Use of next-generation sequencing to detect copy number variants in the molecular diagnosis of familial hypercholesterolemia

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Overview

Introduction

- What is familial hypercholesterolemia (FH) and why is it important?
- What are the causes of FH and how is it being currently diagnosed at the molecular level?

Objective

- Method: How can the molecular diagnosis be potentially improved?

Implications

- What are the implications of this method?

Future Directions

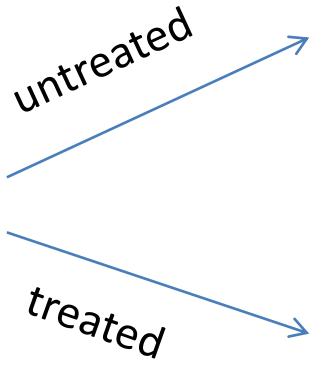
- How can this method be further applied?

Familial Hypercholesterolemia (FH)

- Genetically determined extreme LDL cholesterol
  (LDL-C plasma concentration >95th percentile for age/sex)
- Autosomal dominant inheritance
- Heterozygous FH: Prevalence of ~1 in 250 (Akioyamen LE *et al*. *BMJ Open*. 2017)

  -most common monogenic disorder worldwide

- < 10 % diagnosed globally



- Early onset atherosclerosis causing CVD
  - ↑ risk of MI, stroke
- Effectively lowered LDL-C, ~ normal life expectancy

Familial Hypercholesterolemia (FH)

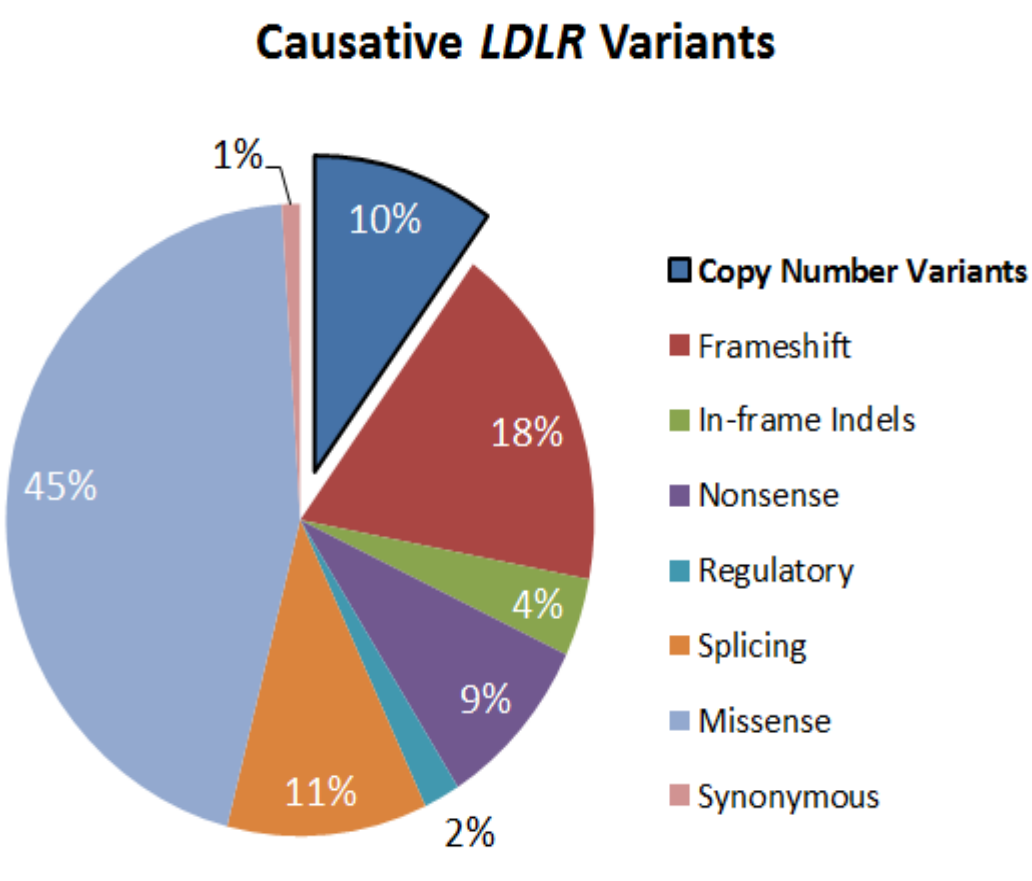
- *LDLR*: loss-of-function variants
- *APOB*: specific protein-altering variants
- *PCSK9*: gain-of-function variants

- **DNA testing a central part of diagnosis worldwide**

Current method:

