Enabling research translation: generating clinical genetic reports to improve the management of cardiovascular disease

Mark Trinder, MSc

MD/PhD student

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Supervisor: Dr. Liam Brunham

Centre for Heart and Lung Innovation

University of British Columbia (Canada)

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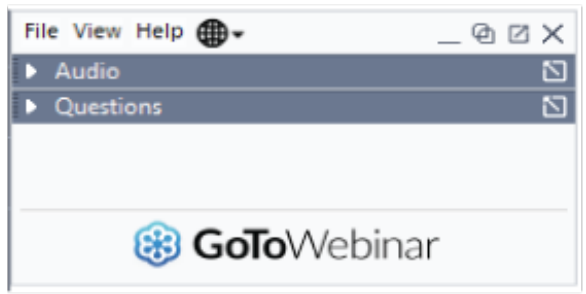

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Overview

Introduction to Familial Hypercholesterolemia.

Methods

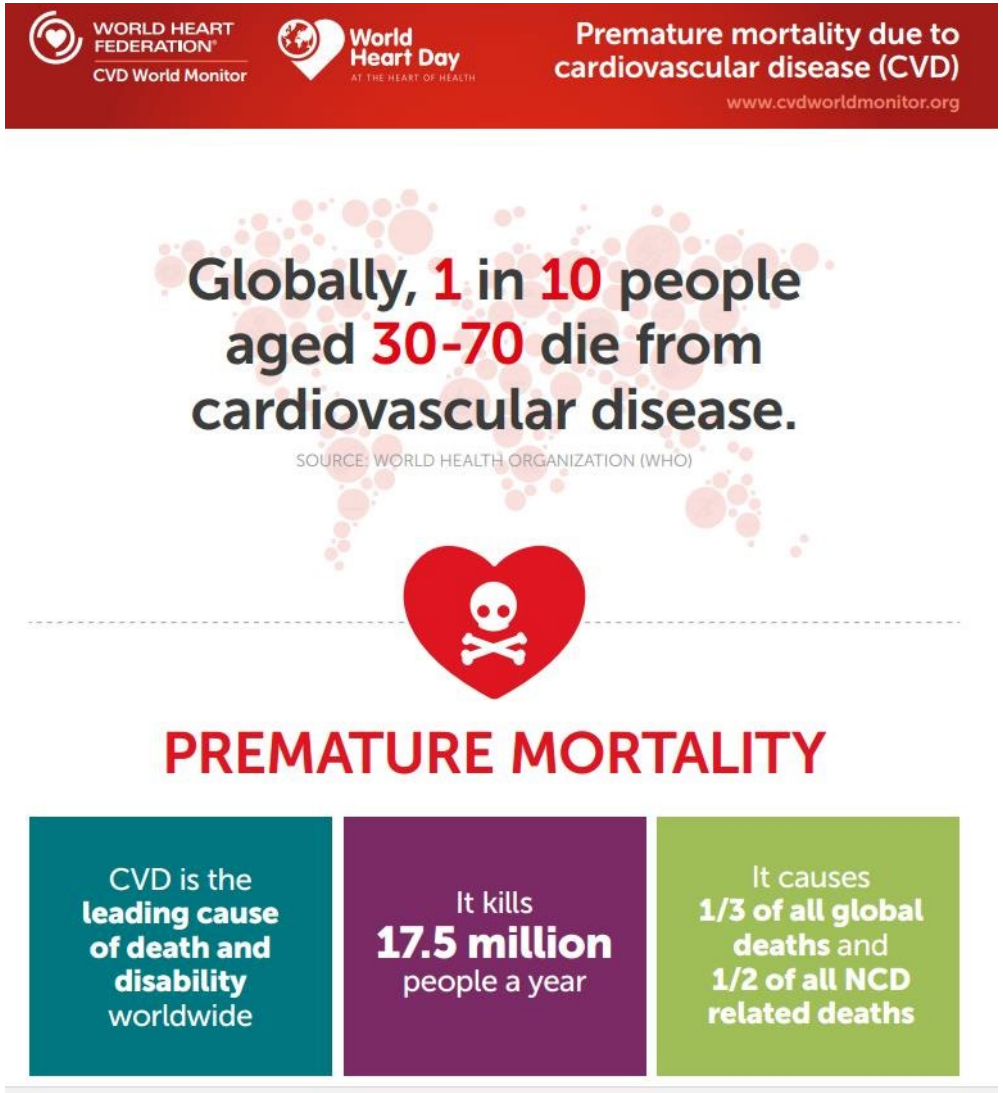
Results

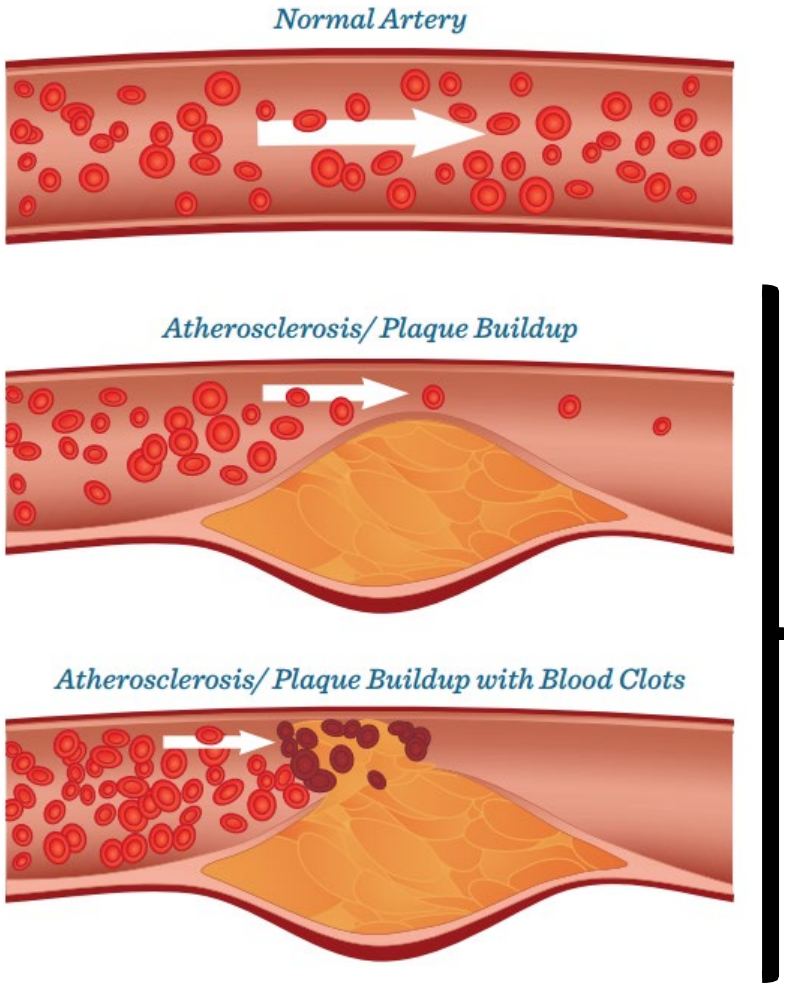
Our lab's preliminary results regarding the cardiovascular risk of monogenic versus polygenic causes of elevated low-density lipoprotein cholesterol.

Translating Results / Methodology to Potential Clinical Use

Conclusions

Cardiovascular disease and low-density lipoprotein cholesterol (LDL-C).



