A Complex Rearrangement in the *LDLR* Gene in a Patient with Familial Hypercholesterolemia and Severe Coronary Artery Disease

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Introduction

Familial hypercholesterolemia (FH) is one of the most prevalent monogenic inherited diseases, with an estimated frequency of ~1:220 worldwide[1]. However, current estimates show that 90% of cases are undiagnosed[2]. FH is characterized by lifelong elevated levels of low-density lipoprotein (LDL)-cholesterol and, if left untreated, leads to aggressive and premature coronary artery disease. Pathogenic variants in the LDL receptor (*LDLR*) gene are the most common cause of autosomal dominant FH, and detection of *LDLR* pathogenic variants aids in the diagnosis. Numerous single nucleotide variants, including frameshift, nonsense, missense, and splice site variants, that cause the loss of *LDLR* function have been associated with FH. Structural variants involving the promoter region, single or multi-exon deletions, and duplications have also been reported.

Here we report a heterozygous, complex structural rearrangement involving the promoter region and exon 1 of the *LDLR* gene (NM_000527.4:c.[-203_67+26delins AGG; 67+3241_67+4720delins(-192_33)inv]) identified by next-generation sequencing (NGS) technology. This variant was identified in a patient who was enrolled in the FH Foundation's PAGENT (Patient Acceptance of GENetic Testing) study[3]. The patient was affected with FH and severe coronary artery disease without significant family history. While large deletions (~9-16 kb) including the promoter region and exon 1 of the *LDLR* gene have been reported in the literature[4-6], the variant described here appears to be novel. Due to the sequence inversion encompassing the 5'UTR and part of exon 1, this variant is unlikely to be detected by methodologies that target coding sequences at a low sampling density, such as aCGH and MLPA.

Methods

Individuals participating in the CASCADE FH Registry Patient Portal and with a clinical diagnosis of FH were invited to opt in for *APOB, LDLR* and *PCSK9* gene testing at Color. Variant calling, analysis, and reporting focused on the complete coding sequence and adjacent intronic sequence of the primary transcript(s).

Laboratory procedures were performed at the Color laboratory under CLIA and CAP compliance. Briefly, DNA was extracted, enriched for select regions using SureSelect XT probes, and then sequenced using NovaSeq 6000 instrument. Sequence reads were aligned against human genome reference GRCh37.p12, and variants were identified using a suite of bioinformatic tools designed to detect single nucleotide variants, small insertions and deletions (indels, 2-50 bp), and large structural variants (> 50 bp).

Variants were classified according to the American College of Medical Genetics and Genomics 2015 guidelines for sequence variant interpretation[7]. All variant classifications were signed out by a board-certified medical geneticist or pathologist.

References

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Results

Case Study

- A Caucasian patient was enrolled in the FH Foundation's PAGENT study.
- The patient was diagnosed with FH (LDL-C 420 mg/dl) following an emergency quadruple bypass surgery at the age of 32. Despite the severe phenotype observed in the patient, he had previously gone without a proper diagnosis and treatment plan for several years.
- The patient's family history lacked any documented cases of hypercholesterolemia, cardiovascular disorders, or sudden unexplained death. Both parents of the patient had slightly elevated cholesterol levels, for which lifestyle modifications were recommended.
- A complex, heterozygous structural rearrangement encompassing the promoter region and exon 1 of the *LDLR* gene was identified in the patient.
- The Color bioinformatics NGS pipeline called multiple deletions and insertions of varying size, as well as two inversions calls at ~3.3 kb and ~5 kb depending on the read direction, within a 5.0 kb region encompassing the promoter region and exon 1 of the *LDLR* gene of the patient.
- The variant was determined to be NM_000527.4:c.[-203_67+26delins AGG; 67+3241_67+4720 delins(-192_33)inv].
- Genetic testing has been offered to the parents but has not yet been performed.

Figure 2. IGV image representing the complex rearrangement of the *LDLR* promoter and exon 1.

Analysis of both paired and soft-clipped reads revealed a complex rearrangement, which includes a 225 bp sequence reverse complementary to c.-192_33. Red bars indicate the deletion in the 5'UTR and exon 1 (c.-203_67+26) and the deletion of ~1.5 kb sequence in intron 1 (c.67+3241_67+4720). Note that reads were randomly down-sampled to increase visibility in this image.

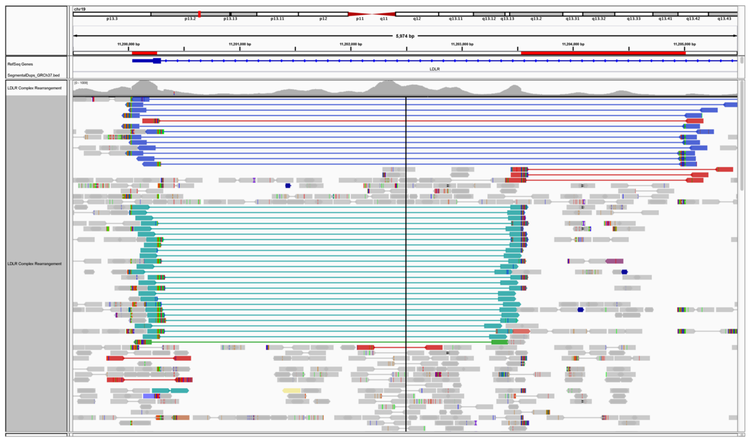


Figure 1. Normalized coverage plot showing the deletion of the *LDLR* promoter and exon 1 region.

