Atherosclerosis 240 (2015) 190–196

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Clinical experience of scoring criteria for Familial Hypercholesterolaemia (FH) genetic testing in Wales

K. Haralambos [a], S.D. Whatley [b], R. Edwards [b], R. Gingell [b], D. Townsend [b], P. Ashfield-Watt [a], P. Lansberg [c], D.B.N. Datta [b], I.F.W. McDowell [a, *]

[a] Wales Heart Research Institute, Cardiff University School of Medicine, University Hospital of Wales, Cardiff CF14 4XN, UK
[b] Wales FH Service c/o Cardiff and Vale University Health Board, UK
[c] CTMM-TraIT Center for Translational Molecular Medicine, High Tech Campus 84, 5656 AG Eindhoven, The Netherlands

ARTICLE INFO

Article history:
Received 28 October 2014
Received in revised form
15 February 2015
Accepted 5 March 2015
Available online 6 March 2015

Keywords:
Familial Hypercholesterolaemia
Genetics
Cholesterol
Diagnosis
Criteria

ABSTRACT

Background/Objective: Familial Hypercholesterolaemia (FH) is caused by mutations in genes of the Low Density Lipoprotein (LDL) receptor pathway. A definitive diagnosis of FH can be made by the demonstration of a pathogenic mutation. The Wales FH service has developed scoring criteria to guide selection of patients for DNA testing, for those referred to clinics with hypercholesterolaemia. The criteria are based on a modification of the Dutch Lipid Clinic scoring criteria and utilise a combination of lipid values, physical signs, personal and family history of premature cardiovascular disease. They are intended to provide clinical guidance and enable resources to be targeted in a cost effective manner.
Methods: 623 patients who presented to lipid clinics across Wales had DNA testing following application of these criteria.
Results: The proportion of patients with a pathogenic mutation ranged from 4% in those scoring 5 or less up to 85% in those scoring 15 or more. LDL-cholesterol was the strongest discriminatory factor. Scores gained from physical signs, family history, coronary heart disease, and triglycerides also showed a gradient in mutation pick-up rate according to the score.
Conclusion: These criteria provide a useful tool to guide selection of patients for DNA testing when applied by health professionals who have clinical experience of FH.

1. Introduction

Familial Hypercholesterolaemia (FH) is a monogenic disorder characterised by increased levels of circulating plasma low-density lipoprotein cholesterol (LDL-C). FH is caused by mutations in the genes affecting the metabolic clearance of LDL-C, most commonly the Low-density Lipoprotein Receptor gene (*LDLR*) but also Apolipoprotein B gene (*APOB*) and Proprotein Convertase Subtilisin/Kexin Type 9 (*PCSK9*).

The widely quoted population frequency of heterozygous FH is 1 in 500 [1] in northern Europe, though more recent studies in The Netherlands [2,3] and in Denmark [4] have suggested that it is more common with estimated frequencies of 1/300, 1/244 and 1/200 respectively.

Untreated FH carries a risk of premature coronary heart disease (CHD) of >50% in men and >30% in women by the age of 60 years [5]. Effective treatment is available to reduce the risk of CHD in FH.

The Wales FH cascade service was launched in December 2010 with the implementation of a diagnostic service for FH, combined with family cascade testing [6] and is now an established clinical service.

Cascade testing is the process of systematically identifying family members of an affected individual (index patient) and then offering testing for the condition. The most efficient method of cascade testing for FH is to offer genotyping to relatives of index patients with an identified mutation [7,8]. This is better than using family cholesterol testing alone, which is non-specific [6,8,9].

The clinical diagnosis of FH in an individual is often not clear cut. Tendon xanthomata (TX) are widely regarded as a specific physical sign, but are often absent and may be difficult to assess, and all the other clinical features of FH are non-specific. A DNA diagnosis provides clarity and can help with an individual's understanding

* Corresponding author.
E-mail address: ian.mcdowell@wales.nhs.uk (I.F.W. McDowell).

http://dx.doi.org/10.1016/j.atherosclerosis.2015.03.003
0021-9150/

and acceptance of their condition [10]. A DNA diagnosis is very helpful for family testing, but is relatively expensive.

It is important to be able to select those patients with hypercholesterolaemia who are most likely to have FH genetic mutations, in order to improve the clinical and cost-effectiveness of genetic cascade testing services for FH. Several sets of criteria have been used to identify patients with FH. The three main criteria are the Simon Broome Criteria [11], the Dutch Lipid Clinic Network Criteria (DLCNC) [12,13], and the US MedPed criteria [14]. Mutation detection rates, sensitivity and specificity vary between the criteria used and how strictly the criteria are applied [15–19].

We have developed an FH scoring system based on a modification of the Dutch Lipid Clinic scoring criteria to guide genetic testing of index patients presenting with hypercholesterolaemia. A numeric score is derived from measures of: family history, physical examination, cardiovascular clinical history, LDL-C and triglyceride concentrations.

We report our experience of applying these criteria in clinical practice as part of the Wales FH service.

