**The dilemma of a young patient with a raised cholesterol concentration**

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Case background

Mr KS, a 26 year old male presented to the lipid clinic at St George’s Hospital in London with a baseline cholesterol concentration of 8.8 mmol/L (340 mg/dL). On clinical examination, there were no tendon xanthomas, nor a family history of hypercholesterolemia or premature death from vascular disease. At this time Mr KS did not meet the Simon Broome criteria (Figure 1) for possible familial hypercholesterolemia (FH), and as the patient had a Body Mass Index (BMI) of 31.5 kg/m2 (target < 25), lifestyle modification was suggested, and a review appointment ordered for 6 months. A Carotid Intima-Medial Thickness (CIMT) measurement was ordered to help assess vascular risk.The results showed no plaque, but a right CIMT of 0.6mm and a left CIMT of 0.7mm, which were both above the 97.5th percentile for his age consistent with early atheromatous change.

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| **Simon Broome Criteria** |
| Diagnose a person with definite FH if they have:* Total Cholesterol > 7.5 mmol/L or LDL-Cholesterol > 4.9 mmol/L
* And tendon xanthomas, or evidence of these signs in a first- or second degree relative
* Or DNA-based evidence of an LDL-Receptor mutation, familial defective Apo B-100 or a PCSK9 mutation
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| Diagnose a person with possible FH if they have:* Total Cholesterol > 7.5 mmol/L or LDL-Cholesterol > 4.9 mmol/L
* And family history of myocardial infarction: aged younger than 50 years in a second degree relative or aged younger than 60 in a first degree relative
* Or family history of raised total cholesterol: > 7.5 mmol/L in an adult first- or second degree relative or > 6.7 mmol/L in a child, brother or sister aged younger than 16 years
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Figure 1: Simon Broome criteria for the diagnosis of Familial Hypercholesterolemia (FH) *(adapted from NICE, 2008)*

Further investigation

The following year, a younger brother (aged 23 years old) presented with a raised cholesterol concentration of 9.6 mmol/L (371 mg/dL). As he was known to have a first degree relative with raised cholesterol he met the Simon Broome criteria for possible FH, and was investigated further.

Consent for genetic testing was obtained from the patient, and DNA extracted from an EDTA whole blood sample, and sent to the molecular genetics laboratory. The DNA was screened using fluorescent sequence analysis to look for mutations in the promoter and coding sequence (including intron-exon boundaries) of the LDL-Receptor gene, PCSK9 (exon 7) and Apo B-100 (part of exon 26) genes. Multiplex Ligation-dependent Probe Amplification (MLPA) analysis was also carried out for the LDL-Receptor gene.

The sequence variant c.1426C>T;p.P476S within exon 10 of the LDL-Receptor gene was identified in the patient. This mutation had previously been identified in the Dutch FH population *(Fouchier et al, 2005)*, but computer predictions performed by the referral lab were equivocal. PolyPhen2 *(Adzhubei et al, 2010)* and Mutation taster *(Schwarz et al, 2014),* predicted the mutation to be pathogenic, whilst SIFT *(Sim et al, 2012)*, Pmut *(http://mmb.pcb.ub.es/PMut/)* and AlignGVGD *(Tavtigian et al, 2005)* predicted it to be non-pathogenic. The mutation was reported as possibly pathogenic and may be consistent with the diagnosis of FH. No further variants, or exon deletions or duplications were identified. A full pedigree study was recommended to see if there was co-segregation of the candidate sequence variant with the phenotype of a raised cholesterol.

Family studies

Fluorescent DNA sequence analysis identified the same LDL-Receptor c.1426C>T;p.P476S variant in Mr KS, their mother, and a sister (Figure 2). The mother had a cholesterol concentration of 7.8 mmol/L (301 mg/dL), and the sister a cholesterol concentration of 9.1 mmol/L (375 mg/dL). The variant was absent in the father who had a cholesterol of 6.6 mmol/L (255 mg/dL). Family studies showed the c.1426C>T;p.P476S mutation did co-segregate with raised cholesterol concentrations within the family (Figure 2).

 Mother Father

 7.8 6.6

 Mr KS Brother Sister

 8.8 9.6 9.1

Figure 2: Family tree showing the cholesterol concentration in mmol/L and mutation status of Mr KS’ family members

Outcome of the case

The LDL-Receptor variant c.1426C>T;p.P476S is a novel mutation thought to be the cause of the families raised cholesterol concentrations. A presumed diagnosis of FH was given and Mr KS was started on Atorvastatin 10 mg daily. He showed a good response and had achieved a cholesterol concentration of 5.1 mmol/L (197 mg/dL) at a recent clinic appointment. His BMI remains raised at 30.1 kg/m2, and weight loss has been advised to further reduce his cholesterol concentration and risk of diabetes.

