Development of a fast and cost-effective genetic diagnostic method for familial hypercholesterolemia in Sweden

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Familial hypercholesterolemia (FH) is an autosomal dominant disease causing elevated levels of low-density lipoprotein cholesterol and carrying a high risk of premature coronary heart disease such as myocardial infarction. The prevalence of FH in Sweden is approximately 1/250. There is an obvious need for improving early detection and subsequent treatment to reduce the risk of premature heart disease or death from cardiac events.

The National Board of Health and Welfare in Sweden has 2015 given a recommendation to genetically investigate FH patients and also their relatives, and thus cost-effective laboratory methods are needed.

In conclusion, our FH analytical strategy detected mutations in approximately one fourth of Swedish patients with suspected FH.

Introduction

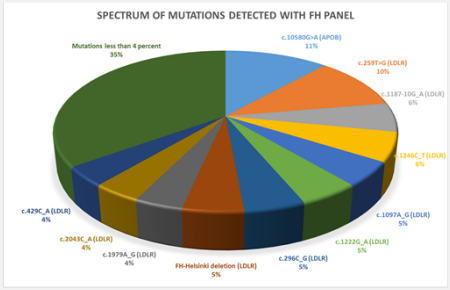
In this nationwide Vinnova funded collaboration we aimed to characterize the FH disease-causing mutation spectrum in Sweden, and to develop a cost-effective diagnostics. This was done through iterative improvement of the analytical strategy, consisting of (i) targeted mutation analysis using a panel based on Agena mass spectrometry-based genotyping; (ii) sequencing of samples failing to show mutations; and (iii) redesign of the panel to include new, recurrently found mutations.

Methods

- Patient material: 1143 FH patients from 14 Swedish hospitals. Dutch Lipid Clinical Network (DLCN) score was available for 482 patients.
- **Genotyping**: Agena Biosciences Iplex Gold, analysed with MA4 MassARRAY mass spectrometer. 113 pathogenic/likely pathogenic mutations in the *LDLR*, *PCSK9* and *APOB* genes were selected. The panel has successfully been validated in proficiency test with the European Molecular Genetics Quality network as well as against samples with known sequence-determined mutations.
- **Sequencing**: Progenika's SEQPRO LIPO amplicon sequencing methodology using Illumina MiSeq instrument. All exons in the *LDLR* gene are analyzed for SNVs and CNVs. The *PCSK9* gene as well as part of exon 26 and 29 in the *APOB* gene are investigated for SNVs. All variations was confirmed by Sanger sequencing or MLPA.
- Reference sequences: NM_000527.4(LDLR), NM_174936.3(PCSK9) and NM_000384.2(APOB)

Results

- DLCN score was available for 482 patients in whom mutations were detected in 27%.
- When restricted to patients with probable or definite FH (DLCN score ≥6; n=309) pathogenic mutations were detected in 35%. The two most prevalent mutations were NM_000384.2(APOB):c.10580G>A(p.Arg3527Gln) and NM_000527.4(LDLR):c.259T>G(p.Trp87Gly). In total, 36 different mutations were detected by the panel in this group of patients. See figure.



- 142 panel negative samples were sequenced with the SEQPPRO-LIPO method, yielding 15 additional mutations in 16 patients.

In conclusion, our FH-panel detected mutations in approximately one fourth of Swedish patients with suspected FH. The chemical cost for analyzing samples with the FH panel is approximately 10% of the chemical cost of sequencing.

The Mutation Analysis Core Facility (MAF) is using the FH panel described above under ISO/IEC 17025 accreditation. MAF is also performing the sequencing.

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