2. Methods

2.1. Development of scoring criteria (Table 1)

These criteria have been adapted from the Dutch Lipid Clinic criteria [9,10], with 5 main modifications. The key differences between these criteria and the Dutch criteria are as follows:

1. In the Welsh criteria, patients score 6 if they or their 1st or 2nd degree relatives have TX. With the Dutch criteria, patients scored 6 if they themselves had tendon xanthomata (TX), but scored only 2 if only their 1st or 2nd degree relatives had TX.
2. A minus score is given for raised triglycerides. This is not a feature of the Dutch criteria, however, their criteria have a caveat saying 'HDL-cholesterol and triglycerides are normal'.
3. A weighting has been applied for the development of CHD at a younger age in the index patient (CHD <45 yrs 4, CHD <50yrs 3, CHD <60yrs 2).
4. Family history of CHD has been weighted to a greater degree. In the Dutch criteria, one point was awarded for CHD in a 1st degree relative under the age of 60. This criterion was modified so that a patient scores one point if their 1st or 2nd degree relative had CHD before the age of 60, and two points if their 1st or 2nd degree relative had CHD before the age of 45.
5. An LDL-C correction factor table used to estimate the pre-treated LDL-C levels of patients on cholesterol lowering medication has been updated to include all available statins with or without ezetimibe. The correction factors were developed from the analysis of 71 original papers that were collated prior to setting up these criteria. See Appendix.

2.1.1. Patients

The study group consisted of 1206 consecutive index patients who had been referred to lipid clinics in Wales over the course of 24 months (between November 2010 and November 2012). The study group does not include relatives of index patients detected by cascade testing. These criteria are only intended for index patients with hypercholesterolaemia. Secondary causes of hypercholesterolaemia were excluded by testing for liver, renal, thyroid function and diabetes. All patients were assessed by one of three specialist FH nurses using the Welsh criteria.

As a starting point, all patients with a score of 6 or greater were offered genetic testing. If a patient scored 5 or less they would not be routinely offered genetic testing, but only if their lipid clinicians (FH nurse and/or consultant lipidologist) considered that there were particular circumstances that might make FH more likely in that patient. Such circumstances might include a family history of early CHD in the absence of other risk factors such as smoking, lack of available history from senior family members (e.g. no contact or adopted), or presence of other non-scoring features such as xanthelasmata or equivocal thickening of Achilles tendons. All such requests for genotyping with a score of 5 or less were vetted by a senior medical advisor to the FH service (DBND or IMcD) before genotyping was performed.

2.1.2. DNA analysis

Samples were analysed in three different genetic laboratories

Table 1
Wales FH service genotype scoring criteria.

Family history	1st/2nd degree relative: • known with premature (<60yrs) CHD • known with premature (<45yrs) CHD • known with LDL-C > 4.9 mmol/l (or total chol > 7.5 mmol/l) • <18 yrs with LDL-C > 4.0 mmol/l (or total chol > 6.7 mmol/l) Please specify relation to index case	 1 2 1 2
Physical examination	• Tendon xanthomata (in patient or 1st/2nd degree relative) • Premature corneal arcus (no score for arcus senilis)	6 4
Clinical history	• Patient with premature CHD (<45 yrs) • Patient with premature CHD (<50 yrs) • Patient with premature CHD (<60 yrs) • Patient with premature (<60yrs) strokes and/or peripheral vascular disease	4 3 2 1
Untreated or corrected LDL- Cholesterol Concentrations (mmol/l)	• LDL-C ≥ 8.5 • LDL-C 6.5–8.4 • LDL-C 5.0–6.4 • LDL-C 4.0–4.9 If untreated LDL- C values are unobtainable see attached sheet (Correction Factor Table) and calculate estimated value.	8 5 3 1
Fasting triglycerides (mmol/l)	• Triglyceride 2.5–3.4 • Triglyceride 3.5–4.9 • Triglyceride ≥ 5.0 Please record in the narrative box any 2° causes that predispose to raised triglycerides, e.g. diabetes.	Minus 2 Minus 3 Minus 4

The highest score is circled from each section and the overall score is obtained by totalling all scores together. If the score is 6 or greater, the patient would be offered genetic testing for FH. If the score was below 6, the criteria form would need to be approved by the FH medical advisor before the patient was offered testing.

over the course of this project [Belfast City Hospital, Progenika Biopharma (Spain) or Bristol Genetics Laboratory] before repatriating the laboratory testing to University Hospital of Wales. The NHS service selected referral laboratories on the basis of service offered and cost. All laboratories provided a high quality genetic testing service for the full *LDLR* gene including copy number variations that detected deletions/duplications and the common mutations in *PCSK9* and *APOB*.

If a DNA variant was found in one of the FH genes this was classified using the 5 class system recommended by the Association for Clinical Genetic Science Guidelines [20]. The classes are as follows: Class 5 is 'clearly pathogenic', Class 4 is 'likely to be pathogenic', Class 3 is 'variant of uncertain significance', Class 2 is 'unlikely to be pathogenic', and Class 1 is 'clearly not pathogenic'.

In this report the term 'mutation' is used to describe class 5 variants only.

2.1.3. Statistics

Statistical analysis was carried out using SPSS version 20. Non-parametric tests (Kruskal–Wallis and Mann Whitney U test) were used to assess the difference in scores between those with different genotype result outcomes (*LDLR vs APOB* vs no variant). The Holm–Bonferroni method [21] was used to adjust for multiple comparisons. Chi square was used to assess the association between categorical variables.

Positive predictive value (PPV) was calculated for different scoring cut-off values using the following formula: PPV True positive/(false positive + true positive) [22]. True positives are defined as those individuals who score above a specified cut-off value and have a pathogenic mutation whereas false positives are those who score above the same cut-off but have no mutation or have a variant classified between 2 and 4 (see Table 3).