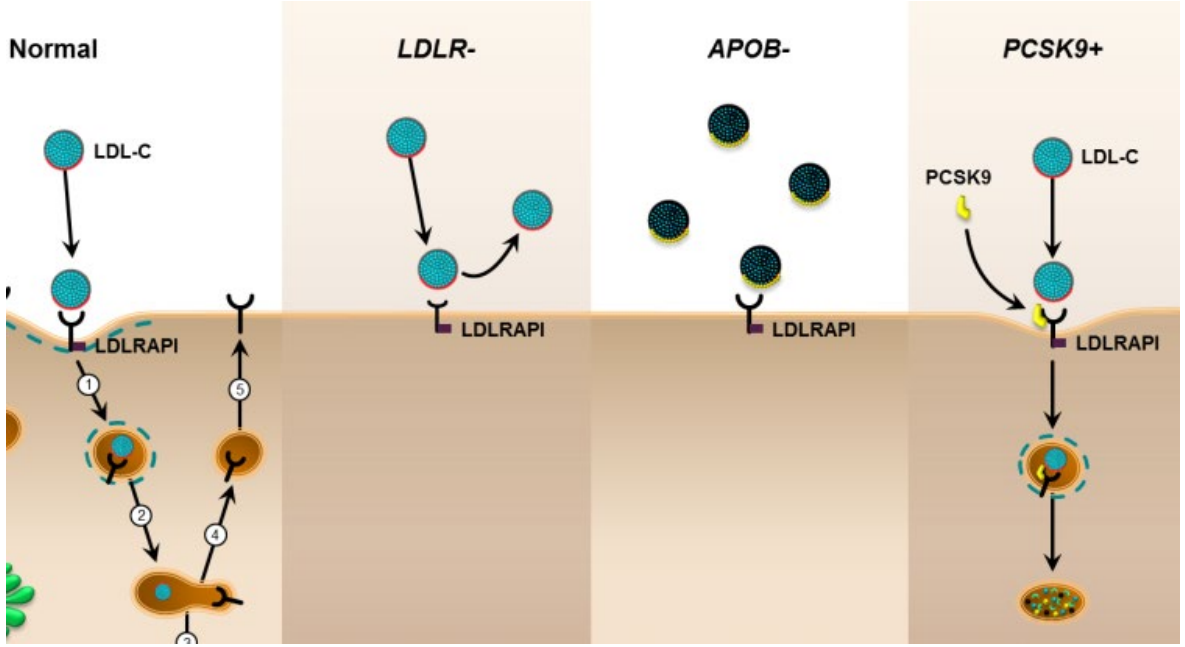


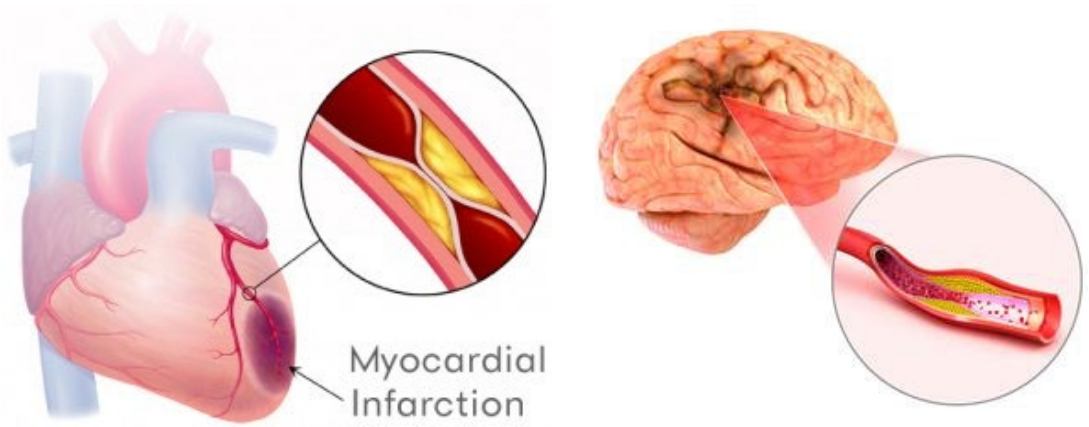
↑ LDL-C

Major, causal risk factor

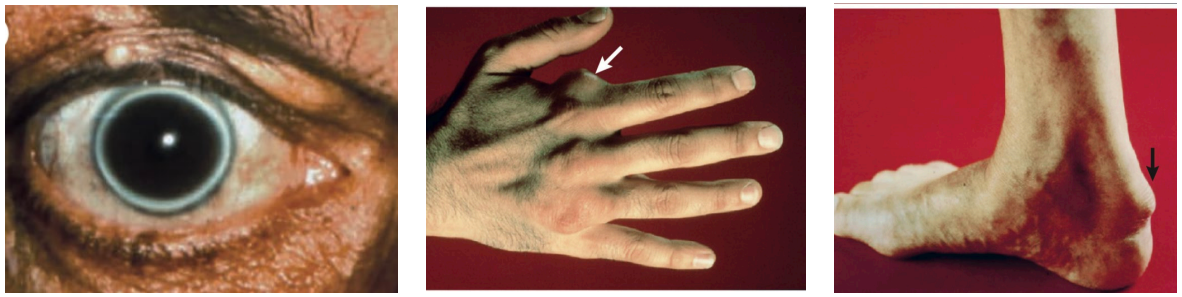
Familial hypercholesterolemia (FH)

- Most common autosomal dominant disorder
  - 1/250 people worldwide
- Characterized by damaging monogenic variants in *LDLR*, *APOB*, or *PCSK9*
  - → ↑ low-density lipoprotein cholesterol (LDL-C)
  - ***→↑ risk of <u>premature</u> coronary artery disease***





FH



Defesche et al. *Nature Reviews* 2017;3(17093):1-20.

- The gold-standard for a diagnosis of FH is DNA testing

- However, this is not frequently done. Instead clinical scoring systems exist:
  - **Dutch Lipid Clinic Network criteria**
  - Simone Broome diagnostic criteria
  - Etc.

Table 1. Dutch Lipid Clinic Network criteria for the diagnosis of heterozygous familial hypercholesterolemia (hFH).[33,34]

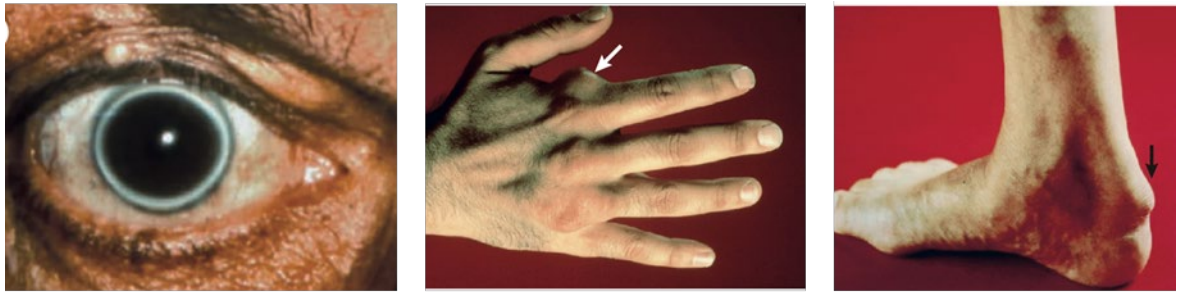
Criteria		Score
Family history	First-degree adult relative with	
	• Premature coronary and/or vascular disease (male < 55 years; female < 60 years)	1
	• LDL-C > 95th percentile for age and gender	1
	• Tendon xanthomata and/or arcus cornealis	2
	First-degree relative < 18 years with LDL-C > 95th percentile for age and gender	2
Clinical history	Patient with premature IHD (ages as above)	2
	Patient with other premature vascular and/or cerebrovascular disease (ages as above)	1
Physical examination	Tendon xanthomata	6
	Arcus cornealis prior to age 45	4
Laboratory analysis	LDL-C (mmol/L)	
	• ≥8.5	8
	• 6.5–8.4	5
	• 5.0–6.4	3
	• 4.0–4.9	1
DNA analysis	Genetic test results confirming functional mutation in *LDLR*, *APOB*, or *PCSK9* gene	8

LDL-C = low-density lipoprotein cholesterol; IHD = ischemic heart disease; *LDLR* = low-density lipoprotein receptor; *APOB* = apolipoprotein B-100; *PCSK9* = proprotein convertase subtilisin/kexin9

Wong et al., 2013 *BCMJ*

Familial hypercholesterolemia (FH)

- It is estimated that ~20 0000 people in British Columbia have FH, however >85% are undiagnosed and undertreated (Benn et al. 2016; Nordestgaard et al., 2013; Wong et al., 2013)



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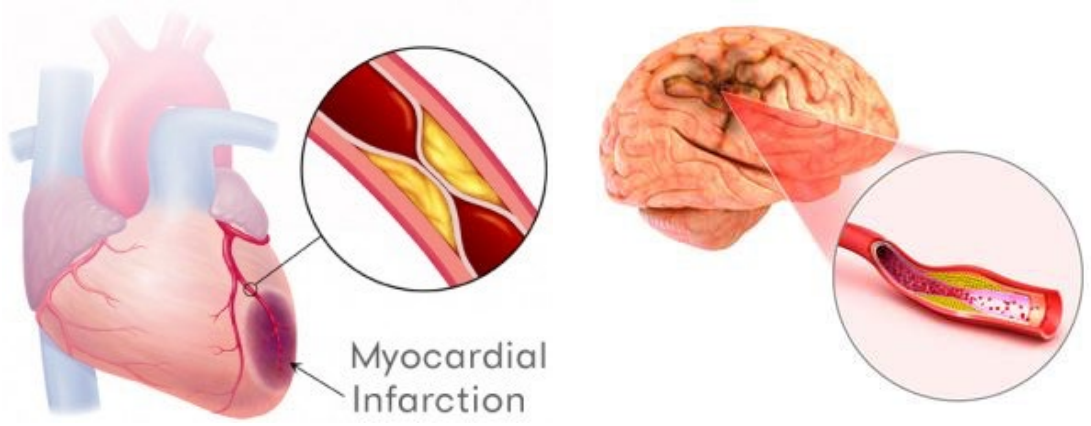
- **Problem**: these individuals have the highest risk for cardiovascular disease and would most strongly benefit from preventative medicine