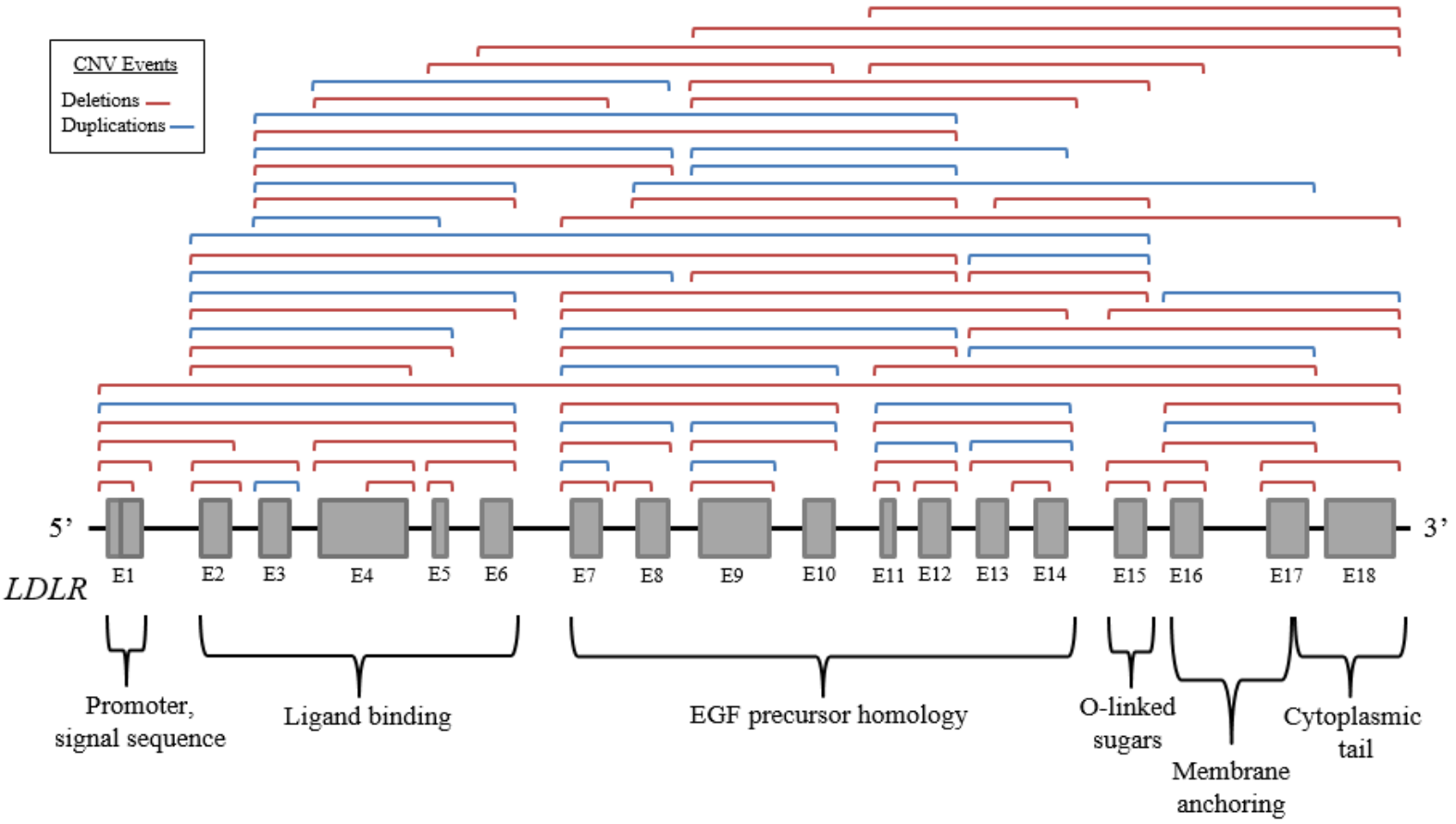
1) <u>Targeted NGS panel</u>
   - small-scale variants
   - *LDLR, APOB, PCSK9*

2) <u>MLPA</u>
   - large-scale CNVs (deletion/duplications of one or more whole exons)
   - *LDLR*



(ClinVar at NCBI, accessed Dec 2017)

Unique *LDLR* CNVs identified in FH patients worldwide



Iacocca MA and Hegele RA. *Curr Opin Lipidol.* 2018

Objective

- To determine the potential of applying bioinformatics to existing NGS data to accurately detect CNVs in *LDLR*, thus removing the need for secondary MLPA analysis