Table 2
LDL-cholesterol correction factor table for patients on statins ± ezetimbe medication.

Statin/dose (mg)	Correction factor
Ezetimibe	
10	1.2
Pravastatin	
10	1.2
20	1.3
40	1.5
Pravastatin + Ezetimibe	
10 + 10	1.5
20 + 10	1.6
40 + 10	1.7
Simvastatin	
10	1.4
20	1.6
40	1.7
80	1.9
Simvastatin + Ezetemibe	
10 + 10	1.9
20 + 10	2.0
40 + 10	2.3
80 + 10	2.4
Atorvastatin	
10	1.6
20	1.8
40	2.0
80	2.2
Atorvastatin + Ezetemibe	
10 + 10	2.0
20 + 10	2.2
40 + 10	2.2
80 + 10	2.5
Rosuvastatin	
5	1.8
10	1.9
20	2.1
40	2.4
Rosuvastatin + Ezetimibe	
10 + 10	2.5
20 + 10	2.7
40 + 10	3.3

For a patient on regular medication for whom a pre-treatment LDL-C was not available, an estimate of untreated LDL-C was obtained by multiplying the measured LDL-C by the appropriate factor. The correction factors calculated were based on analysis of 71 original papers that were reviewed prior to setting up these criteria. See Appendix 1 for table of references.

3. Results

3.1. Patient referral numbers (Fig. 1)

Of 1206 individuals referred to the service over a 24 month period, 547 scored 6 or above and were offered genotyping (522 accepted; 30 declined). Of 659 patients who scored 5 or less, 101 were genotyped making a total of 623 patients genotyped.

The LDL-C correction factor for patients already on statins was applied in 164/623 patients.

3.2. DNA diagnosis in relation to criteria score (Fig. 2 and Table 3)

Overall 231 genetic variants (class 2–5) were identified from 623 patients tested (37%). In 173 patients (28%), the variant was classified as class 5 and in 58 (9%) it was classified between 2 and 4, of which 6 patients (1%) had a class 2 variant.

The calculations of positive predictive value give the proportion of patients who had a pathogenic mutation from this group who had DNA testing (i.e. hypercholesterolaemic patients selected from Welsh Lipid clinic referrals with a clinical suspicion of FH). This ranges from 0.32 to those scoring 6 or above up to 0.85 for those scoring 15 or above. There was a clear gradation in mutation detection rates according to the score.

It is not valid to calculate sensitivity and specificity because not all hypercholesterolaemic individuals with scores less than 6 were genotyped so the overall prevalence of pathogenic mutations has not been established for hypercholesterolaemic patients in Wales lipid clinics.

3.3. Discriminatory capacity of different scoring criteria (Table 4)

LDL-cholesterol was the strongest discriminatory factor. Scores gained from physical signs, family history, CHD and triglycerides also showed a gradient in mutation pick-up rate according to the score.

The identification of mutations (class 5) correlates strongly with the score for LDL-C concentration, with rates of 0%, 4%, 9%, 28% & 59% from those scoring LDL points of 0, 1, 3, 5, 8 respectively.

Out of the 623 patients genotyped, 21% (n = 132) had TX and 5% (n = 29) had equivocal xanthomata (usually recorded as 'thickened Achilles'). Out of the group of patients with TX, mutations were identified in 57% (75 out of 132) and in those who were recorded to have equivocal xanthomata, variants were identified in 38% (11 out

Table 3
Positive predictive value.

Genotype score cut-off	≥6	≥7	≥9	≥11	≥13	≥15
PPV	0.32	0.4	0.53	0.66	0.78	0.85

Positive predictive value (PPV) is shown for different genotype scoring cut-off values. This shows the proportion of patients that fulfil the criteria above each of these cut-offs who have a mutation (Class 5 variant).

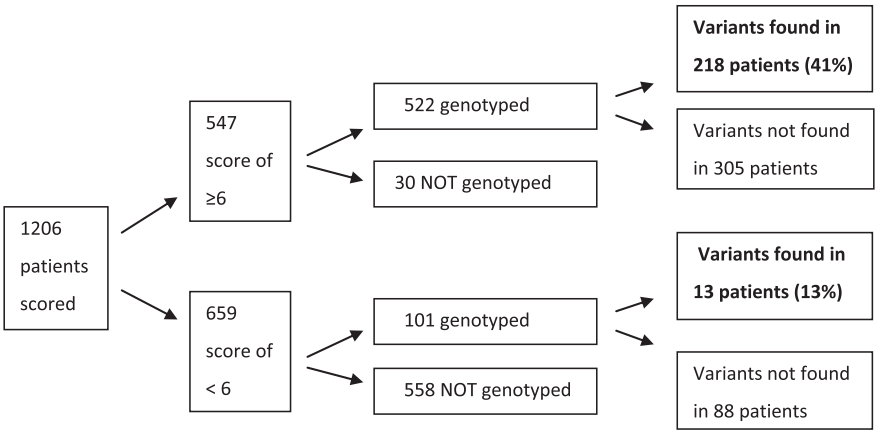

Fig. 1. Patient numbers. All patients were offered testing if score ≥6 but only offered testing if score 5 or less when there were particular clinical circumstances. All DNA variants (classes 2–5) have been included in this diagram.

of 29). Equivocal xanthomata are not scored using these criteria, but if this is written in the 'clinical narrative' section of the genotyping request form, it can be taken into account if the patient scores 5 or less and a decision has to be made as to whether or not they should be tested.