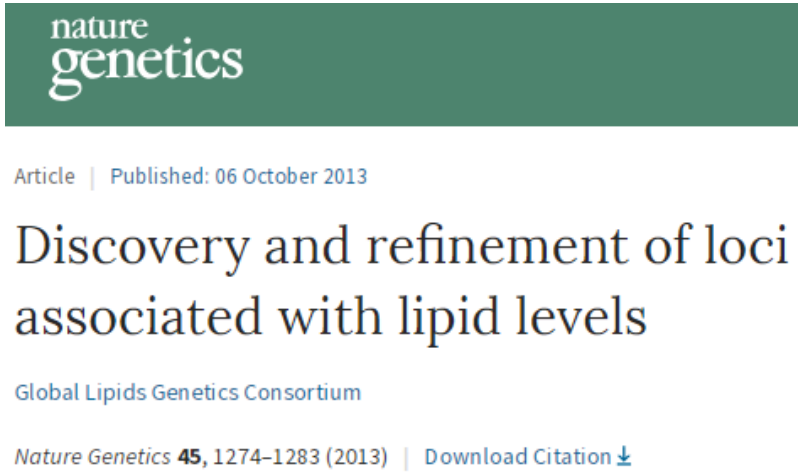


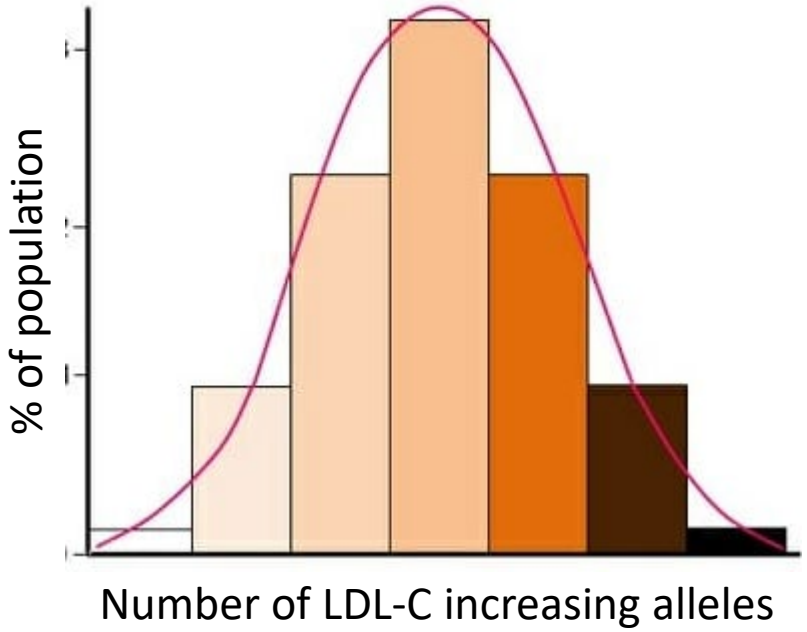
Polygenic causes of hypercholesterolemia

Causes of Hypercholesterolemia

- ~30 – 80 % monogenic FH-causing variants (SNPs, CNVs)
- ~20% polygenic hypercholesterolemia (Talmud et al., 2013; Futema et al., 2015; Wang et al., 2015)

- LDL-C is a polygenic trait





The Question…

Causes of Familial Hypercholesterolemia

- ~30 – 80 % pathogenic FH-causing variants
- ~20% polygenic

- Is the identification of FH-causing variants or polygenic risk clinically meaningful?
  - LDL-C levels alone associate with coronary artery disease risk in patients with FH (Perak et al.; 2016).
  - FH-causing variants associate with coronary artery disease independent of LDL-C (Khera et al., 2016; Tada et al., 2017).

Hypothesis

Clinical FH with*:*

- An FH-causing variant
- and elevated LDL-C polygenic risk scores…

…have greater risk of **premature *coronary artery disease (<55 years old)*** than patients in whom a causative variant is not identified.

Methods: Overview

HF Canada FH Canada
Hypercholestérolémie Familiale Familial Hypercholesterolemia

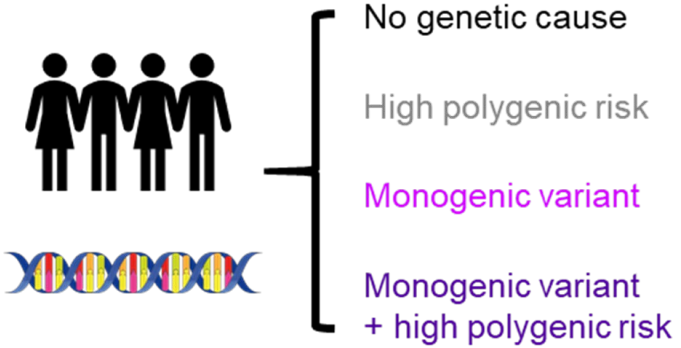
Prospective database of 626 patients clinically diagnosed with heterozygous FH.

Dutch Lipid Network Clinic criteria:

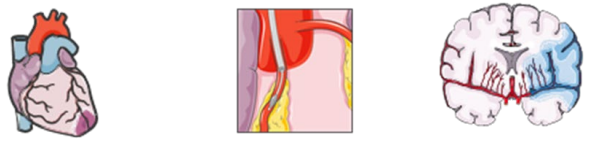
- Possible
- Probable
- Definite

J Lipid Res. 2015 Oct;56(10):1993-2001.

DNA isolated & prepared for lipid-gene next-generation sequencing.



Assess risk for very premature atherosclerotic cardiovascular disease.



Myocardial infarction | Unstable angina Revascularization | Stroke

Methods: Variant annotation

Variants were considered monogenic single-nucleotide FH-causing variants if:

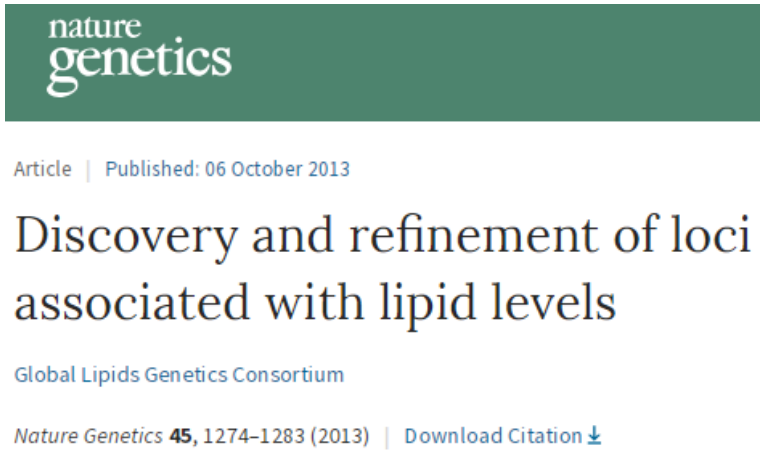
- *LDLR*, *APOB*, *PCSK9, LDLRAP1* genes variants were annotated in ClinVar as pathogenic or likely pathogenic
- Novel LDRL frameshift or nonsense variants
- Novel or ambiguously annotated *LDLR* missense variants were deemed pathogenic by 5 of 6 bioinformatic tools

Variants were considered monogenic CNV FH-causing variants if:

- VarSeq Copy-Number Variation (CNV) Caller application was used to detect structural variants in the *LDLR*, *APOB*, *PCSK9*, and *LDLRAP1* genes **(Iacocca et al. 2017; Journal of Lipid Research)**

LDL-C polygenic score calculations:

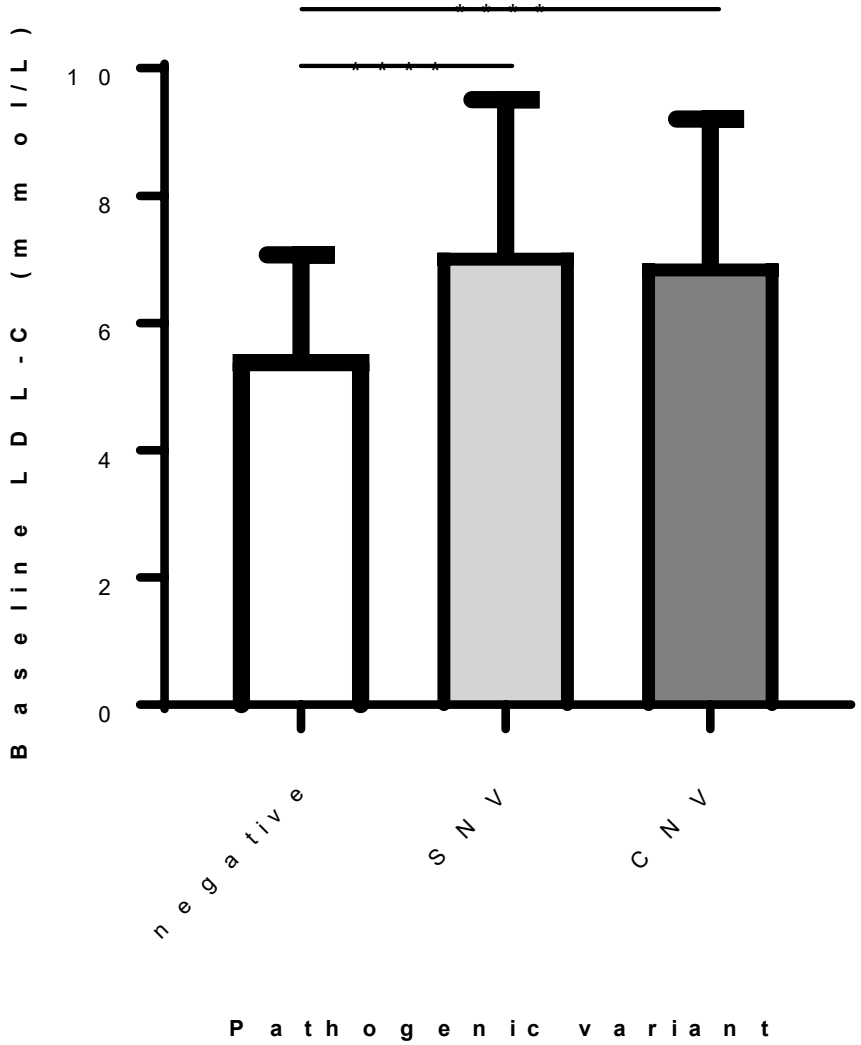
- LDL-C weighted scores were calculated using the effect sizes of 28 SNPs from the genome-wide association study discovery sample
- Polygenic risk scorey = Σ [βx,discovery * SNPxy]

