MLPA process takes approximately three days with consumable costs of £25. These findings of a protocol utilising methods similar to those found in the LIPOchip® platform further support the use of genetic testing to confirm the clinical diagnosis of FH (Taylor et al 2009) (level IV diagnostic evidence).

A New Zealand study investigated the ability of HRM analysis,[9] a genetic method distinct from LIPOchip®, to detect mutations in the LDLR gene. HRM analysis uses slow heating of amplicons[10] and a saturating double-stranded DNA binding dye to generate a measurable fluorescence signal. Laurie and George (2009) analysed 60 DNA samples from individuals previously identified as heterozygous for mutations by DHPLC[11] and DNA sequencing. Melting curves of amplicons from these samples were then compared to curves resulting from a normal homozygous DNA samples. In samples heterozygous for mismatch mutation, heteroduplex[12] amplicons melt at lower temperatures than homoduplex[13] DNA found in homozygous samples, producing measurable differences in melting curves.[14] Of the 54 sequence variants finally examined, 52 (96%) were detected by HRM analysis,[15] a rate comparable to DHPLC used in the initial analysis. Accordingly, HRM analysis does not approach so close to 100 per cent sensitivity as LIPOchip®. However, mutation scanning is a compromise between the speed and cost of analysis, and sensitivity. The ability to melt a plate of 96 samples in 10 minutes makes HRM 100 times faster than DHPLC (Laurie & George 2009) (level III-2 diagnostic evidence).

[9] High-resolution melting analysis.
[10] DNA pieces formed by natural or, as in this case, artificial amplification processes such as the polymerase chain reaction (PCR).
[11] Denaturing high performance liquid chromatography.
[12] Double-stranded DNA in which strands originate from different parent molecules.
[13] Converse to heteroduplex DNA, the strands originate from the same parent molecule.
[14] The binding affinity between strands of DNA is proportional to sequence divergence. Since heteroduplex strands have greater sequence divergence than homoduplex strands, binding affinity and melting point are lower.
[15] Six exon 4 mutations did not provide usable melting data, possibly due to the high guanine-cytosine content of the exon 4b amplicon.

OTHER ISSUES

Results of a case-series (level IV prognostic evidence) have suggested a role for LIPOchip® beyond diagnosis of FH. This study recruited 436 FH patients and 268 healthy subjects to investigate possible correlation between LDLR defects and intima media thickness[16] of the common carotid artery, an established surrogate maker for cardiovascular disease. LDLR defects in known FH patients were classified by severity into two main groups using standard criteria following LIPOchip® analysis. Measures of carotid artery thickness were then taken for patients and healthy controls. Carotid artery thickness was compared between patients and controls, and between patients displaying different severities of LDLR mutation. Significant differences were found in both comparisons. Investigation of whether LIPOchip® could determine elevated risk of cardiovascular disease among clinically identical patients, thereby targeting individuals in need of more intensive pharmacotherapy for FH, is warranted (Junyent et al 2010).

2010 SUMMARY OF FINDINGS

Studies to date continue to show that LIPOchip® is an efficient tool in the diagnosis of FH among high risk populations, certainly favourable above clinical diagnosis alone. More studies comparing different genetic methods for FH determination may aid clinicians to make informed decisions about which tests provide the most accurate and efficient results. Long term outcomes have not been investigated; however, LIPOchip® has performed as an inexpensive technology in the detection of FH.

2010 HEALTHPACT ACTION:

There is an obvious clinical need for the accurate and rapid diagnosis of familial hypercholesterolaemia, which the LIPOchip® technology appears to satisfy. Clinicians may choose to adopt this maturing technology as a first choice diagnostic tool in place of the more labour intensive DNA sequencing and therefore no further review on behalf of HealthPACT is required. HealthPACT has recommended that this summary be disseminated to the jurisdictions and to the Human Genetics Advisory group.