There was a reduced likelihood of detecting a mutation in those tested with elevated triglycerides ($p = 0.035$). Patients with triglycerides ≥2.5 mmol/l (scoring minus 2 or 3) had a mutation pick-up rate of 14% compared to those with normal triglyceride (below 2.5 mmol/l) for whom the proportion was 29%.

This data was also analysed using a single cut off value for raised triglycerides of 3 mmol/l. In this cohort, those who had triglycerides ≥3 mmol/l had a mutation pick up rate of 10% and those who had concentrations below 3 mmol/l had a pick up rate of 29%.

In the Welsh criteria additional weighting was given for very early onset CHD in the patient (CHD <45 yrs 4 points, CHD <50yrs 3 points, CHD <60yrs 2 points), whereas in the Dutch criteria only a score of 2 (CHD: men<55 yrs, women <60 yrs) was attributed. In both sets of criteria, a score of 1 was gained for stroke and/or peripheral vascular disease <60 yrs. In our dataset, using the Welsh criteria, the early age of onset of CVD was significantly associated with the presence of a mutation ($p = 0.02$). In individuals with very early onset CVD (scores of 3 or 4) 37% received a genetic diagnosis compared to only 20% with later onset of CVD (scores of 1 or 2).

In addition to the additional weighting for premature CHD in the patient themselves, the Welsh criteria added the ability to score higher when a 1st or 2nd degree relative had CHD before the age of 45. A score of 2 was attributed compared to a score of 1 on the Dutch criteria Patients who scored 2 in this section had a high mutation pick up rate of 43% (46 out of 106 tested).

3.4. Simon Broome criteria

Prior to genetic testing, 20% of the cohort was classified as 'Simon Broome Definite (SBD)', 65% as 'Simon Broome Possible (SBP)' and 15% did not fulfil the Simon Broome clinical criteria. All SBD patients were tested scoring a minimum 9 and a maximum of 20. However, only some of SBP patients were tested as these patients can score as low as 4 and therefore are not eligible using an eligibility threshold of 6. The mutation pick up rate of SBD patients in this cohort was 60%, for SBP patients it was 21% and for those not meeting SB it was 14%.

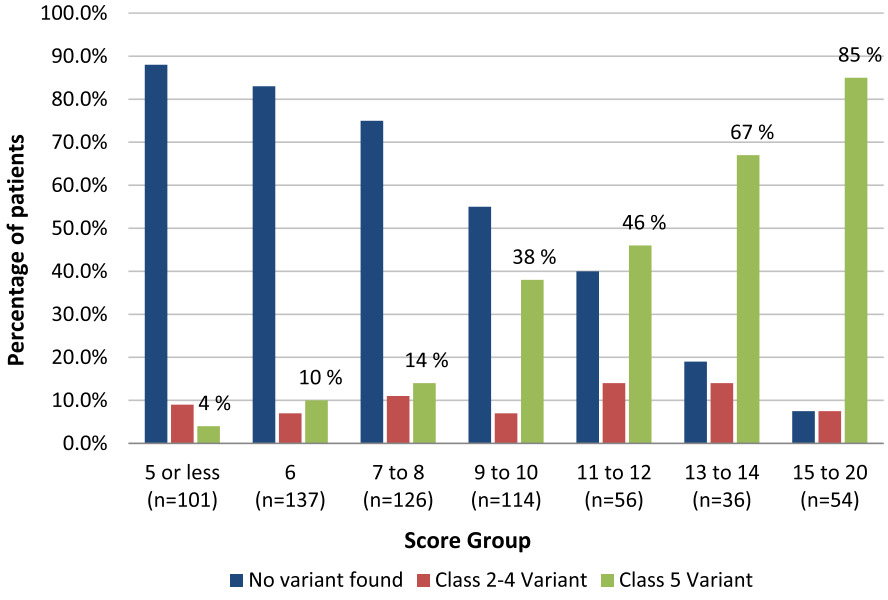

Fig. 2. DNA diagnosis in relation to criteria score. The percentage figures refer to the percentage of patients within a group that have a class 5 variant.

Table 4
Discriminatory capacity for different scoring criteria.

Score	Number tested	Class 5 variants detected (% pick up rate and total number)	Class 2–5 variants detected (% pick up rate and total number)
		Family history	
2	106	43% (46)	57% (60)
1	466	24% (114)	33% (154)
0	50	24% (12)	32% (16)
		Physical examination: tendon xanthomata and corneal arcus	
6 [TX (+/-CA)]	132	57% (75)	65% (86)
0 (equivocal TX)	29	38% (11)	41% (12)
4 (CA alone)	96	27% (26)	34% (33)
0	396	18% (73)	29% (113)
		Clinical history	
4 (CHD <45)	64	39% (25)	50% (32)
3 (CHD <50)	40	33% (13)	45% (18)
2 (CHD <60)	75	16% (12)	25% (19)
1 (TIA/PVD <60)	9	56% (5)	62% (5)
0	435	27% (118)	36% (157)
		Untreated LDL-cholesterol concentrations	
8 (≥8.5)	141	59% (83)	70% (99)
5 (6.5–8.4)	252	28% (71)	37% (93)
3 (5.0–6.4)	182	9% (17)	19% (34)
1 (4.0–4.9)	45	4% (2)	9% (4)
0	2	0	0% (0)
		Fasting triglycerides (minus score)	
−4 (≥5.0)	0	0	0% (0)
−3 (3.5–4.9)	13	15% (2)	31% (4)
−2 (2.5–3.4)	46	15% (7)	20% (9)
0	565	29% (164)	39% (218)

This table shows the number/percentage of patients with a genetic variant across categories for each individual scoring criterion. This is stratified between class 5 and class 2–5 variants.