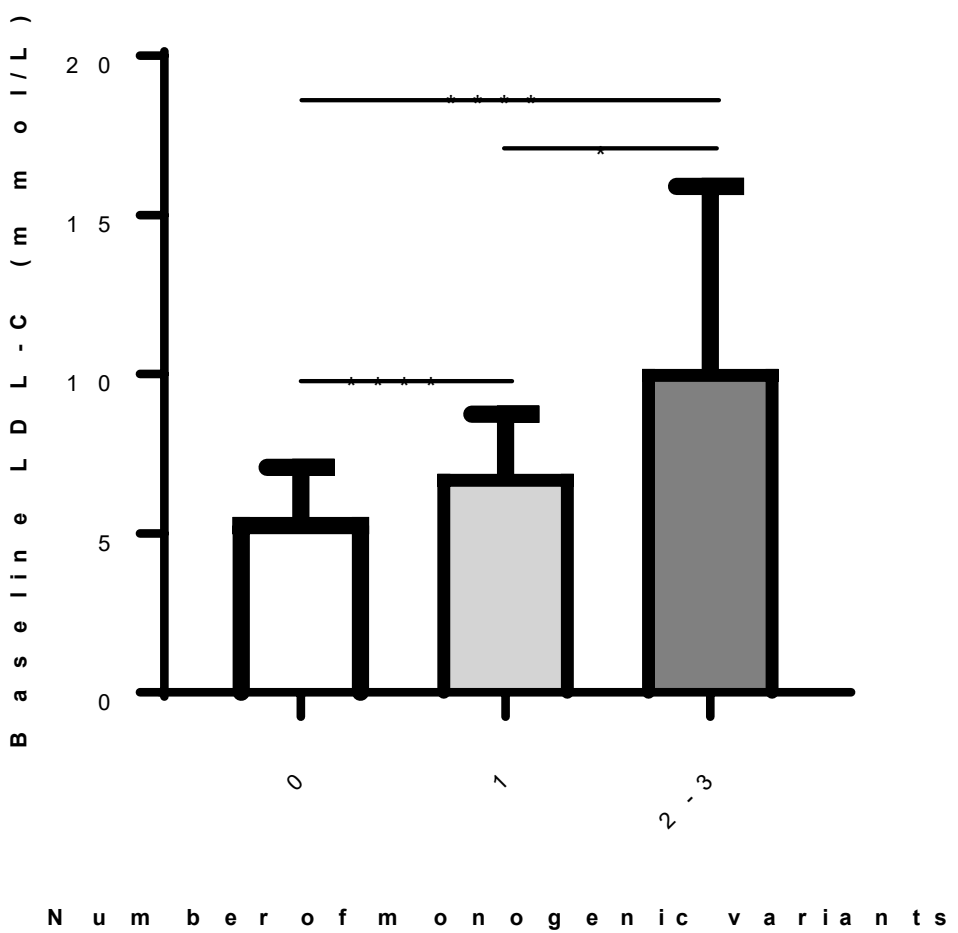


Results: Patient characteristics

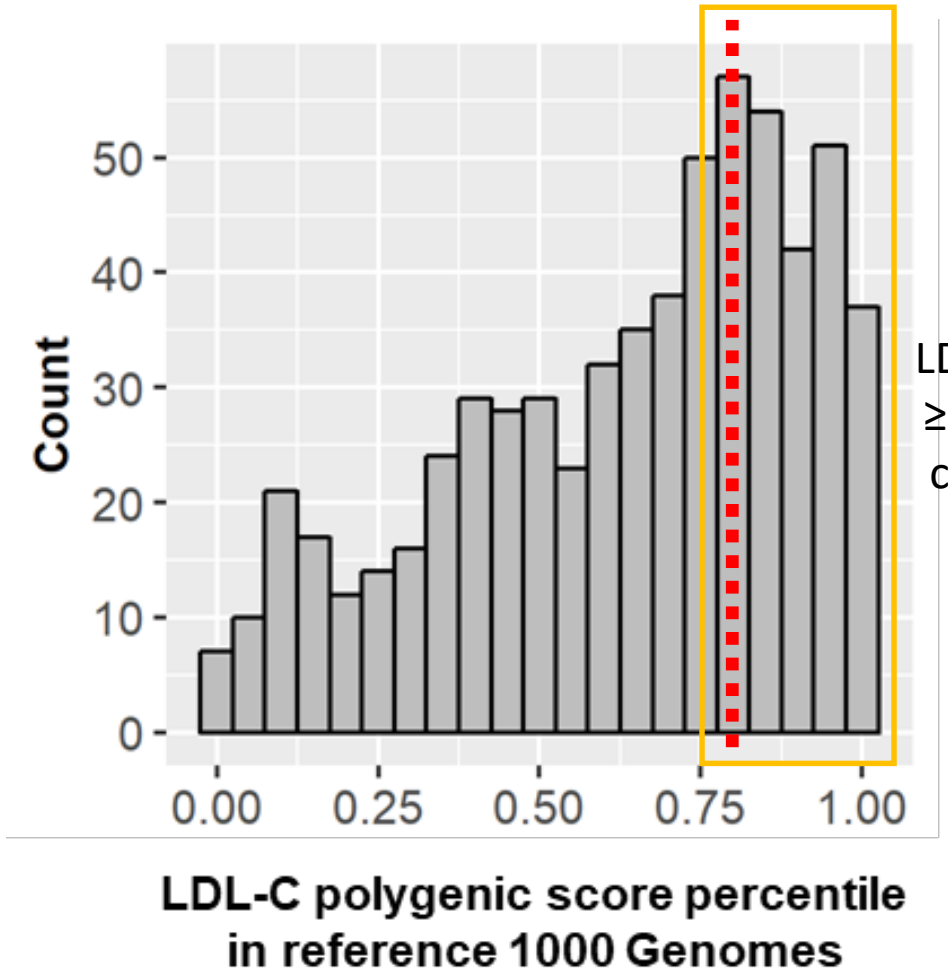
Demographics	Measure	Value
n	No.	626
Age (years)	Mean (SD)	46.2 (15.1)
Sex (female)	No. (%)	317 (50.6)
DLCNC score	Median (IQR)	7 (5 - 11)
Baseline lipids		
Total-C (mmol/L)	Mean (SD)	8.34 (2.18)
LDL-C (mmol/L)	Mean (SD)	6.19 (2.11)
HDL-C (mmol/L)	Mean (SD)	1.40 (0.43)
TG (mmol/L)	Mean (SD)	1.62 (0.88)
Lipoprotein(a) (mg/L)	Median (IQR)	282 (102 – 755)
Lipid-lowering medication	No. (%)	127 (21.9)
Medical history		
Current smoker	No. (%)	25 (4.0)
Hypertension	No. (%)	153 (24.4)
Diabetes mellitus	No. (%)	53 (8.5)
FHx CAD	No. (%)	421 (67.2)