Methods

Study subjects

- 388 individuals from Canada with a clinical diagnosis of at least 'probable' FH per the DLCN criteria

Next-generation sequencing (NGS)

- LipidSeq
- 73 genes, including *LDLR, APOB, PCSK9* and *LDLRAP1, APOE, STAP1, ABCG5, ABCG8, LIPA*

CNV analysis by MLPA

- Multiplex PCR method
- Assay of promoter and all 18 exons in *LDLR*

CNV analysis by NGS data

- Bioinformatics applied to existing NGS data
- VarSeq CNV Caller: Depth of coverage analysis

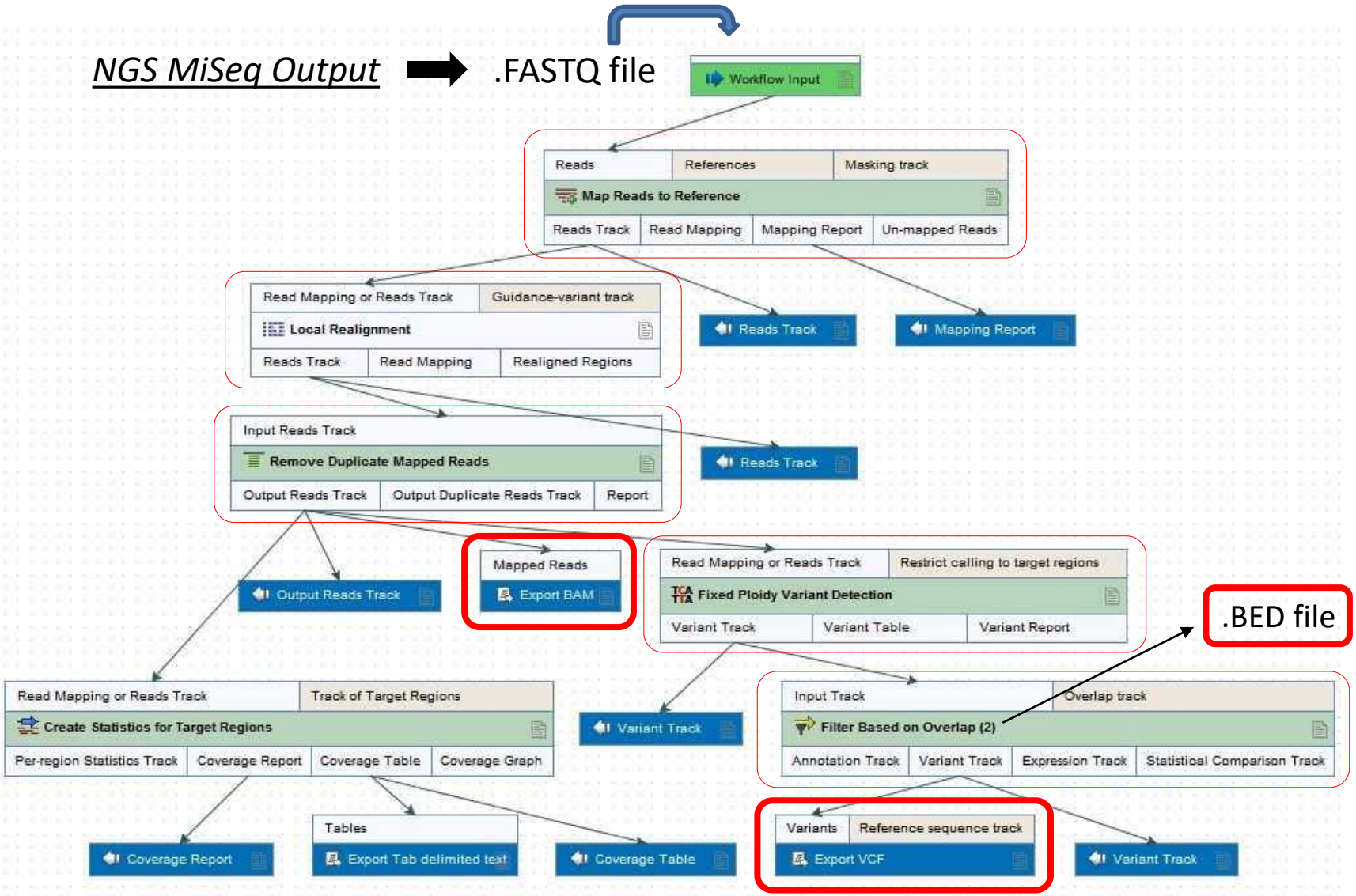
Methods: NGS Panel

LipidSeq Panel NGS

- 73 lipid metabolism-related genes, including all FH-associated genes *LDLR, APOB, PCSK9* and *LDLRAP1, APOE, STAP1, ABCG5, ABCG8, LIPA*
  - All exons, 150 bp at intron/exon boundaries, ~250 bp of 5'UTR
  - 178 SNP loci
- Library prep: Nextera Rapid Capture Custom Enrichment kit (Illumina)
- Platform: MiSeq (Illumina) – 2 x 150 bp paired-end chemistry
- Avg. 300-fold coverage per base