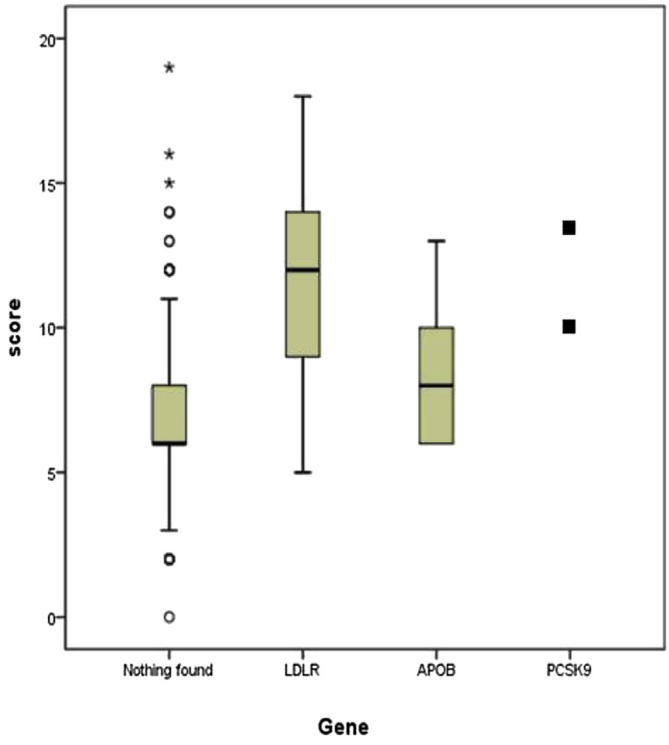

Fig. 3. Comparison of the score for different genes. Box and whisker plot to show the distribution of scores where no variant was found compared to patients with a mutation (class 5 variant) in *LDLR* and in *APOB*. The 2 patients with a *PCSK9* mutation have been added as black squares. The inner line within the boxes corresponds to the median and bars span 95% of the values. Circles and stars represent outliers (>1.5 × IQR) and extreme outliers (>3 × IQR) respectively.

3.5. DNA variants identified in patients with low scores

Out of the 101 patients who scored 5 or below and were genotyped, a class 5 variant was identified in 4 patients and a class 2–4 variant was identified in 9. All 13 of these variants were identified within *LDLR* gene. These low scoring individuals were considered to be eligible for testing on clinical grounds as judged by the FH medical advisor due to the presence of other un-scored features that were thought to make the diagnosis of FH more likely.

The clinical grounds quoted for these 13 patients include: 'thickening of the Achilles' (2 patients) 'xanthelasmas' (4 patients) and younger age. The mean age of the patients with a genetic variant in the group scoring 5 or less was 43 and the mean age of genotype positive patients overall was 51 years.

Out of the 231 patients where a class 2–5 variant was identified, 25 had APOB variants (11%). There was a higher proportion of *APOB* variants in patients who scored exactly 6 (36%), but none were found in those genotyped who scored lower than 6.

3.6. Comparison of the score for different genes (Fig. 3)

The score for those with *APOB* and *LDLR* mutations were significantly higher than those with no mutation ($p = 0.017$ and $p < 0.001$ respectively). In addition, the score for those with *APOB* mutations was significantly lower than those with *LDLR* mutations ($p < 0.001$). There were only 2 subjects with *PCSK9* mutations both of whom had a high score (13, 16).

4. Discussion

These data show that there is a strong correlation between the clinical score and the likelihood of identifying a mutation.

Overall approximately one quarter of variants detected are currently assigned to classes 2–4. Many of these variants will be reclassified following further investigation so that some will be

converted to a pathogenic status (class 5). Therefore, the positive predictive value estimates would increase with reclassification of some variants as pathogenic.

FH caused by *APOB* mutations is known to have a slightly milder phenotype [23] and *PCSK9* a more severe phenotype [24], which is consistent with the results reported here. Some studies have shown that patients with *APOB* mutations have a low score on the Dutch Criteria and that this score is not significantly different to the scores of those with no mutation [25]. However, the results in this study identified a significantly higher score ($p = 0.017$) in those with an *APOB* mutations compared to those where no mutation was identified. This indicates that these criteria are capable of selecting patients with *APOB* mutations for genetic testing, from those who present to a lipid clinic with hyperlipidaemia.

In our clinical cohort the 25 *APOB* variants made up a high proportion of the patients who scored exactly 6 (36% compared to 11% overall), but *ABOB* variants were not found in any of the 101 patients who scored less than 6. However, only a selected group of patients who scored below 6 were genotyped, so it is possible that some lipid clinic patients with *APOB* variants were not genotyped and therefore may have been missed. It is also possible that other individuals with lesser degrees of hypercholesterolaemia may be missed because they are not referred to the lipid clinic in the first place.

A patient with an LDL-C $\geq$ 8.5 mmol/l scores 8, therefore is eligible for genotyping without taking any of the other criteria into account. This appears justified because the results show that LDL-C was the most important single determinant of an FH causing mutation.

