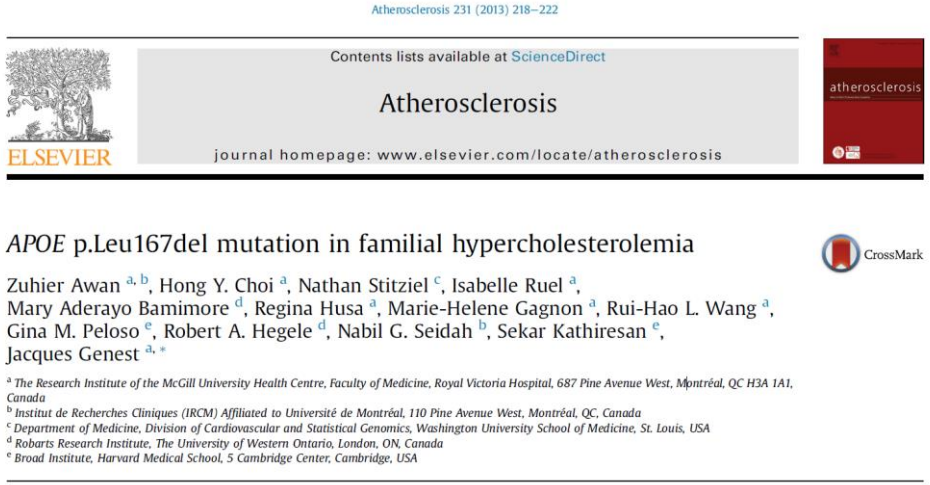
# APOE p.Leu167del mutation in familial hypercholesterolemia