Mr KS’ mother and two siblings were also prescribed atorvastatin to lower their cholesterol concentrations. As FH shows autosomal dominant inheritance, all three siblings were offered genetic counselling due to a 50% risk of transmitting their c.1426C>T;p.P476S mutation to any offspring.

Learning points

The term FH encompasses a group of genetic disorders characterised by elevated total and LDL-Cholesterol concentrations. The cholesterol is raised from birth, which results in the early development of atherosclerosis and coronary heart disease *(NICE, 2008)*. There is a greater than 50% risk of coronary heart disease in men by the age of 50 years and at least 30% in women by 60 years *(NICE, 2008)*.

The majority of FH patients have mutations in the LDL-Receptor, which may include intron changes (sequence changes and splice mutations) and partial or total exon deletions. Other types of FH include Apo B-100 mutations, PCSK9 mutations and the rarest LDL-Adaptor Protein 1 (LDLRAP1) mutations. Approximately 1400 unique mutations have been identified worldwide, of which 200 have been reported in the UK population *(NICE, 2012)*.

The prevalence of heterozygous FH in the UK population is estimated to be 1 in 500, which means approximately 110,000 people are affected *(NICE, 2008)*. Homozygous FH is much rarer, with only 1 case per million in the UK population *(NICE, 2008)*. Homozygous FH is associated with early death from coronary heart disease and symptoms usually appear during childhood *(NICE, 2008)*.

This case describes the investigation of a 26 year old male who was found to have an elevated cholesterol concentration. Young patients with raised cholesterol pose a common dilemma for clinicians. Risk stratification scores yield an artificially low risk estimate, causing FH patients to be missed. Conversely a significant proportion of patients with elevated cholesterol have polygenic rather than monogenic hypercholesterolemia. Various strategies have been proposed for genetic testing in patients considered to be at risk of FH. In all cases they are based on raising the prior probability before proceeding to genetic testing. The two most accepted are the use of a clinical score, either a numeric scoring system as developed from the FH cohort in the Netherlands *(Van Aalst-Cohen et al, 2006)* or the Simon Broome criteria *(Marks et al, 2003)*. An alternative is to use non-invasive imaging in addition. Carotid scanning allows CIMT measurements to be performed and for direct visualisation of arterial plaques. A CIMT above the 75th percentile or the presence of plaque raises the probability of mutation detection.

The laboratory approach for FH diagnosis is also problematic. Screening for only the relatively common FH mutations will miss patients with novel mutations and the correct diagnosis not made for an eminently treatable condition. The use of a microarray to detect the commonest mutations, followed by full sequencing has been proposed, but a technology evaluation considered full sequencing by next generation sequencing to be more clinically and cost effective. By providing a DNA diagnosis at an asymptomatic stage and prescribing effective lipid lowering therapy, the UK National Health Service (NHS) saves costs and improves quality of life for patients and their families *(NICE, 2012)*. With the development of fully automated genetic testing platforms, the cost of next generation sequencing is also progressively falling *(NICE, 2012)*.

As this case illustrates, identification of a mutation alone may not be sufficient to diagnose FH. Mathematical modelling of the sequence changes can be informative but due to the variety of programs available and their conflicting results, predictions are often unhelpful. Family segregation studies are needed for any novel mutation to determine whether the mutation is causative.

The case also shows the importance of reviewing the family history at each clinic visit. In this case, an elevated cholesterol concentration in a sibling led to further investigation and discovery of the c.1426C>T;p.P476S FH mutation. Without this investigation, Mr KS’ weight could have been blamed for his raised cholesterol. By confirming a diagnosis of FH, statins were prescribed, which effectively reduced the cholesterol concentration. The diagnosis allowed other family members to be screened and identified. NICE (2008) guidance recommend statins should be considered in all FH patients to reduce LDL-C to greater than 50% from baseline. For Mr KS, statins reduced his calculated LDL- from 8.4 to 4.5 mmol/L. The case shows the importance of genetic testing in making a definite FH diagnosis.

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Take away messages

1) A raised cholesterol is a common clinical finding but standard risk prediction algorithms significantly underestimate the risk in young patients.

2) Genetic testing may identify a previously undocumented sequence variant.

3) Lack of prior knowledge of the sequence variant combined with mathematical modelling can produce diagnostic uncertainty.

4) Family studies assessing phenotype plus genotype may be required for diagnosis.

5) Genetic testing is ultimately required for diagnosis but may be insufficient on its own.