The LDL-C correction factor table (Table 2) for patients on cholesterol lowering medication proved to be useful in clinical practice. This is because patients referred for evaluation were often on lipid lowering medication and records of the pre-treatment values for LDL-C were not always available, even if a value for total cholesterol was known.

The weighting for triglyceride appears to be useful for identifying patients that should be genotyped and in addition, it shows that it was appropriate to not completely exclude subjects with raised triglyceride. This is consistent with data from Civeira et al. [26] who reported that 16% of subjects previously classified as familial combined hyperlipidaemia on the basis of raised triglyceride were shown to have an FH mutation.

Rather than having multiple different cut offs for raised triglycerides, an alternative would be a single cut off. Based on these data we would suggest the most suitable single cut off would be $\geq$3 mmol/L with a negative correction of −2. When the data was analysed with this cut off it resulted in good discrimination with mutation pick up rates of 10% and 29% in those with triglycerides $\geq$3 mmol/l and those <3 mmol/l respectively. Therefore this could be considered as a simplification for future use.

TX are considered a classical physical sign of FH, however they may be absent or difficult to diagnose with confidence, even for experienced lipid clinicians. That said, the presence of TX in genetically confirmed FH patients is associated with a significant increase in cardiovascular risk [27,28]. The prevalence of TX in our cohort of mutation positive individuals (43%) is similar to that reported elsewhere (44%) [29]. The inclusion of TX as a criterion in our scoring system is supported by the observation that, of those patients who presented with TX, 57% were found to be mutation positive.

In the Welsh criteria, the weighting given for very early onset CHD in the patient or a 1st or 2nd degree relative gave a greater discriminatory capacity compared to the Dutch criteria which indicates that this is a useful modification to these criteria.

In this cohort there were 97 patients who did not fulfil Simon Broome criteria due to either a lack of family history, or an LDL-C of 4.9 or less. Mutations were identified in 14% of these patients and would have been missed if Simon Broome criteria had been used as a selection for genotyping. The lowest score a patient who fulfils the criteria for SBP is 4. Thus not all SBP patients are eligible for genetic testing based on a threshold of 6. For this reason the mutation pick up rate of SBP patients in the Welsh lipid clinic population cannot be accurately calculated, because only the higher scoring ones were tested.

In clinical practice, corneal arcus is a physical sign that is commonly linked with FH and is more frequently detected than TX [30]. It is generally thought that corneal arcus develops at a younger age than TX, and for that reason the scoring criteria only scores corneal arcus (4 points) in the absence of TX. Of the 96 patients who were TX negative and CA positive, variants were found in 34% (33 out of 96) indicating that corneal arcus on its own is a relatively non-specific diagnostic sign.

The mutation negative group contains some individuals who had very high scores using these criteria including some patients with LDL-C greater than 8.5 and/or TX. It is possible that these patients could harbour mutations that were not detected by the laboratory methods used at the time of testing. Also some patients may have a more complex polygenic basis for hypercholesterolaemia [31]. Further testing using next generation sequencing or other laboratory approaches may be warranted in this high-scoring mutation negative group.

If the score is very high then the decision to offer genotyping is straightforward. However, a larger number of patients have a less severe clinical phenotype and these patients are those where it is most difficult to decide about genotyping. Further possible refinements of these criteria could be considered as discussed below.

Age-, gender-, and population-specific cholesterol ranges are an option [14,17,18]. It is known that genotype proven family members with FH have differing LDL-C values by age and gender [32] and this will also apply to index patients. This is particularly relevant for younger individuals with FH who have less elevated cholesterol, no physical signs and no personal or family history of cardiovascular disease (yet) and thus may not score highly enough to be offered testing. The criteria are currently not suitable for patients who are under 18 years of age.

Refinements are also possible for the assessment of the extent of premature cardiovascular disease in the index patient. Clarke and colleagues have reported that measures of atherosclerotic burden using carotid intimal medial thickness or coronary calcium score, can be used for this purpose, but these techniques are not widely available in a routine clinical setting [16].

The presence of xanthelasmas was not attributed a score in the current version of the criteria. However, xanthelasmas were recorded in 4 of the 13 patients who scored less than 6 and where a variant was identified. A possible refinement to the scoring system could be to add in xanthelasma to the 'physical examination' section and this could be scored 2, but only in the absence of TX or CA. In a cohort of FH patients in Finland, Xanthelasmas were found in 32% [33], in Norway 8% [34] and in Australia and New Zealand FH guidelines, xanthelasma are quoted as occurring in 12% of FH patients [35].

5. Conclusions

These results show that an integrated score combining lipid values, physical signs, personal and family history of premature CVD, is a useful guide for selecting patients for FH genetic testing. Individually, many of the criteria are insensitive or non-specific diagnostic markers for FH, but when combined they are a useful diagnostic tool. They can be successfully applied in a practical

setting and encourage a consistent approach across different clinics and individuals, while still allowing for some application of clinical judgement. These criteria can be used to target resources to those individuals and families that are most likely to benefit from FH genetic testing.

Acknowledgements

This project has been supported by Cardiff University and the Wales FH Service. We are pleased to acknowledge our collaboration with the FH service in Netherlands who were willing to provide us with information about the Dutch FH scoring criteria. We acknowledge assistance from the medical department of Merck Sharpe and Dohme in the compilation of LDL-C correction factors for patients on treatment with ezetimibe with and without a statin, based on a literature review of 71 clinical trials. We acknowledge the support and input of lipid clinic consultants and other members of the Wales FH service.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2015.03.003.