Johansen CT *et al. J Lipid Research.* 2014
Hegele RA *et al. Curr Opin Lipidol.* 2015

Methods: CLC Genomics Workbench



Dilliott AA *et al. J Vis Exp.* 2018

VarSeq CNV Caller Requirements

1) Patient sample

- .BAM file
- .VCF file

2) Matched reference controls (*N*= 30 to 50)

- .BAM file
- .VCF file

3) .BED file

Results

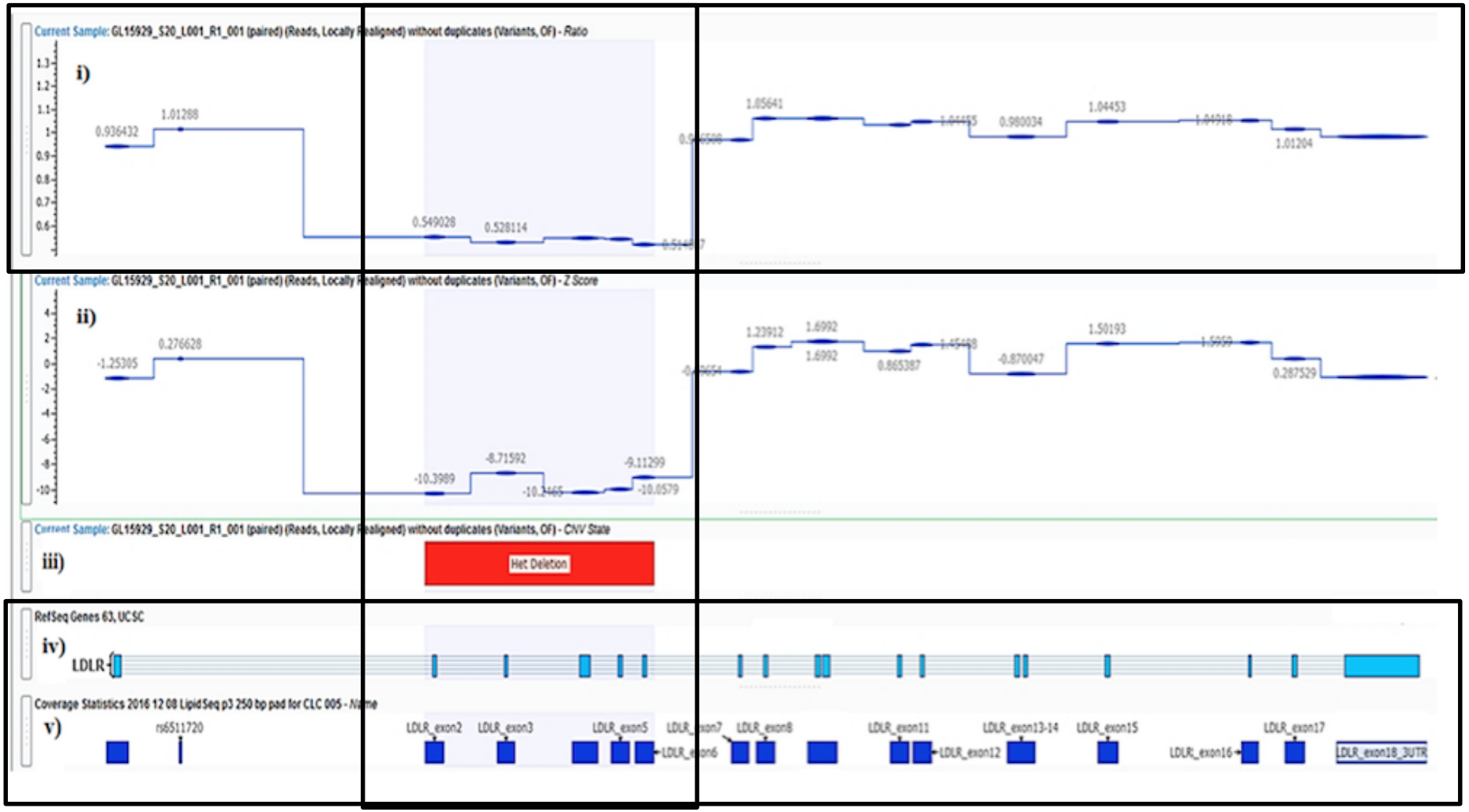
MLPA	
Type	**Region**
Het. Deletion	Promoter-Exon 1 (n=22)
Het. Deletion	Promoter-Exon 2 (n=2)
Het. Deletion	Promoter-Exon 6
Het. Deletion	Exons 2-3
Het. Deletion	Exons 2-6
Duplication	Exons 2-6
Het. Deletion	Exons 3-6
Het. Deletion	Exons 5-6
Duplication	Exon 7
Duplication	Exons 11-12
Het. Deletion	Exons 11-12
Het. Deletion	Exons 13-14
Het. Deletion	Exons 13-15
Het. Deletion	Exons 16-18
Het. Deletion	Exons 17-18 (n=2)