2010 LIST OF STUDIES INCLUDED

Total number of studies	4
Level III-1 diagnostic evidence	1
Level III-2 diagnostic evidence	1
Level IV diagnostic evidence	1
Level IV prognostic evidence	1

[16] Thickened intima media of arterial blood vessels is a hallmark of atherosclerotic plaque deposits resulting from high levels of circulating blood cholesterol.

2010 REFERENCES:

Alonso, R., Defesche, J. C. et al (2009). 'Genetic diagnosis of familial hypercholesterolemia using a DNA-array based platform', *Clin Biochem,* 42 (9), 899-903.

Junyent, M., Gilabert, R. et al (2010). 'Impact of low-density lipoprotein receptor mutational class on carotid atherosclerosis in patients with familial hypercholesterolemia', *Atherosclerosis,* 208 (2), 437-441.

Laurie, A. D. & George, P. M. (2009). 'Evaluation of high-resolution melting analysis for screening the LDL receptor gene', *Clin Biochem,* 42 (6), 528-535.

Leren, T. P., Finborud, T. H. et al (2008). 'Diagnosis of familial hypercholesterolemia in general practice using clinical diagnostic criteria or genetic testing as part of cascade genetic screening', *Community Genet,* 11 (1), 26-35.

Marks, D., Thorogood, M. et al (2003). 'Comparing costs and benefits over a 10 year period of strategies for familial hypercholesterolaemia screening', *J Public Health Med,* 25 (1), 47-52.

Marks, D., Wonderling, D. et al (2000). 'Screening for hypercholesterolaemia versus case finding for familial hypercholesterolaemia: a systematic review and cost-effectiveness analysis', *Health Technol Assess,* 4 (29), 1-123.

Taylor, A., Martin, B. et al (2009). 'Multiplex ligation-dependent probe amplification analysis to screen for deletions and duplications of the LDLR gene in patients with familial hypercholesterolaemia', *Clin Genet,* 76 (1), 69-75.

Wonderling, D., Umans-Eckenhausen, M. A. et al (2004). 'Cost-effectiveness analysis of the genetic screening program for familial hypercholesterolemia in The Netherlands', *Semin Vasc Med,* 4 (1), 97-104.

PRIORITISING SUMMARY (2009)

REGISTER ID: **000419**

NAME OF TECHNOLOGY: **LIPOCHIP**

PURPOSE AND TARGET GROUP: **GENETIC DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLAEMIA**

STAGE OF DEVELOPMENT (IN AUSTRALIA):

☒	Yet to emerge	☐	Established
☐	Experimental	☐	Established *but* changed indication or modification of technique
☐	Investigational	☐	Should be taken out of use
☐	Nearly established		

AUSTRALIAN THERAPEUTIC GOODS ADMINISTRATION APPROVAL

☐	Yes	ARTG number
☒	No	
☐	Not applicable	

INTERNATIONAL UTILISATION:

COUNTRY	LEVEL OF USE		
	Trials Underway or Completed	Limited Use	Widely Diffused
Spain			✓

2009 IMPACT SUMMARY:

Progenika-Biopharma markets the LIPOchip platform, which is designed to diagnose familial hypercholesterolaemia (FH). FH can result in premature death and is often undiagnosed until a coronary event occurs. The LIPOchip platform is a tiered system with a microarray based first tier coupled with genetic sequencing. This system is aimed at providing a definitive diagnosis (genotype) which is not possible using standard diagnosis based on clinical symptoms (phenotype). FH if diagnosed early can be treated with existing therapies.

2009 BACKGROUND

Familial hypercholesterolaemia is a common but under-diagnosed disorder that results in an almost 100-fold increased risk of coronary artery disease (CAD) (Bates et al 2008). FH follows an autosomal dominant pattern of inheritance, with offspring having a 50 per cent chance to inherit the disorder. Homozygotes usually have a more severe phenotype than heterozygotes, but symptom severity also results from the specific mutation(s) in each subject (Emery et al 2007).