References

[1] J.L. Goldstein, H.H. Hobbs, M.S. Brown, Familial hypercholesterolaemia, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), The Metabolic Basis of Inherited Disease, McGraw Hill, New York, 1995, pp. 1981–2030.

[2] P.J. Lansberg, S. Tuzgöl, M.A. van de Ree, J.C. Defesche, J.J. Kastelein, Higher prevalence of familial hypercholesterolemia than expected in adult patients of four family practices in Netherlands, Ned. Tijdschr. Geneeskd. 144 (2000) 1437–1440.

[3] M. Benn, G.F. Watts, A. Tybjaerg-Hansen, B.G. Nordestgaard, Familial hypercholesterolemia in the Danish general population: prevalence, coronary artery disease, and cholesterol-lowering medication, J. Clin. Endocrinol. Metab. 97 (2012) 3956–3964.

[4] B. Sjouke, D.M. Kusters, I. Kindt, J. Besseling, J.C. Defesche, E.J. Sijbrands, J.E. Roeters van Lennep, A.F. Stalenhoef, A. Wiegman, J. de Graaf, S.W. Fouchier, J.J. Kastelein, G.K. Hovingh, Homozygous autosomal dominant hypercholesterolaemia in the Netherlands: prevalence, genotype-phenotype relationship, and clinical outcome, Eur. Heart J. (2014), http://dx.doi.org/10.1093/eurheart/ehu058. First published online 28 Feb 2014.

[5] N.J. Stone, R.I. Levy, D.S. Fredrickson, J. Verter, Coronary artery disease in 116 kindred with familial type II hyperlipoproteinemia, Circulation 49 (1974) 476–488.

[6] B.N. Datta, I.F.W. McDowell, A. Rees, Integrating provision of specialist lipid services with cascade testing for familial hypercholesterolaemia, Curr. Opin. Lipidol. 21 (2010) 366–371.

[7] National Institute of Health and Clinical Excellence Clinical Guidelines and Evidence Review for Familial Hypercholesterolaemia: Identification and Management of Adults and Children with Familial Hypercholesterolaemia, 2008 (Clinical Guideline 71).

[8] National Institute of Health and Care Excellence, Elucigene FH20 and LIPOchip for the Diagnosis of Familial Hypercholesterolaemia (NICE Diagnostics Guidance 2), 2011.

[9] P. Sharma, D. Boyers, C. Boachie, F. Stewart, Z. Miedzybrodzka, W. Simpson, M. Kilonzo, P. McNamee, G. Mowatt, Elucigene FH20 and LIPOchip for the diagnosis of familial hypercholesterolaemia: a systematic review and economic evaluation, Health Technol. Assess. 17 (17) (2012), http://dx.doi.org/10.3310/hta16170.

[10] I. McDowell, M. Watson, D. Townsend, K. Featherstone, K. Parham, S. Whatley, An evaluation of DNA diagnostics for familial hypercholesterolaemia including a study of personal and family implications [abstract], Atherosclerosis 194 (2007) 282.

[11] Risk of fatal coronary heart disease in familial hypercholesterolaemia. Scientific Steering Committee on behalf of the Simon Broome Register Group, BMJ 303 (1991) 893–896.

[12] World Health Organization, Familial Hypercholesterolemia - Report of a Second WHO Consultation, World Health Organization, Geneva, Switzerland, 1999 (WHO publication no.WHO/HGN/FH/CONS/99.2).

[13] J.C. Defesche, Familial hypercholesterolaemia, in: D.J. Betteridge (Ed.), Lipids and Vascular Disease, Martin Dunitz, London, 2000, pp. 65–76.

[14] R.R. Williams, S.C. Hunt, M.C. Schumacher, R.A. Hegele, M.F. Leppert, E.H. Ludwig, P.N. Hopkins, Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics, Am. J. Cardiol. 72 (1993) 171–176.

[15] D. Damgaard, M.L. Larsen, P.H. Nissen, J.M. Jensen, H.K. Jensen, V.R. Soerensen, L.G. Jensen, O. Faergeman, The relationship of molecular genetic to clinical diagnosis of familial hypercholesterolemia in a Danish population, Atherosclerosis 180 (1) (2005) 155–160.

[16] R.E. Clarke, S.T. Padayachee, R. Preston, Z. McMahon, M. Gordon, C. Graham, M.A. Crook, Wierzbicki, Effectiveness of alternative strategies to define index case phenotypes to aid genetic diagnosis of familial hypercholesterolaemia, Heart 99 (3) (2013) 175–180.

[17] F. Civeira, E. Ros, E. Jarauta, N. Plana, D. Zambon, J. Puzo, J.P. Martinez de Esteban, J. Ferrando, S. Zabala, F. Almagro, J.A. Gimeno, L. Masana, M. Pocovi, Comparison of genetic versus clinical diagnosis in familial hypercholesterolemia, Am. J. Cardiol. 102 (9) (2008) 1187–1193.

[18] R. Huijgen, B.A. Hutten, I. Kindt, M.N. Vissers, J.J. Kastelein, Discriminative ability of LDL-cholesterol to identify patients with familial hypercholesterolemia: a cross-sectional study in 26,406 individuals tested for genetic FH, Circ. Cardiovasc. Genet. 5 (3) (2012) 354–359.