Results: LDL-C levels versus monogenic variants

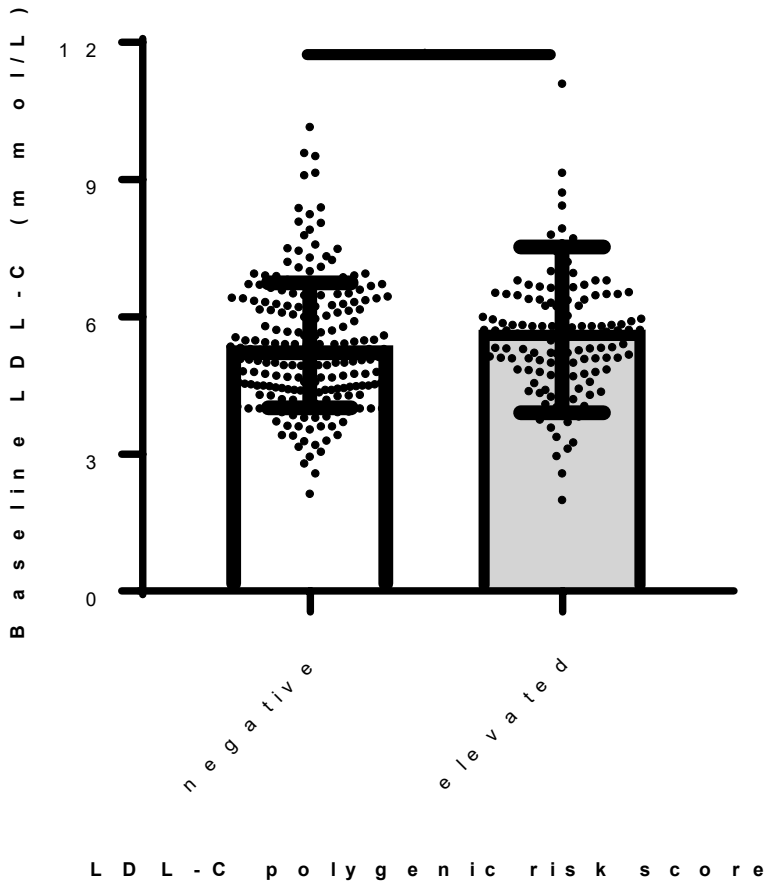




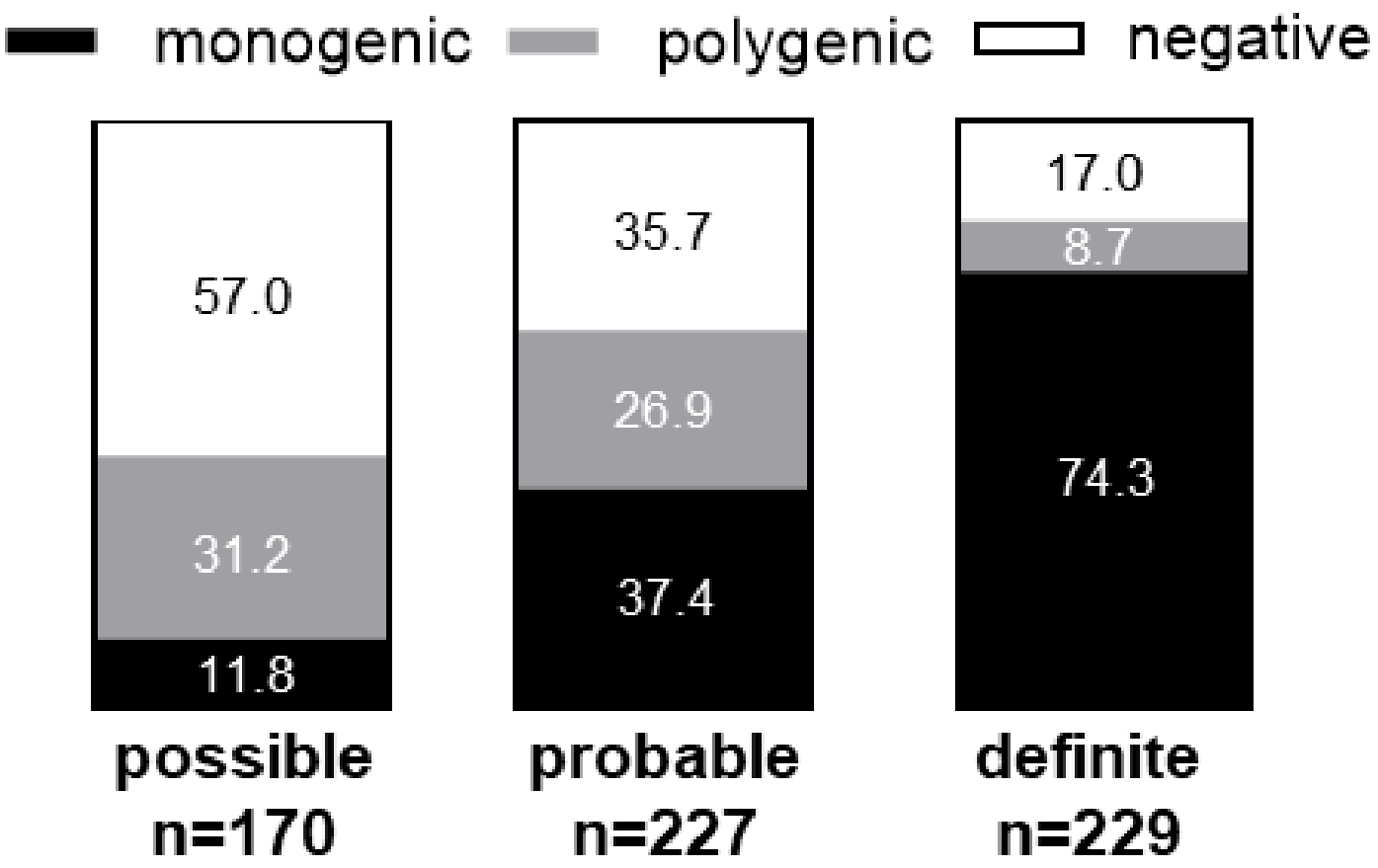
Results: LDL-C levels versus polygenic status



LDL-C polygenic score ≥80th percentile was considered elevated



Results:



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Clinical history	Patient with premature IHD (ages as above)	2
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Physical examination	Tendon xanthomata	6
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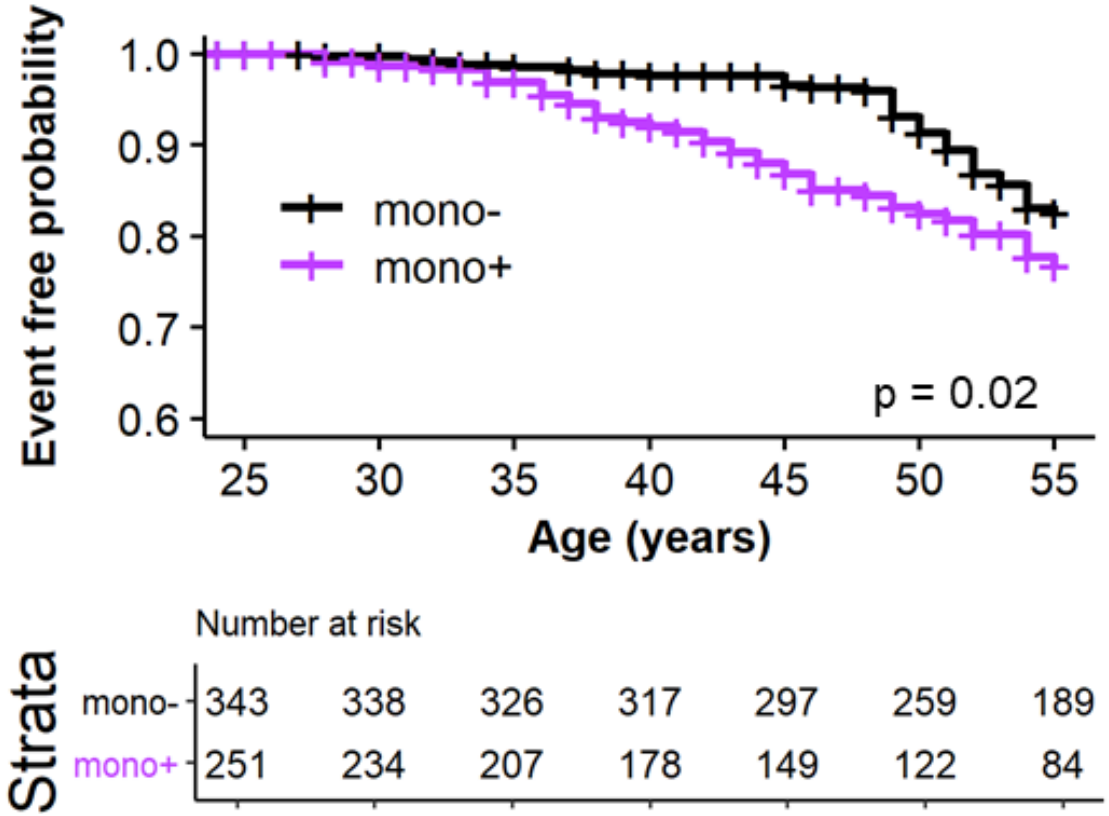
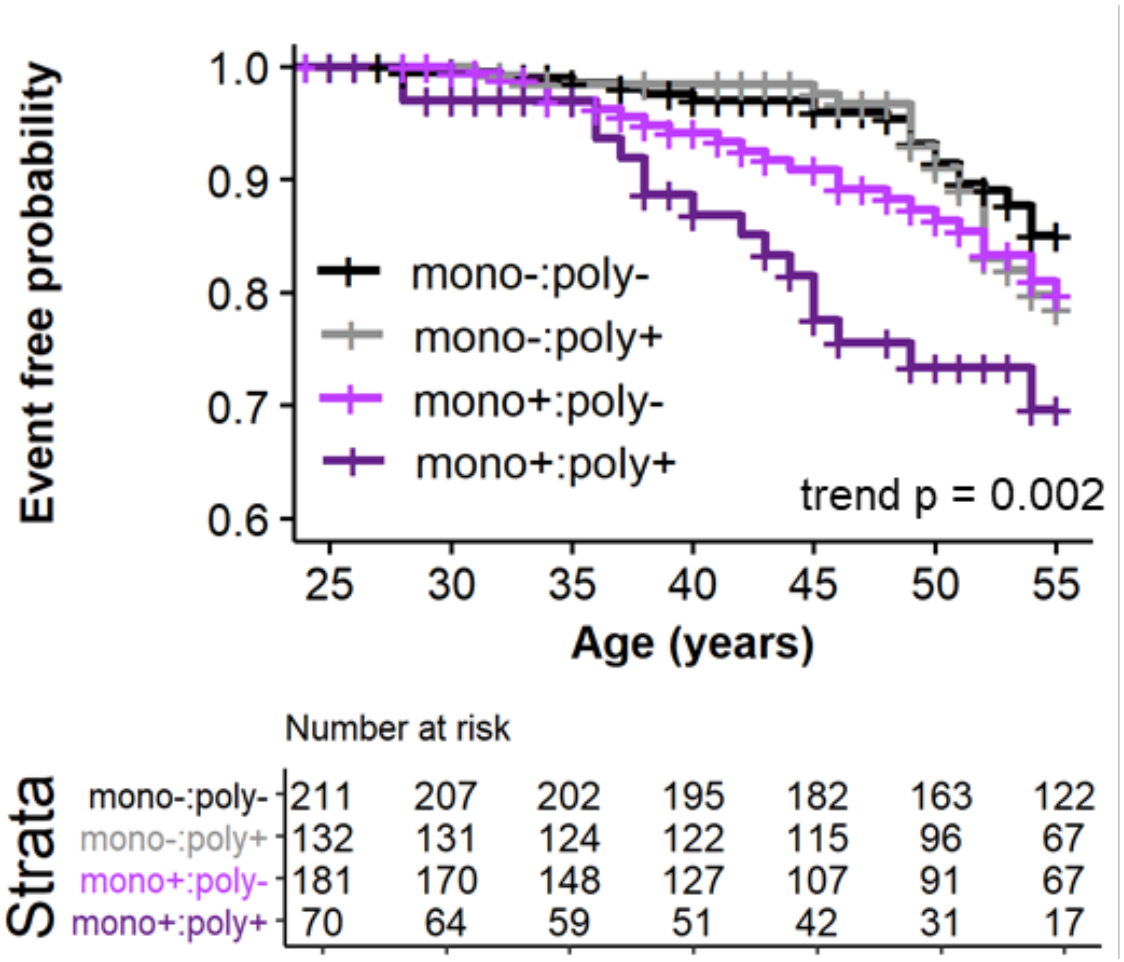
Wong et al., 2013 *BCMJ*

