Extended genetic diagnosis of Familial Hypercholesterolemia (FH) using next-generation sequencing

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Introduction

- Familial Hypercholesterolemia (FH) is a major risk factor for coronary artery disease and is caused by mutations in the genes coding for the low-density lipoprotein receptor (*LDLR*), apolipoprotein B (*APOB*) and proprotein convertase subtilisin/kexin 9 (*PCSK9*).
- Routine genetic diagnosis of FH is often limited to sequencing *LDLR* followed by partial sequencing of *APOB* and *PCSK9* in cases with no *LDLR* mutations. This is mainly due to the large size of *APOB* and rarity of *PCSK9* mutations which makes Sanger sequencing inefficient.

Methods

- DNA from 20 patients with 31 known mutations including single-nucleotide coding and promoter variants, insertions, deletions, indels and large copy-number variants were used (**Table 1**).
- Libraries were made using Ion AmpliSeq™ technology to enrich coding sequence as well as 25 bp flanking intronic regions of *LDLR*, *APOB* and *PCSK9*, which targets ~33 Kb of genomic DNA (**Figure 1**).
- Sequencing was performed using Ion-PGM™ sequencing platform (Life technologies™) as shown in **Figure 1**.
- The sequence data were analyzed using SeqNext software (JSI medical systems).
- Newly identified variants were confirmed by Sanger sequencing.

Results

- Libraries were pooled in two pools with 10 libraries in each pool. In total two sequencing runs were performed using Ion 318™ chip kit.
- An average coverage of >1000x per bp target was obtained (**Figure 2**).
- Enrichment pattern was similar in two different runs.
- We could successfully identify all previously detected mutations.
- Interestingly, we also identified additional 8 rare variants including *PCSK9* p.Cys679X and 7 *APOB* variants (e.g. p.Arg532Trp, p.Asp1113His, p.Lys3076Met, etc.), some of which were already reported in the literature to be functional, while others predicted to be functional using *in silico* prediction models.
- SeqNext was successful in identification of all variants including small indels and large CNVs (**Figure 3**).
- As an example, a newly identified *PCSK9* truncating mutation in a patient with compound heterozygosity for two previously identified *LDLR* mutations may help to explain unexpected low LDL-C levels in heterozygous carriers in the extended pedigree (**Figure 4**).
- Exon1 of the *APOB* and 3 exons from *PCSK9* were not completely covered and had to be separately sequenced by Sanger sequencing.

Conclusions

- Our study suggests a fast, cost-effective and accurate approach for extended genetic diagnosis of FH which can increase the yield of FH diagnosis.
- The unbiased approach of complete sequencing of all three FH genes may improve phenotype-genotype correlation studies in extended pedigrees and may help explaining unexpected phenotypes often seen in families with dyslipidemia and referred to as phenocopy or incomplete penetrance of the phenotype.

Acknowledgements

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Table 1: Distribution of identified variants in current strategy compared to NGS strategy

Mutation type	Identified by Sanger sequencing	Identified by NGS (SeqNext software)
Single Nucleotide mutations	11	11 + **8**
Insertion	5	5
Deletion	7	7
Indel	2	2
Promoter mutation	1	1
Large CNV (>Kb)	5	5
Total	**31**	**39**

Figure 1: Ion Ampliseq™ library prep (left) and Ion-PGM sequencing strategy (right)