FH is a genetic disorder resulting mainly from mutations in the low-density lipoprotein receptor (LDLR) gene that encodes the LDL receptor protein, or the apolipoprotein B (ApoB) gene. Other mutations in different genes are known but are very rare. Although over 1,000 mutations have been identified, many are rare or family specific. FH is rarely diagnosed until a coronary event occurs in a proband,which may result in other family members being screened for their FH status. The clinical symptoms of FH manifest due to the impaired uptake of cholesterol, specifically LDL, resulting in high circulating cholesterol levels and a subsequent increased risk of cardiovascular disease. Standard diagnosis of FH is performed using family history and blood lipid examination. This results in a *phenotypic* diagnosis of the patient which, due to other causes of hypercholesterolaemia, may not correlate with their genetic FH status.

The LIPOchip platform is based on a tiered mutation identification algorithm. Initially the patient is assessed using a microarray based mutation detection system. The microarray is designed to detect 203 mutations in the LDLR gene and four mutations in the ApoB gene. The subject's genetic material is extracted from a blood sample and assessed with a microarray. If the patient has any of the 207 mutations the subject is deemed FH positive. If the subject is negative their sample is subjected to a second tier of large genetic rearrangement analysis[17]. If this tier is also negative then a third tier consisting of sequencing of the LDLR gene to assess the subject for novel mutations. If this step is also negative then the subject is deemed FH negative.

2009 Clinical Need and Burden of Disease

FH is a cause of premature cardiac death, with most cases not diagnosed until the primary cardiac event. It is estimated that 5-10 per cent of CAD before the age of 55 results from FH. It is thought that there may be 40,000 FH cases in Australia, with only 20 per cent correctly diagnosed and less than 10 per cent are treated with existing therapy. The prevalence of FH in the general population is thought to be 1 in 500, with sub-populations of certain ancestries having a higher prevalence, up to 1 in 70 for those of Afrikaaner origins (Emery et al 2007).

2009 Diffusion

The LIPOchip is not in use within Australia at the time of writing.

2009 Comparators

The standard diagnostic method for FH is based on clinical testing and family history. The criteria for the standard diagnosis of FH are:[18]:

[17] This analysis will detect if the subject's genetic material has undergone any large scale rearrangements or deletions. This type of mutation would be expected to produce a negative result in the first tier as the subject's target sequence is missing or significantly damaged.

[18] Adapted from Emery, J., Barlow-Stewart, K. et al (2007). 'Genetics and preventive health care', *Aust Fam Physician*, 36 (10), 808-811.

a. Known FH DNA mutation;
b. Tendon xanthomas in patient or first/second degree relative;
c. Family history CHD <50 years of age in second degree relative or <60 years of age in first degree relative;
d. Family history of cholesterol >7.5 mmol/L in first or second degree relative;
e. Cholesterol >7.5 mmol/L (adult) or >6.7 mmol/L (age <16 years); and
f. LDL-C >4.9 mmol/L (adult) or >4.0 mmol/L (age <16 years).

Definite FH: (e or f) + a
Probable FH: (e or f) + b
Possible FH: (e or f) + (c +d)

It is recommended that when a FH proband is diagnosed that cascade screening of relatives occurs as there is a 50 per cent chance of first degree relations being FH positive(Emery et al 2007).

2009 Safety and Effectiveness Issues

Several studies have used the LIPOchip to diagnose FH affected individuals. The LIPOchip has several versions with the array being updated yearly to be able to detect newly discovered mutations, and as such only the most recent studies are included.

A study using the LIPOchip investigated 825 consecutive subjects attending three Spanish lipid clinics (level IV diagnostic evidence). Subjects were suspected of having FH due to: very high familial total or LDL cholesterol levels; possible family history of premature CAD; and possible tendon xanthomas[19]. The version of the LIPOchip used screened for 203 mutations in the LDLR gene and four in ApoB. Subjects who had negative results were subsequently assessed for large genetic rearrangement analysis. If they were negative for this also, then genetic sequencing of the promoter, 18 exons and flanking introns was performed to uncover new mutations. Overall 459 subjects were found to have FH-causing mutations. Those who were positive for mutations were found to be more likely to have a family history of tendon xanthomas (OR 7.779 (95% CI [3.639–16.712])), more likely to have tendon xanthomas (OR 3.675 (95 % CI [1.583–8.528]), and more likely to be female (OR 1.966 (95 % CI [1.059–3.649])). No problems were reported with using the LIPOchip (Civeira et al 2008b).