Results: Patient characteristics

Demographics	Measure	mono- : poly -	mono- : poly+	mono+ : poly -	mono+ : poly+	p
n	No.	217	134	198	77	-
Age (years)	Mean (SD)	52.2 (12.8)	51.7 (11.3)	39.4 (15.7)	37.5 (14.3)	<0.0001
Sex (female)	No. (%)	100 (46.1)	60 (44.8)	116 (58.6)	41 (53.2)	0.02
DLCNC score	Median (IQR)	6 (4 – 7)	6 (5 – 7)	10 (7 – 14)	12 (8 – 16)	<0.0001
Baseline lipids						
Total-C (mmol/L)	Mean (SD)	7.63 (1.56)	7.97 (2.01)	9.12 (2.60)	8.95 (1.96)	<0.0001
LDL-C (mmol/L)	Mean (SD)	5.38 (1.37)	5.72 (1.81)	7.08 (2.54)	7.05 (1.91)	<0.0001
HDL-C (mmol/L)	Mean (SD)	1.45 (0.47)	1.43 (0.39)	1.37 (0.40)	1.30 (0.41)	0.02
TG (mmol/L)	Mean (SD)	1.79 (0.83)	1.76 (0.91)	1.44 (0.90)	1.37 (0.80)	<0.0001
Lipoprotein(a) (mg/L)	Median (IQR)	304 (102 – 827)	277 (101 – 681)	264 (104 – 735)	303 (104 – 667)	0.9
LLM	No. (%)	39 (14.0)	27 (20.1)	49 (24.7)	22 (28.6)	0.02
Medical history						
Current smoker	No. (%)	8 (3.7)	6 (4.5)	7 (3.5)	4 (5.2)	0.8
Hypertension	No. (%)	69 (31.8)	41 (30.6)	28 (14.1)	15 (19.5)	0.0001
Diabetes mellitus	No. (%)	25 (11.5)	8 (6.0)	14 (7.1)	6 (7.8)	0.2
FHx CAD	No. (%)	155 (71.4)	89 (66.4)	122 (61.6)	55 (71.4)	0.3