The copy ratio graph shows the read depth across the *LDLR* gene for 50 bp segments, normalized against a representative set of comparable samples. The segmentation method 'flasso' (CNVkit) identified the 1.5 kb deletion in intron 1. In addition, two segments representing the small deletion flanking the 225 bp inversion of exon 1 were detected at copy ratios below normal.

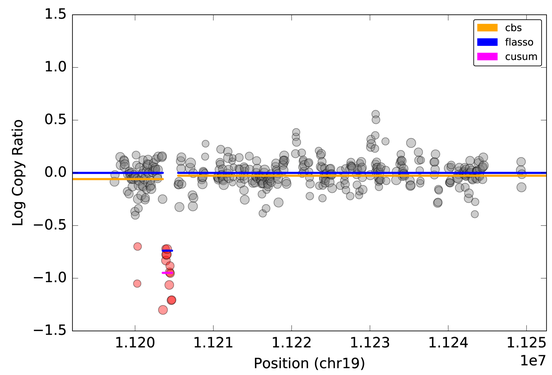
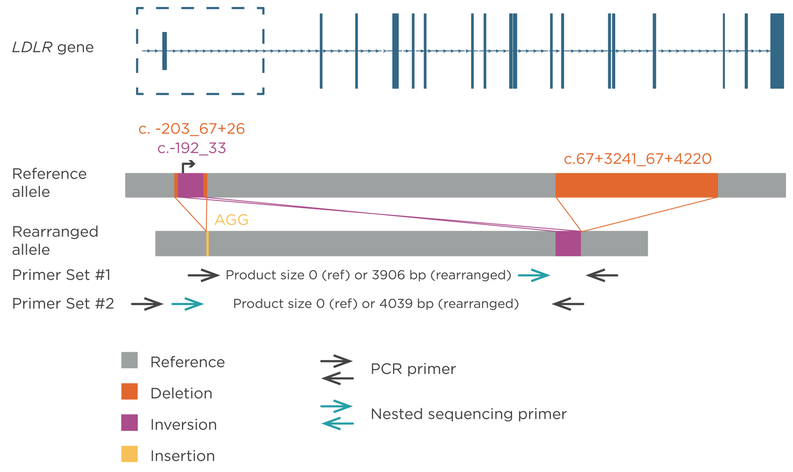


Figure 3. Secondary confirmation by PCR and Sanger sequencing.

Two sets of primers specific to this rearrangement were designed:
- Primer Set #1 uses a forward primer overlapping the 5' breakpoint (c.-211_-203 and first 11 bp of the rearrangement).
- Primer Set #2 uses a reverse primer targeting the inverted sequence (c.-192_33inv).



PCR fragment analysis confirmed the approximate size of the rearrangement, as well as the absence of a PCR product in control DNA. Sanger sequences based on the forward, reverse, and nested primers confirmed the order and breakpoints of the constituents of the complex rearrangement in the patient's sample.

Conclusions

- A complex rearrangement in the *LDLR* gene was identified by NGS in a patient affected with FH and coronary artery disease. This variant is unlikely to be detected by methodologies that target coding sequences at a low sampling density, such as aCGH and MLPA.
- This case study underscores the clinical utility of preventive genetic testing to obtain a definitive FH diagnosis and discover disease-causing pathogenic variants that would not be predicted based on family history alone.
- The lack of significant disease history in the patient's family suggests that the pathogenic variant may be associated with highly variable penetrance or cholesterol-lowering genetic variants in other members of the family. Further family testing may help identify additional carriers, who may be at increased risk for cardiovascular disorders.
- It is also possible that the detected *LDLR* pathogenic variant arose *de novo* in the patient. *De novo* pathogenic variants in the *LDLR* gene have been documented in several FH cases in the literature[8-12]. A preventive genetic testing allows for detection of rare *de novo* pathogenic variants in the absence of clear family history of disease.
- FH and associated cardiovascular disease are largely preventable with treatment begun early in life[13]. Yet, FH remains underdiagnosed and undertreated, which is reflected in this case study.
- There is currently not a systematic approach for family cascade screening of FH in the US. The FH Foundation initiated the CASCADE FH Registry and aims to address the gap in diagnosis and care. The PAGENT study will assess the uptake of genetic testing by clinically diagnosed FH patients participating in the CASCADE FH Registry Patient Portal and, subsequently, their first-degree relatives. All subjects will be offered, at no cost, sequencing of key genes responsible for the majority of FH cases: *LDLR, APOB,* and *PCSK9*.