CNVs in *LDLR* detected by MLPA

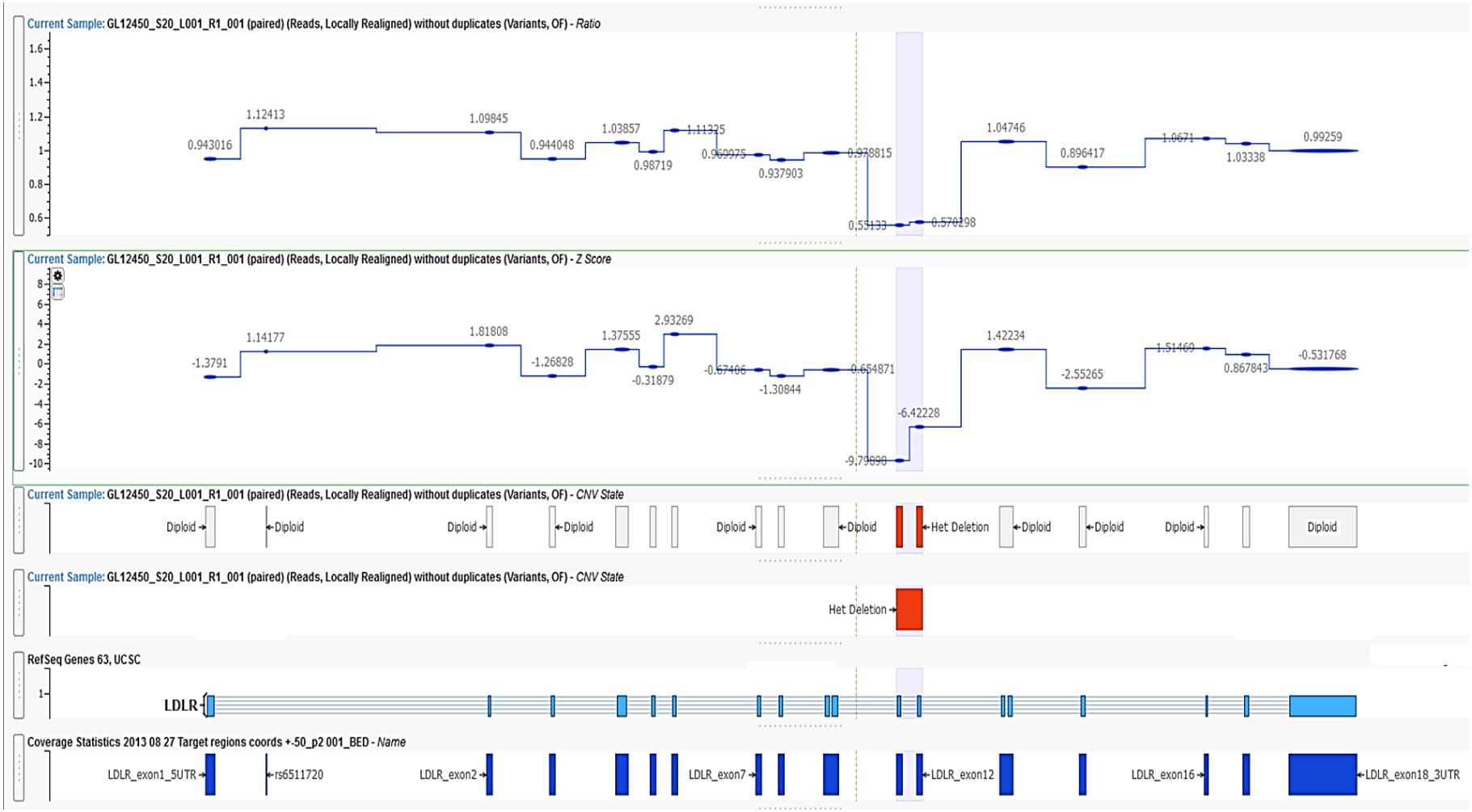
- 38 of 388 (9.8%) FH patients were CNV positive