Awan *et al.*, 2013



Atherosclerosis 231 (2013) 218–222

Contents lists available at ScienceDirect

Atherosclerosis

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Lisbon, 2015

# Familial hypercholesterolemia

## Autosomal dominant hypercholesterolemia - ADH

Cutaneous xanthomas and xanthelasmas

Low density lipoprotein-cholesterol (BAD) ⬆

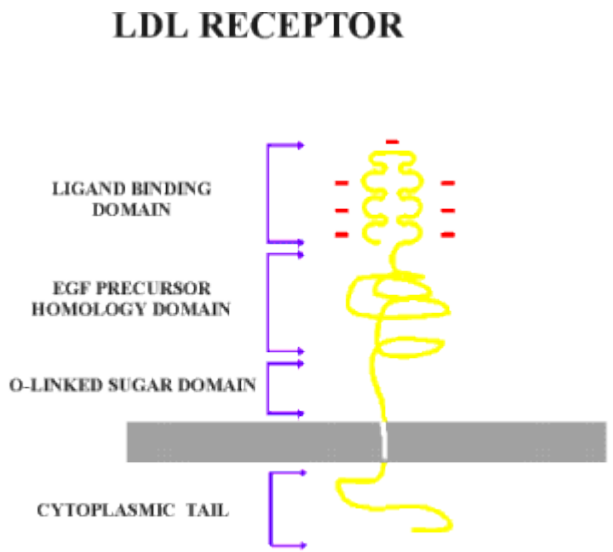
Premature coronary artery disease

**LDLR gene – primary genetic basis of disease**

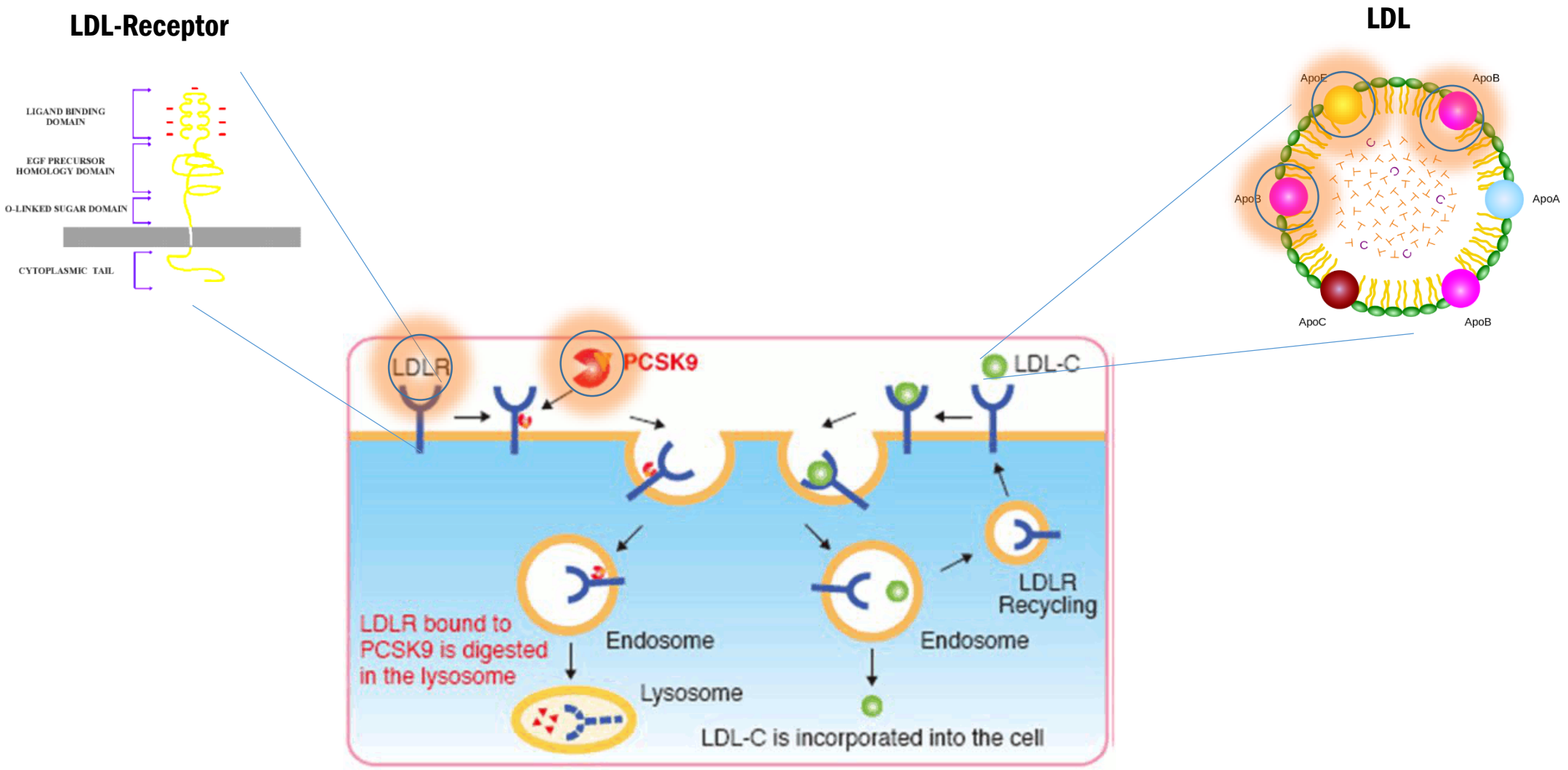
APOB

PCSK9

20% of patients do not have identifiable mutations in these genes!



# LDL intake by cells



# APOE

**Multi-functional glycosilated protein (34 KDa)**

**Secreted in brain, liver, kidney, adrenals, adipocytes, macrophages and imune cells**

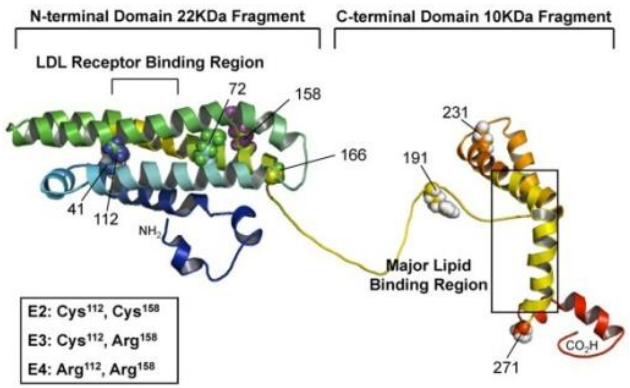
**Key component of all lipoproteins, especially:**