[19] M. Harada-Shiba, H. Arai, T. Okamura, K. Yokote, S. Oikawa, A. Nohara, T. Okada, T. Ohta, H. Bujo, M. Watanabe, A. Wakatsuki, S. Yamashita, Multicenter study to determine the diagnosis criteria of heterozygous familial hypercholesterolemia in Japan, J. Atheroscler. Thromb. 19 (11) (2012) 1019–1026.

[20] Y. Wallis, S. Payne, C. McAnulty, D. Bodmer, E. Sistermans, K. Robertson, D. Moore, S. Abbs, Z. Deans, A. Devereau, Practice Guidelines for the Evaluation of Pathogenicity and the Reporting of Sequence Variants in Clinical Molecular Medicine (Approved September 2013), Association for Clinical Genetic Science (ACGS), Dutch Society of Clinical Laboratory Specialists (VKGL), 2013.

[21] S. Holm, A simple sequentially rejective multiple test procedure, Scand. J. Stat. 6 (2) (1979) 65–70.

[22] S. Mallett, S. Halligan, M. Thompson, G.S. Collins, D.G. Altman, Interpreting diagnostic accuracy studies for patient care, BMJ 2 (2012) 345–399.

[23] S.E. Humphries, T. Cranston, M. Allen, H. Middleton-Price, M.C. Fernandez, V. Senior, E. Hawe, A. Andrew Iversen, R. Wray, M.A. Crook, A.S. Wierzbicki, Mutational analysis in UK patients with a clinical diagnosis of familial hypercholesterolaemia: relationship with plasma lipid traits, heart disease risk and utility in relative tracing, J. Mol. Med. 84 (2006) 203–214.

[24] R.P. Naoumova, I. Tosi, D. Patel, C. Neuwirth, S.D. Horswell, A.D. Marais, C. van Heyningen, A.K. Soutar, Severe hypercholesterolemia in four British families with the D374Y mutation in the PCSK9 gene: long-term follow-up and treatment response, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 2654–2660.

[25] L. Palacios, L. Grandoso, N. Cuevas, E. Olano-Martín, A. Martinez, D. Tejedor, M. Stef, Molecular characterization of familial hypercholesterolemia in Spain, Atherosclerosis 221 (2012) 137–142.

[26] F. Civeira, E. Jarauta, A. Cenarro, A.L. García-Otín, D. Tejedor, D. Zambón, M. Mallen, E. Ros, M. Pocoví, 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) (2008) 1546–1553.

[27] D.M. Oostweveer, J. Versmissen, M. Yazdanpanah, T.H. Hamza, E.J.G. Sijbrands, Differences in characteristics and risk of cardiovascular disease in familial hypercholesterolemia patients with and without tendon xanthomas: a systematic review and meta-analysis, Atherosclerosis 207 (2) (2009) 311–317.

[28] S. Bertolini, L. Pisciotta, C. Rabacchi, A.B. Cefalù, D. Noto, T. Fasano, A. Signori, R. Fresa, M. Averna, S. Calandra, Spectrum of mutations and phenotypic expression in patients with autosomal dominant hypercholesterolemia identified in Italy, Atherosclerosis 227 (2013) 342–348.

[29] D. Bhatnagar, Diagnosis and screening for familial hypercholesterolaemia: finding the patients, finding the genes, Ann. Clin. Biochem. 43 (Pt 6) (2006) 441–456.

[30] A.F. Winder, J.C. Jolleys, L.B. Day, P.F. Butowski, Corneal arcus, case finding and definition of individual clinical risk in heterozygous familial hypercholesterolaemia, Clin. Genet. 54 (6) (1998) 497–502.

[31] P.J. Talmud, S. Shah, Futema M. Whittall, P. Howard, J.A. Cooper, S.C. Harrison, K. Li, F. Drenos, F. Karpe, H.A. Neil, O.S. Descamps, C. Langenberg, N. Lench, M. Kivimaki, J. Whittaker, A.D. Hingorani, M. Kumari, S.E. Humphries, Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolaemia: a case-control study, Lancet 381 (9874) (2013) 1293–1301.

[32] B. Starr, G. Hadfield, B.A. Hutten, P.J. Lansberg, T.P. Leren, D.H. Damgaard, A.W. Neil, S.E. Humphries, Development of sensitive and specific age- and gender specific low-density lipoprotein cholesterol cutoffs for diagnosis of first-degree relatives with familial hypercholesterolaemia in cascade testing, Clin. Chem. Lab. Med. 46 (6) (2008) 791–803.

[33] P.V. Koivisto, U.M. Koivisto, T.A. Miettinen, K. Kontula, Diagnosis of heterozygous familial hypercholesterolemia DNA analysis complements clinical examination and analysis of serum lipid levels, Arterioscler. Thromb. Vasc. Biol. 12 (1992) 584–592.

[34] T.P. Leren, T. Manshaus, U. Skovholt, T. Skodje, I.E. Nossen, C. Teie, S. Sørensen, K.S. Bakken, Application of molecular genetics for diagnosing familial hypercholesterolemia in Norway: results from a family-based screening program, Semin. Vasc. Med. 4 (1) (2004) 75–85.

[35] The Cardiac Society of Australia and New Zealand, Guidelines for the Diagnosis and Management of FH, The Cardiac Society of Australia and New Zealand, 2013. http://www.csanz.edu.au/wp-content/uploads/2013/12/Familial_Hypercholesterolemia_2013.pdf.