Iacocca MA, Wang J, *et al. J Lipid Res.* 2017

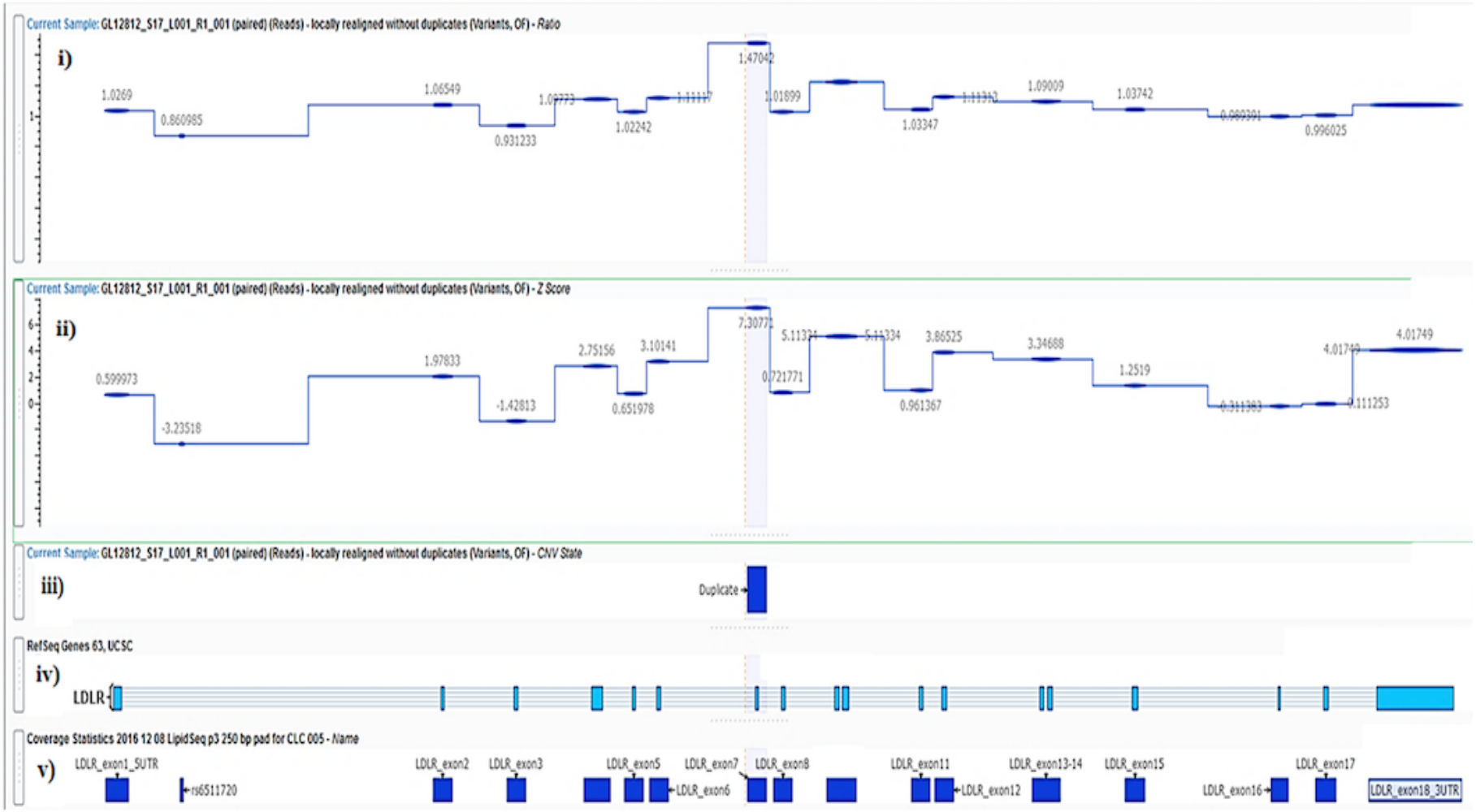
Ex) VarSeq NGS data output: *LDLR* Exons 2- 6 heterozygous deletion