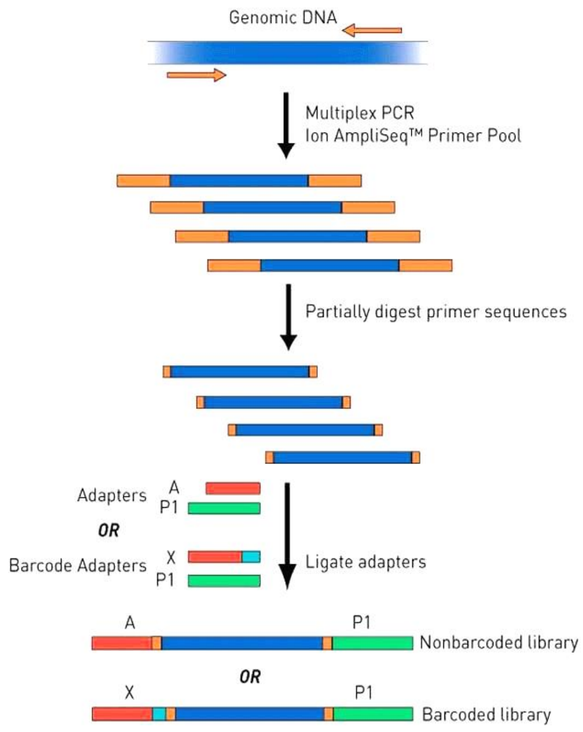


Figure 2: Average coverage per exon of the target genes in pool 1 (blue) and pool 2 (red)

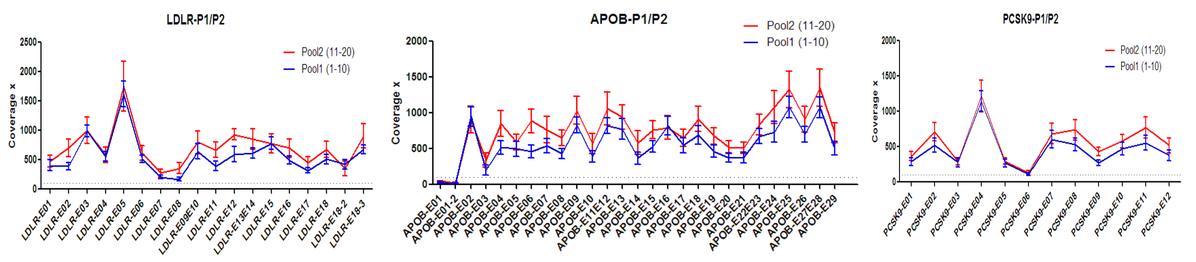
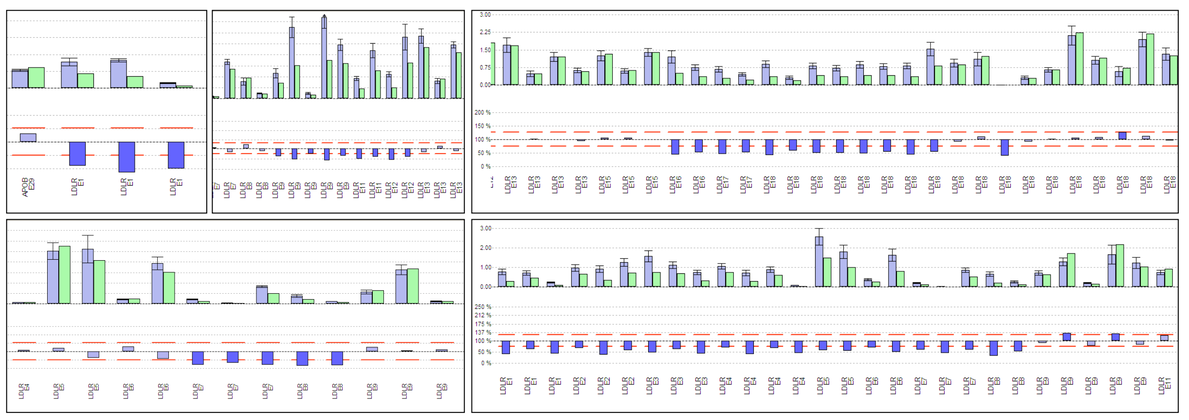
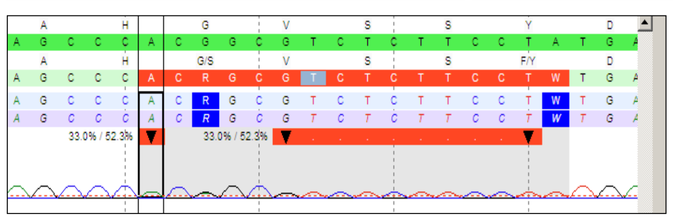


Figure 3: Identification of all CNVs and indels by SeqNext software





Indel ACGGCGTCTCTTCCTA ->CAGCT (het)
c.1379_1394delinsCAGCT

Figure 4: Identification of additional variants may help in explaining unexpected phenotypes seen in extended pedigrees