Results: Genetics and premature cardiovascular risk





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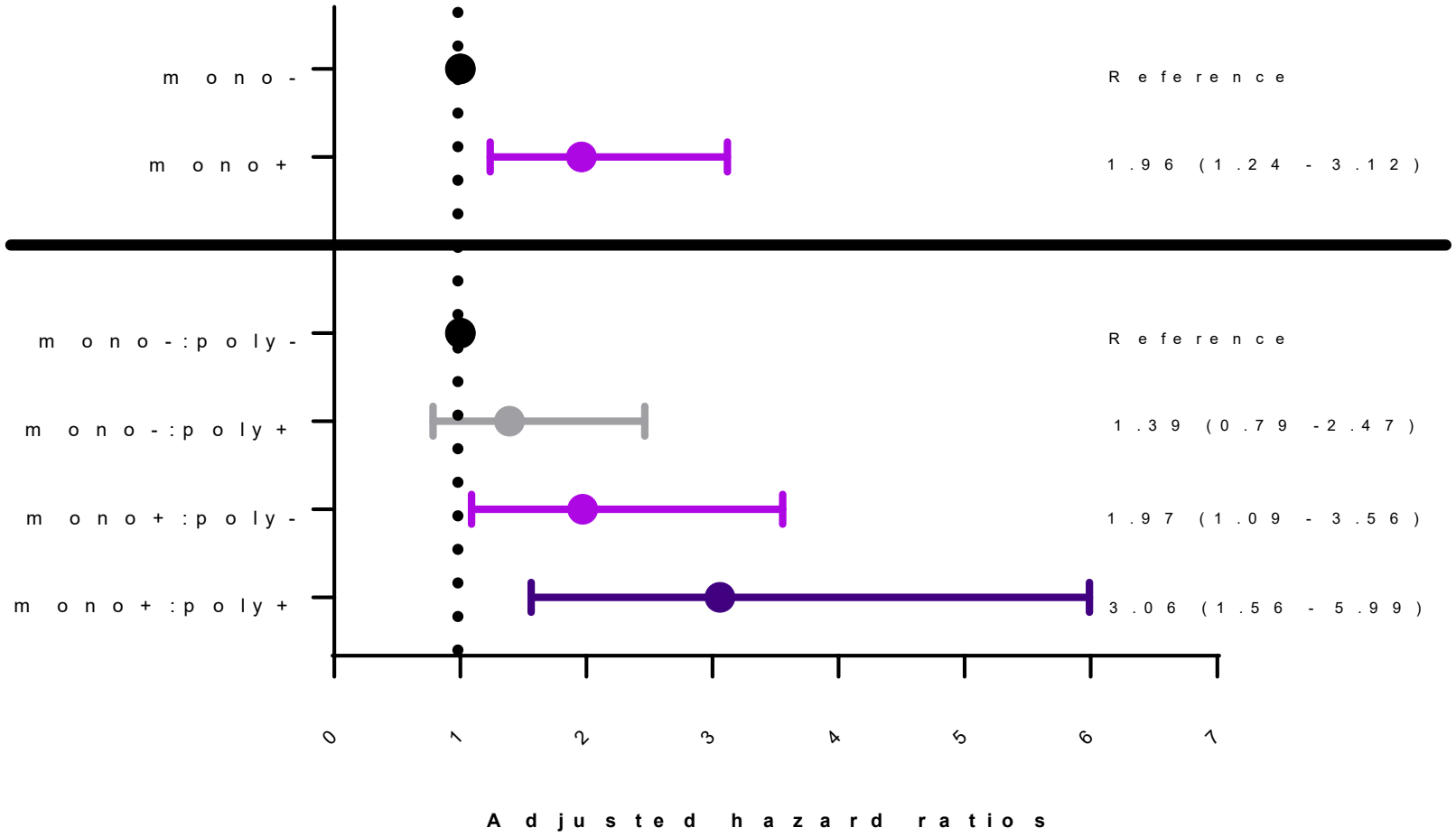
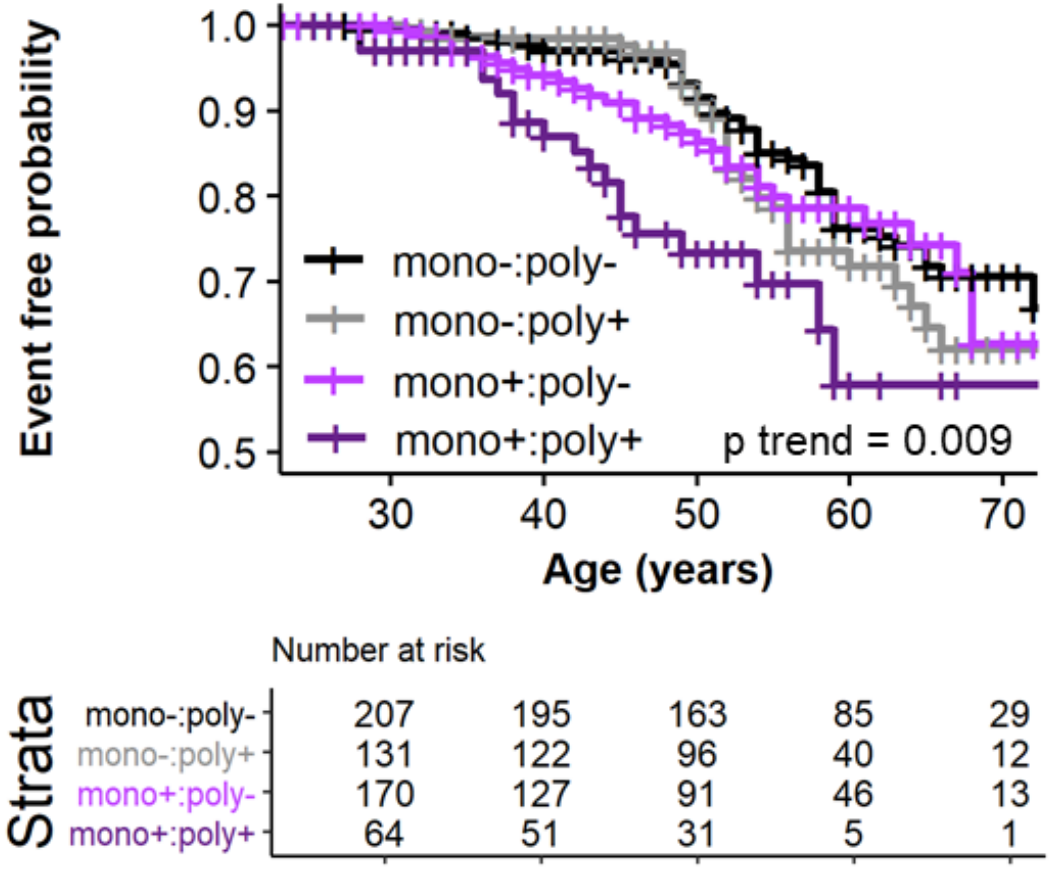
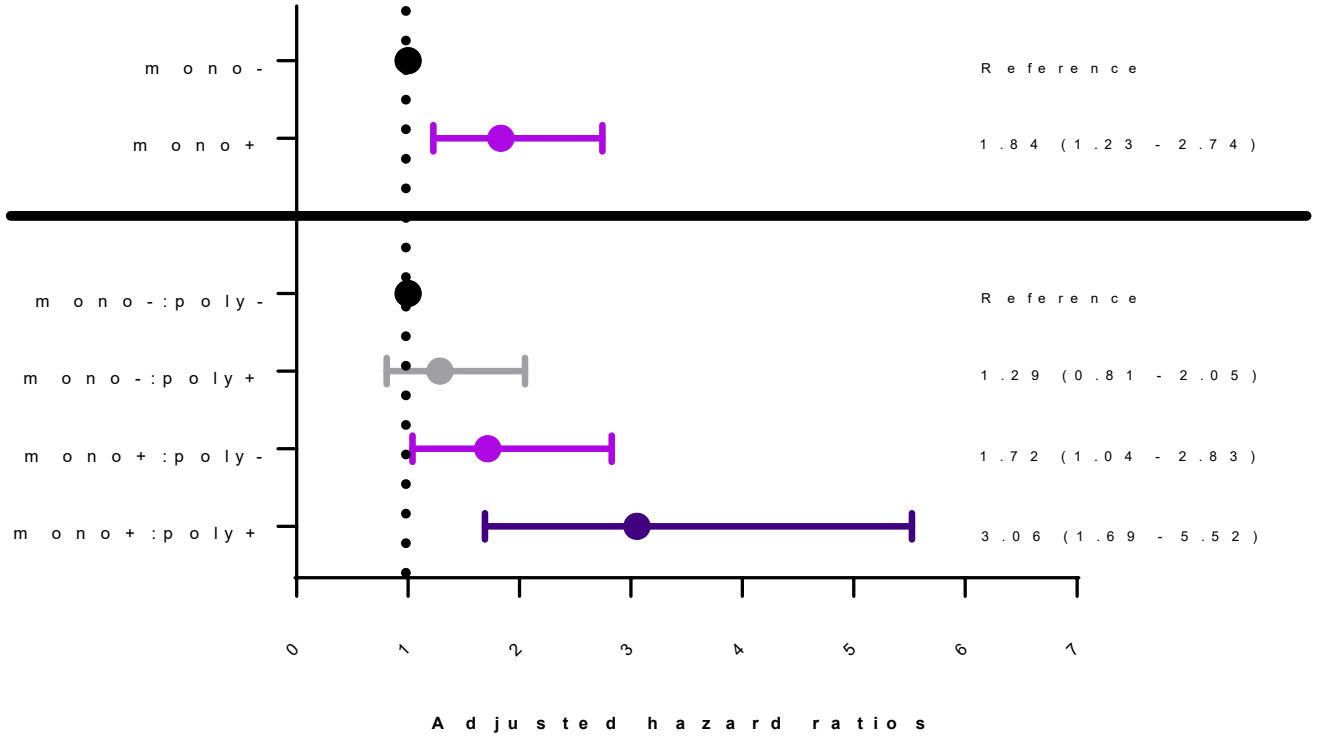


Adjusted for age, sex, LDL-C, diabetes, & hypertension

Results: Genetics and overall cardiovascular risk

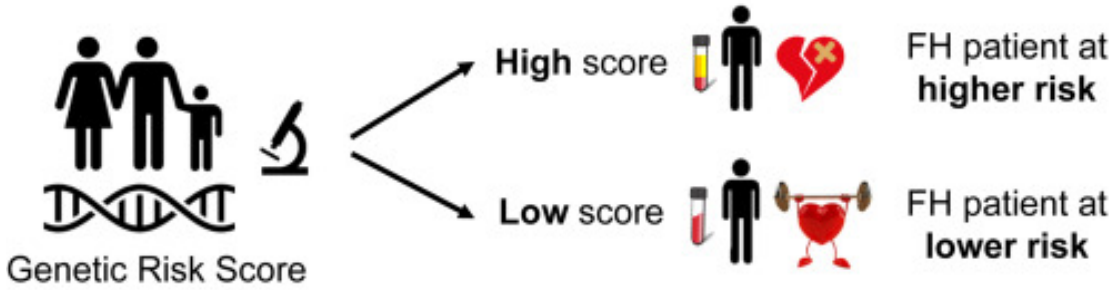




Adjusted for age, sex, LDL-C, diabetes, & hypertension

Preliminary Summary

- We identified 22 monogenic FH-causing variants in the *LDLR* gene not reported in ClinVar → potentially novel
- An elevated LDL-C polygenic score acted as a risk enhancer in patients with monogenic FH
- Genetic testing for monogenic and polygenic causes of FH provides important prognostic information that is independent of LDL-C levels.



Paquette et al. 2017, *Journal of Clinical Lipidology*

Making Clinical Use

- VarSeq software suite includes VS Clinical
  - ACMG/AMP joint guidelines for variant interpretation provide a set of criteria to score variants

Sequencing data

HEALTHY HEART PROGRAM – PREVENTION CLINIC: TARGETED LIPID GENE SEQUENCING

St. Paul's Hospital, Room 180, 1081 Burrard Street, Vancouver British Columbia V6K 1Y6 | Phone: 604-806-8591 | Fax: 604-806 8590

CASE ID : FH005071301

Patient Name :

Gender : Female

Date of Birth : 6/5/1990

SAMPLE INFORMATION

SAMPLE SITE	Centre for Heart and Lung Innovation	AVG. READ DEPTH	504x
SAMPLE TYPE	Saliva	COLLECTION DATE	7/21/2016
COLLECTION METH…	Oragene•DNA, catalog no: OG-500	RECEIPT DATE	11/15/2017
PANEL COVERAGE	88.67%	REPORT DATE	3/4/2019

RESULTS

POSITIVE. Variants with an established link to Familial Hypercholesterolemia detected. The patient's low-density lipoprotein cholesterol polygenic risk score was in the 0.64 percentile relative to the 1000 Genomes European superpopulation reference (calculated from 28 single nucleotide polymorphisms).