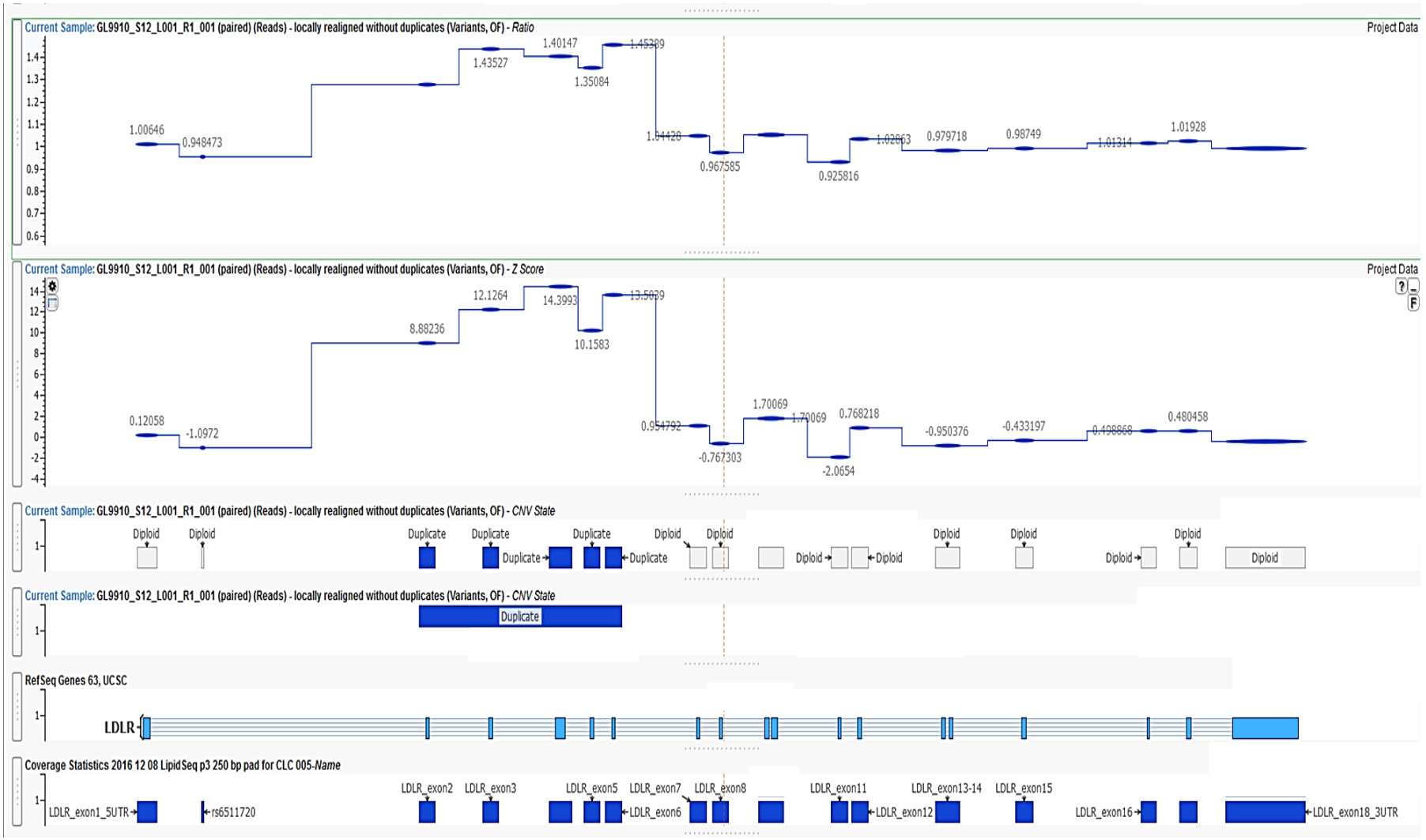
Ex) VarSeq NGS data output: *LDLR* Exons 11-12 heterozygous deletion



Ex) VarSeq NGS data output: *LDLR* Exon 7 duplication



Ex) VarSeq NGS data output: *LDLR* Exons 2-6 duplication



Results

Concordance

		MLPA Result	
		CNV	Diploid
NGS + VarSeq Result	Positive	True Positives **38**	False Positives **0**
	Negative	False Negatives **0**	True Negatives **350**

Sensitivity: 100%

Specificity: 100%

Iacocca MA, Wang J, *et al. J Lipid Res.* 2017

Implications

- Use of a single platform (NGS) for detection of both small and large-scale DNA variants

- Reduced costs, resources, analysis time associated with the routine molecular diagnosis of FH
  - MLPA: $80 USD per sample - $31,000 USD for this cohort of 388 samples