- **Chylomicrons and chylomicron remnants**

  Triglyceride-rich

- **VLDL**
- **IDL**

**APOE**




**Catabolism by interaction with receptors from LDLR superfamily:**

**LDLR – also apoB/E receptor**
**LRP1**
**LRP8 (apoE receptor 2)**

# APOE polymorphisms

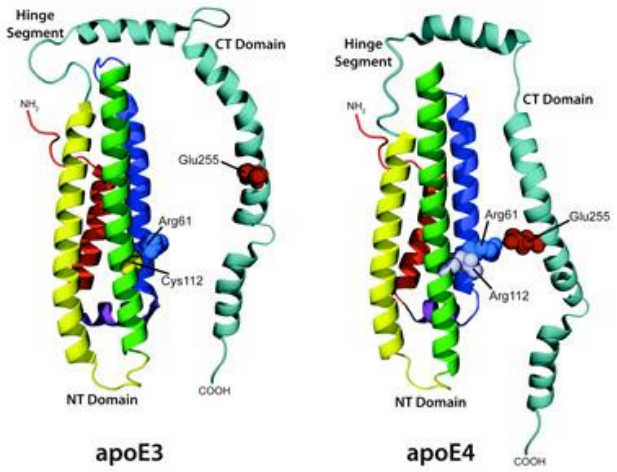
**3 polymorphisms in APOE gene:**

**apoE3** Neutral allele (~79% frequency)

**apoE4**

**apoE2** → Binds with much less affinity to the LDLR → Type III dyslipidemia

Homozigosity in 0.5 % of population




**GWAS – apoE strongly associated with LDL-C levels**

**Link to p.Leu167del and ADH phenotype**

# Methods – patient selection

**1** **Cascade screening:**
**Patients with FH from McGill University Health Centre (MUHC)**

More than:

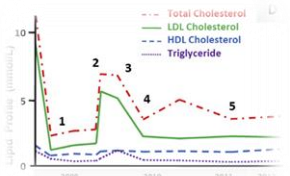
Total cholesterol 7 mmol/L
LDL-C 5mmol/L
Triglycerides 3 mmol/L

**Suspected FH**

**Data collected for 3 years**
(and data prior to study from medical records)

**2** **Plasma samples (150 uL) – HPLC for lipoprotein fractions**

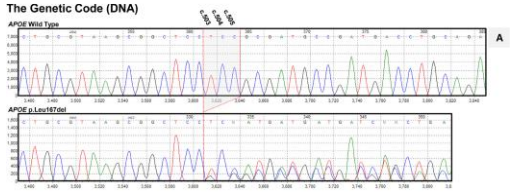
**Total cholesterol and triglycerides measured**



# Methods – patient selection

**3** **Sequencing of all exons from:**

- **LDLR**
- **PCSK9**
- **LDLRAP1**
- **Part of exon 26 of APOB**




Selection of **APOE p.Leu167del** mutation
Then found in large French family

***In silico*:**

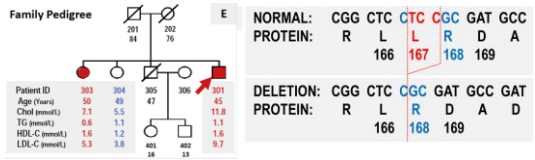
- **Common CNVs at 5' region of LDLR**
- **Excluded SNPs in non-coding regions (or not expected to impair function)**
  PolyPhen-2 ans SIFT softwares

**FILTERS (Removal):**

- Variants unlikely to be causal in the LDL-C GWAS
- Variants with a MAF > 5%
- Synonymous or intronic variants
- Variants not in dominant model of transmission

# Methods – mutation analysis

**4** **Exome sequencing -> Comparison between:**







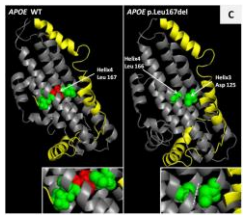
**Proband family** ⟷ **39 patients with ADH from clinic in Ontario**