A second study by the same researchers investigated the overlap of FH with familial combined hyperlipidaemia (FCH)[20]. The population consisted of 143 FCH patients

[19] Subcutaneous depositions of fat associated with tendons, especially the Achilles tendon, or those of the hands and feet. Caused by severe hypercholesterolaemia.
[20] The diagnostic criteria for FCH are equivocal and overlap with those of FH, and include hyperlipidaemia and hypercholesterolaemia. FCH is linked with obesity and diabetes, which is not necessarily true for the general FH positive population. The authors therefore aimed to definitively

from two Spanish lipid clinics. These subjects could be classified as FH clinically and thus were investigated using the definitive genetic analysis provided by the LIPOchip. Mutations in the LDLR gene were discovered in 28 (19.6 %) of FCH subjects. This indicated that there is some diagnostic overlap of these two disease types and may explain why some FH patients present with high triglycerides (Civeira et al 2008a) (level IV diagnostic evidence).

As the identification of genetic mutations is the definitive diagnostic method for FH the LIPOchip studies presented here were unable to be compared against a suitable control. The clinical diagnosis of FH is certainly less accurate than genetic methods. Because the LIPOchip was not able to be compared to a gold standard it is not possible to comment on the effectiveness of the system, that is the FH cases missed due to rare or undetected mutations. As the system now stands it seems to be a useful tool for the definitive diagnosis of FH subjects in very high risk populations. Larger and longitudinal studies are required to assess the effectiveness of the LIPOchip.

2009 COST IMPACT

The manufacturer was contacted for pricing details but at the time of publication there was no response.

ETHICAL, CULTURAL OR RELIGIOUS CONSIDERATIONS

No issues were identified/raised in the sources examined.

OTHER ISSUES

According to the Progenika-Biopharma website a clinical trial is planned in the USA.

2009 SUMMARY OF FINDINGS

The LIPOchip seems to function well in populations at high risk of FH, although longer term outcomes of patients diagnosed by the LIPOchip are not available as yet. As no better standard exists than genetic diagnosis, the level of misdiagnoses is not apparent. Comparisons with other genetic methods for diagnosis of FH are needed.

2009 HEALTHPACT ACTION:

A number of diagnostic genetic tests for familial hypercholesteraemia are likely to come on to the market in the near future. HealthPACT recommends that all genetic tests for FH, including the LIPOchip, be monitored for further information in 12-months time.

NUMBER OF INCLUDED STUDIES

Total number of studies

Level IV Diagnostic evidence 2

diagnose subjects who may be clinically diagnosed as FH positive but in fact be FH negative genetically and therefore more likely to misclassified FCH positive subjects instead.

2009 REFERENCES:

Bates, T. R., Burnett, J. R. et al (2008). 'Detection of familial hypercholesterolaemia: a major treatment gap in preventative cardiology', *Heart Lung Circ,* 17 (5), 411-413.

Civeira, F., Jarauta, E. et al (2008a). 'Frequency of low-density lipoprotein receptor gene mutations in patients with a clinical diagnosis of familial combined hyperlipidemia in a clinical setting', *J Am Coll Cardiol,* 52 (19), 1546-1553.

Civeira, F., Ros, E. et al (2008b). 'Comparison of genetic versus clinical diagnosis in familial hypercholesterolemia', *Am J Cardiol,* 102 (9), 1187-1193, 1193 e1181.

Emery, J., Barlow-Stewart, K. et al (2007). 'Genetics and preventive health care', *Aust Fam Physician,* 36 (10), 808-811.

SEARCH CRITERIA TO BE USED:

DNA Mutational Analysis

Receptors, LDL/ blood

Cholesterol, LDL/ blood

Receptors, Lipoprotein/ blood