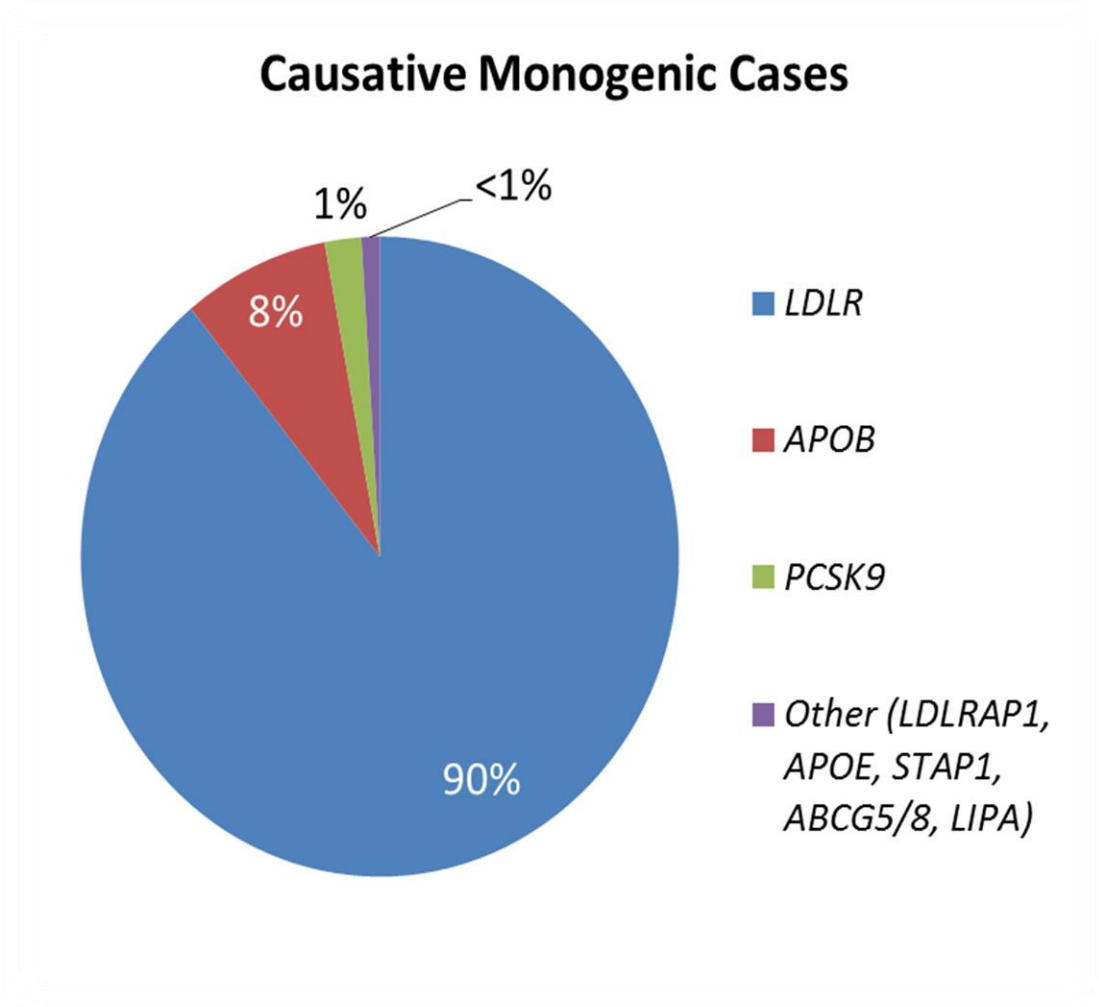
- Expanding CNV screening to all FH-associated genes on a given NGS panel at no extra cost

  LipidSeq: *APOB*, *PCSK9* and *LDLRAP1, APOE, STAP1, ABCG5/8, LIPA*

  ↳ further accounting for all genetic abnormalities capable of defining FH cases

Future Directions

- <u>Novel CNV screening in additional FH-associated genes</u>



Conclusion

- FH is the most prevalent monogenic disorder worldwide affecting ~1 in 250 individuals
- DNA testing increasingly becoming a central part of diagnosis; current procedure often includes targeted NGS followed by MLPA
- In analysis of 388 FH patient samples, there was 100% concordance in *LDLR* CNV detection between MLPA and NGS method
- Suggests MLPA is dispensable, significantly reducing associated costs, resources, analysis time
- All genes on a given NGS panel assessed for CNVs concurrently; allows for novel CNV screening in additional FH genes at no extra cost

  - promoting more widespread assessment of CNVs across diagnostic laboratories
  - potential for discovery of novel genetic mechanisms for FH
  - increasing molecular diagnostic yield

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