Proband and 2 sisters — No mutations in ApoE

**Excluding mutations in:**

- **LDLR**
- **PCSK9**
- **APOB**

***In silico*:**



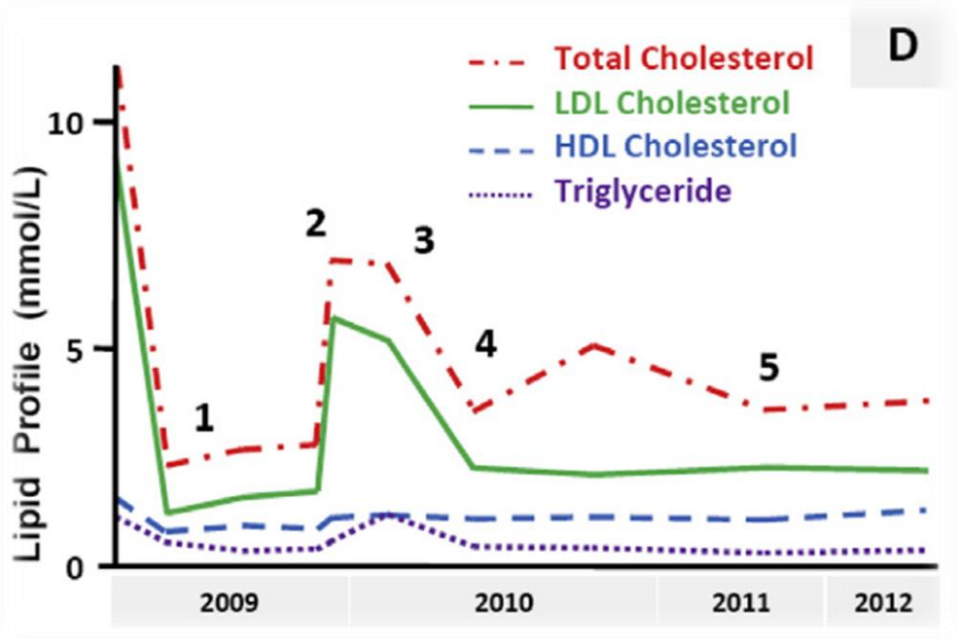

**Computer prediction and modeling – impact of mutation on protein function and structure modeling**
(PROVEAN, I-TASSER and PyMol)

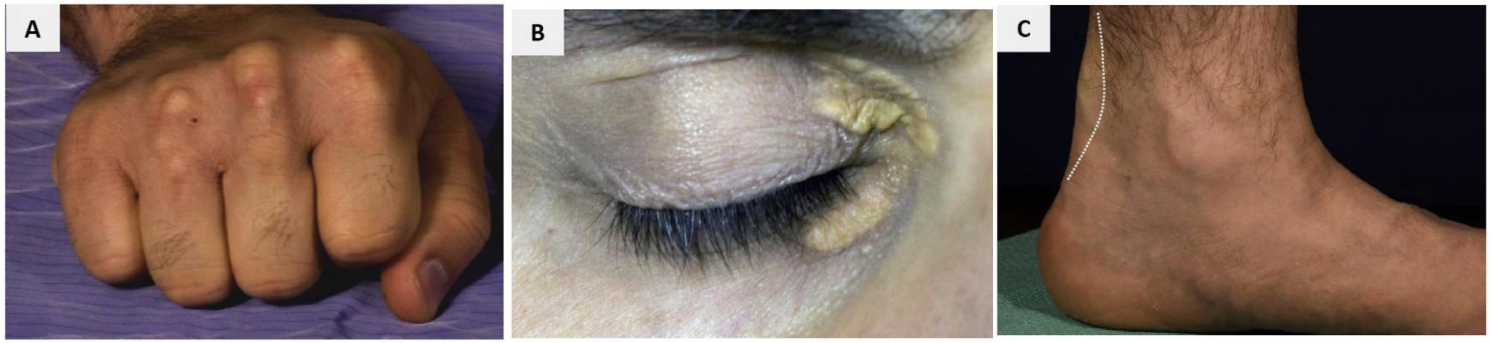
# Proband –> (Index case)

43 year-old man, Italian origin

Acute coronary syndrome

Tendinous xanthomas, xanthelasmas, Achilles tendons xanthomas, elevated cholesterol and LDL-C levels




**Reference values:**
Total cholesterol 7 mmol/L
LDL-C 5mmol/L
Triglycerides 3 mmol/L

# Results

## Sequencing of LDLR, PCSK9, LDLRAP1 and exon 26 of APOB

- **No known mutation was identified**
- **Polymorphisms found in non-coding regions or not expected to be deleterious**

## APOE gene was not considered for the initial screening

- **Only a report from the same year (Mardual et. al.) identified this gene as causal**