AFFECTED GENES

PRIMARY FINDINGS

No variants specified

INTERPRETATION

The splice donor variant c.313+1G>A in LDLR (NM_000527.4) has not been reported previously as a pathogenic variant nor as a benign variant, to our knowledge. The c.313+1G>A variant is novel (not in any individuals) in 1000 Genomes. The c.313+1G>A variant is a loss of function variant in the gene LDLR, which is intolerant of Loss of Function variants, as indicated by the presence of existing pathogenic loss of function variant NP_000518.1:p.Met1? and 460 others. There are 496 downstream pathogenic loss of function variants, with the furthest variant being 746 residues downstream of the variant c.313+1G>A. The patient's phenotype or family history is highly specific for a disease with a single genetic etiology. For these reasons, this variant has been classified as Pathogenic.

RECOMMENDATIONS

This result is consistent with a molecular diagnosis of heterozygous Familial Hypercholesterolemia. The patient's first degree relatives have a 50% chance of also carrying this DNA variant and should be screened for Familial Hypercholesterolemia.

SUMMARY

INDIVIDUAL VARIANT INTERPRETATIONS DISPLAY

REFERENCES DISPLAY

ADDITIONAL INFORMATION DISPLAY

DRAFT REPORT

*** DISCLAIMER ***
AS THE PATIENTS' ATTENDING PHYSICIAN YOU ARE RESPONSIBLE TO PROVIDE ANY APPROPRIATE COUNSELING OF FOLLOW-UP IN RELATION TO THESE RESULTS.

THESE DATA WERE GENERATED FROM A RESEARCH PROTOCOL AND DO NOT REPRESENT A CLINICALLY-VALIDATED DIAGNOSTIC TEST.

Population Criteria: 2

Pathogenic:

PM2 — Moderate

Absent from controls in population catalogs

Unanswered

Evidence for "No"

Comment

Benign:

BS1 — Strong

Allele frequency is greater than expected for disorder

Unanswered

Evidence for "No"

Comment

Gene Impact Criteria: 1

Pathogenic:

PVS1 — Very Strong

Null variant in a gene where LOF is a known mechanism of disease

Unanswered

Evidence for "Yes"

Comment

Benign:

No Criteria Found

Studies Criteria:

Pathogenic:

>	PS3 Strong	Well-established functional studies supportive of a damaging effect on the gene or gene product	Uncertain	Comment
>	PP5 Supporting	Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation	Uncertain	Comment
>	PS1 Strong	Same amino acid change as a previously established pathogenic variant	Uncertain	Comment

Benign:

>	BS3 Strong	Well-established functional studies show NO damaging effect on the gene or gene product	Uncertain	Comment
>	BP6 Supporting	Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation	Uncertain	Comment

Clinical Criteria:

Pathogenic:

PP4	Supporting	Patient's phenotype or family history is highly specific for a disease with a single genetic etiology	Uncertain	Comment
PP1	Supporting	Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease	Uncertain	Comment

Benign:

BP5	Supporting	Variant found in a case with an alternate molecular basis for disease	Uncertain	Comment
BS4	Strong	Lack of segregation in affected members of a family	Uncertain	Comment

Interpretation

Exclude Variant From Evaluation:

☐ Dismiss/Fail Variant

Include in Reports (Record Sets):

☑ Primary Findings ☐ Secondary Findings

Classification:

Pathogenic

For Disorder:

Hypercholesterolemia, Familial

Inheritance / Variant Type:

Autosomal Dominant / Heterozygous

Interpretation:

The splice donor variant c.313+1G>A in LDLR (NM_000527.4) has not been reported previously as a pathogenic variant nor as a benign variant, to our knowledge. The c.313+1G>A variant is novel (not in any individuals) in 1000 Genomes. The c.313+1G>A variant is a loss of function variant in the gene LDLR, which is intolerant of Loss of Function variants, as indicated by the presence of existing pathogenic loss of function variant NP_000518.1:p.Met1? and 460 others. There are 496 downstream pathogenic loss of function variants, with the furthest variant being 746 residues downstream of the variant c.313+1G>A. The

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PANEL COVERAGE	88.67%	REPORT DATE	3/4/2019

RESULTS

POSITIVE. Variants with an established link to Familial Hypercholesterolemia detected. The patient's low-density lipoprotein cholesterol polygenic risk score was in the 0.64 percentile relative to the 1000 Genomes European superpopulation reference (calculated from 28 single nucleotide polymorphisms).

AFFECTED GENES

PRIMARY FINDINGS

No variants specified

INTERPRETATION

The splice donor variant c.313+1G>A in LDLR (NM_000527.4) has not been reported previously as a pathogenic variant nor as a benign variant, to our knowledge. The c.313+1G>A variant is novel (not in any individuals) in 1000 Genomes. The c.313+1G>A variant is a loss of function variant in the gene LDLR, which is intolerant of Loss of Function variants, as indicated by the presence of existing pathogenic loss of function variant NP_000518.1:p.Met1? and 460 others. There are 496 downstream pathogenic loss of function variants, with the furthest variant being 746 residues downstream of the variant c.313+1G>A. The patient's phenotype or family history is highly specific for a disease with a single genetic etiology. For these reasons, this variant has been classified as Pathogenic.

RECOMMENDATIONS

This result is consistent with a molecular diagnosis of heterozygous Familial Hypercholesterolemia. The patient's first degree relatives have a 50% chance of also carrying this DNA variant and should be screened for Familial Hypercholesterolemia.

SUMMARY

INDIVIDUAL VARIANT INTERPRETATIONS DISPLAY

REFERENCES DISPLAY

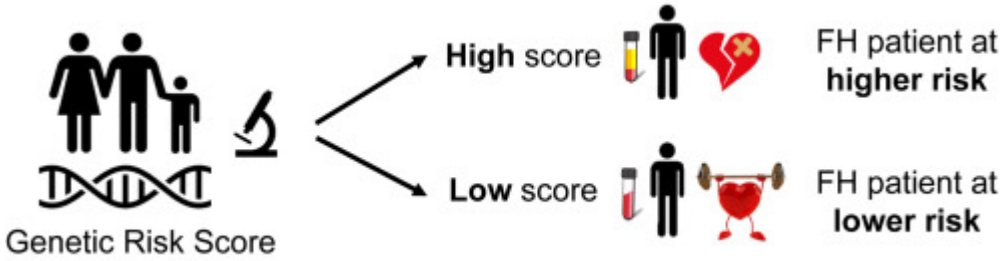
ADDITIONAL INFORMATION DISPLAY

DRAFT REPORT

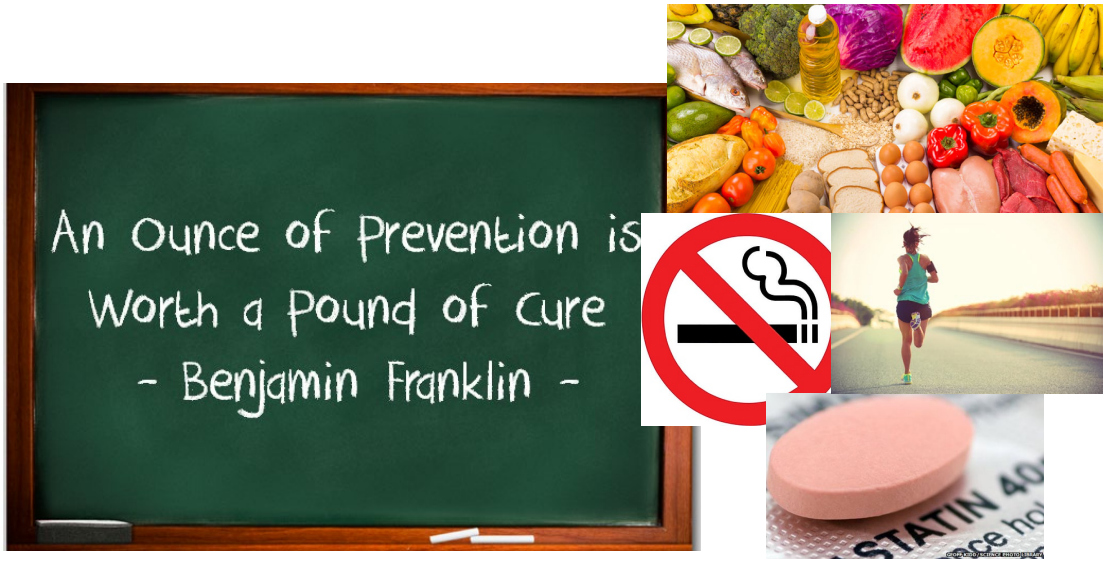
Conclusions

Identifying genetic causes of FH is clinically meaningful:

- Assessing cardiovascular disease risk
- Initiate cascade screening → primary prevention
- Identification of monogenic FH-causing variants identifies patients at greatest CVD risk, in which the use of the more intensive lipid-lowering therapies may result in the greatest absolute reduction in risk.



Paquette et al. 2017, *Journal of Clinical Lipidology*



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