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Project: Investigation of the role of founder effect in familial hypercholesterolaemia patients with LDLR gene mutations

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Sponsor: Canterbury Health Laboratories

Introduction:

Familial hypercholesterolaemia is an autosomal dominant condition caused by mutations in the low density lipoprotein (LDL) receptor (LDLR) gene, resulting in a defective or absent LDL receptor. The LDL receptor is a transmembrane protein that mediates the uptake of cholesterol-rich LDL by binding to the ApoB protein found on the surface of LDL molecules. The LDLR gene has 18 exons distributed across 45 kb on chromosome 19 and over 900 different mutations have been described. Canterbury Health Laboratories (CHL) perform genetic analysis screening for LDLR mutations in patients attending the Christchurch Hospital Lipid Disorders Clinic, with cascade screening being performed on relatives of patients in which an LDLR mutation is identified. This program has so far detected over 200 index patients with LDLR mutations. A number of LDLR mutations have been found in multiple index patients. There are two reasons why several apparently unrelated patients may have the same LDLR mutation: (i) they could have arisen independently through separate mutation events, or (ii) it could be due to identity by descent where by a single mutation event occurred in a common ancestor that was then passed onto each index case (this is also referred to as a "founder effect").

Aim:

The aim of the project was to investigate whether multiple instances of the same LDLR mutation observed in apparently unrelated index patients in the Canterbury cohort is due to identity by decent and a founder effect or the result of independent mutation events.

Method:

The project involved constructing haplotypes (a set of DNA variants that are inherited together and can be used to trace inheritance) for the various LDLR mutation alleles common in the cohort by using short tandem repeat (STR) markers that flank the LDLR gene. The first step was to analyse CHL's database of patients with LDLR mutations to identify mutations present in multiple index cases. A multiplex polymerase chain reaction (PCR) was then used to amplify the STR markers from the patient DNA samples. Fragment analysis, which measures the length of the PCR products, was performed on each amplified sample to determine the STR alleles present for each patient. Phasing analysis, which is a method of determining which alleles are found together on the same chromosome, was then performed to construct haplotypes of the LDLR mutation allele for each index case. This was done by comparing the alleles of each member in a family to determine which STR alleles were found on the same chromosome as the LDLR mutation allele, creating a haplotype. These haplotypes were then able to be compared between the different index cases with the same LDLR mutation, which allowed for the identification of any instances of identity by descent.

In addition, 50 random patient samples were also analysed to genotype the panel of STR markers to obtain allele frequency data for the different STR markers.

Results:

In total we tested 76 index cases (and their family members) who had at least one of the 24 LDLR mutations we investigated. It was found that for 14 of these mutations there were common haplotypes among the different index cases with the same mutation. These mutations were 1813C>T, 313+1G>A, D461N, EX15_18DEL, D206E, D154N, E219K, V408M, G544A, L578S, 2312-3C>A, E119K, 1206DELCT and Ex2_12DUP. This strongly suggests identity by decent, where a single mutation event has occurred in a common ancestor shared by these index cases. No common haplotypes were found for the other 10 mutations (Ex2_8DUP, Q357P, R60C, E80K, G322S, W462R, D471N, D679N, V806I3 and F509L). It is therefore likely that these mutations arose independently by separate mutation events. For the two LDLR mutations observed at the highest frequency in the cohort, D206E (9 index cases) and D461N (7 index cases), there were two different haplotypes observed for each. The D206E mutation is a well-known LDLR mutation present in the Dutch-Afrikaner population in South Africa and the surnames of the patients representing one of the D206E haplotypes suggest this is the Afrikaner D206E haplotype. A number of index patients were found to have the same alleles for 4 of the 5 markers as one of the common haplotypes but not an exact match. This may be due to either recombination between that particular marker and the LDLR gene, or could be due to slippage during DNA replication, in which the DNA polymerase adds an extra STR repeat or fails to add enough STR repeats during DNA replication. Looking at the allele frequency we can show that there is a very high chance that identity by descent does explain their LDLR mutation, even though their haplotypes do not fully match the common haplotype. These results can give us an idea of the frequency of new mutations occurring in the LDLR gene. We found that the most commonly seen mutations show identity by descent which indicates that there are fewer separate mutational events that underlie the spectrum of LDLR mutations in the cohort. This would indicate there is a lower rate of de novo mutations in LDLR than if the mutations were recurrent.

Conclusion:

We found that there was strong evidence for identity by decent to explain the multiple occurrences of 14 of the mutations included in the study, indicating that there is a founder effect for these mutations in New Zealand. There was no evidence for identity by decent for the other 10 mutations we studied, suggesting they arose through separate mutation events.