## Exome sequencing on 3 individuals from the family – index and 2 sisters

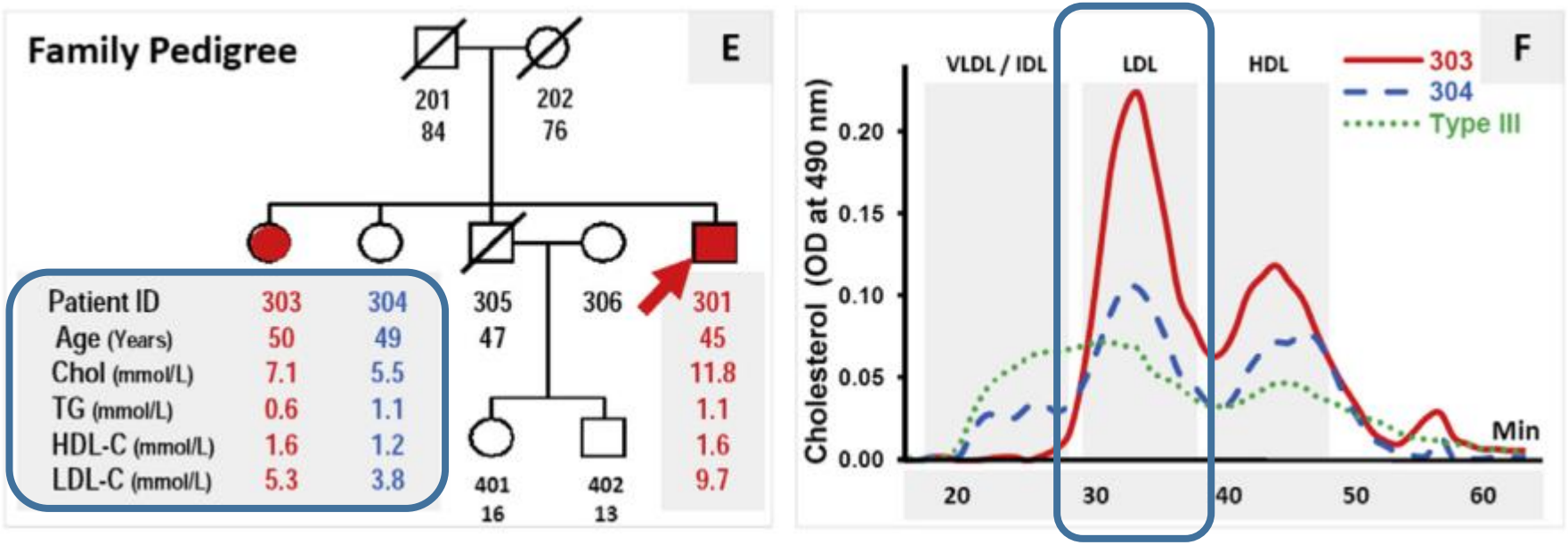
- **54,000 variants identified**
- **49 missense mutations, 1 frameshift, 2 in-frame deletions in 52 genes**

**APOE p.Leu167del**

**Recently discovered in a large family with FH (in France, previous study)**

# Family study

**Proband and 1 sister (303) were heterozygous for the APOE p.Leu167del mutation**



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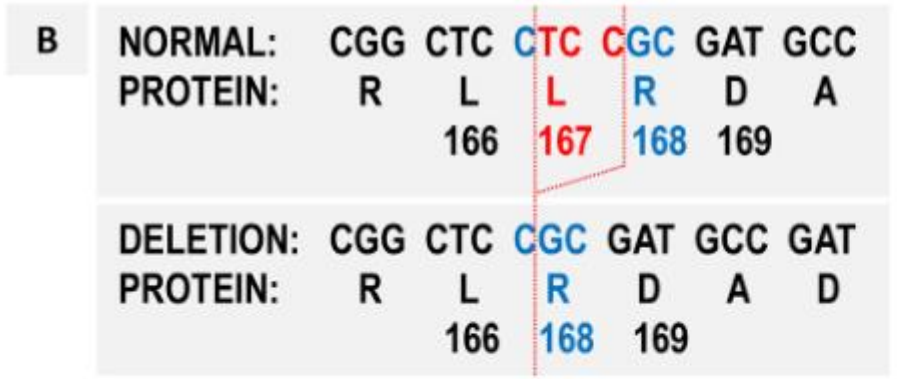
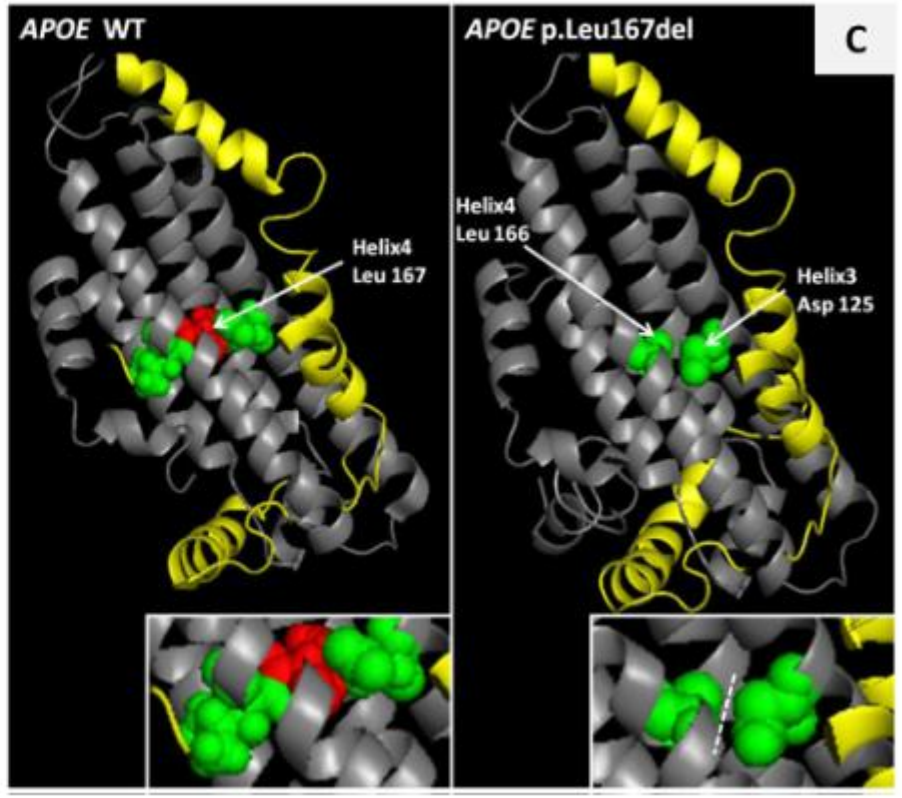


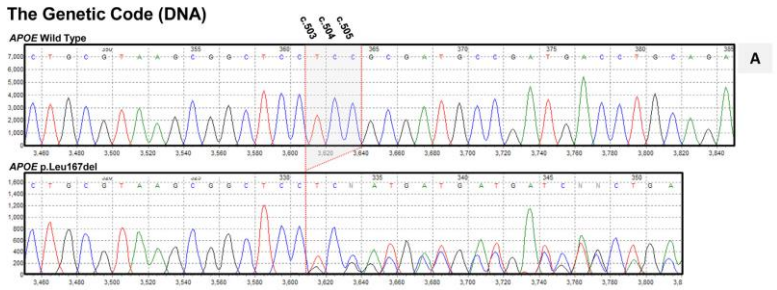

- In-frame deletion of TCC in exon 4

- Highly conserved motif

- PROVEAN: -7.51 -> deleterious to the protein structure

- Leucine zipper disruption

- Decrease in LDL clearance



# Discussion

Ligand for other LDL-R related peptide (LRPs) in the LDLR family that assist in LDL clearance

**May also interfere with LDL internalization (binding with LRP6)**

Associated with various clinical phenotypes:

- Classical ADH, hypertriglyceridemia with splenomegaly and sea-blue histiocytosis and familial combined hyperlipidemia

**Diversity of phenotypes not seen in this case**

- **Reason unclear: different genetic background gene-gene interactions, gene-environment interactions, epigenetic and other non-mendelian interacting effects**

# Conclusions

**Confirmed 4th locus in ADH**

- LDLR
- APOB
- PCSK9
- **APOE**

**Association between p.Leu167del and ADH identified by next-generation exome sequencing**

**Private mutation**

**Impact on large registries of FH patients, specialized medical care**

**Cascade gene screening in high risk families to prevent vascular disease**

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BioISI
Biosystems and Integrative Sciences Institute

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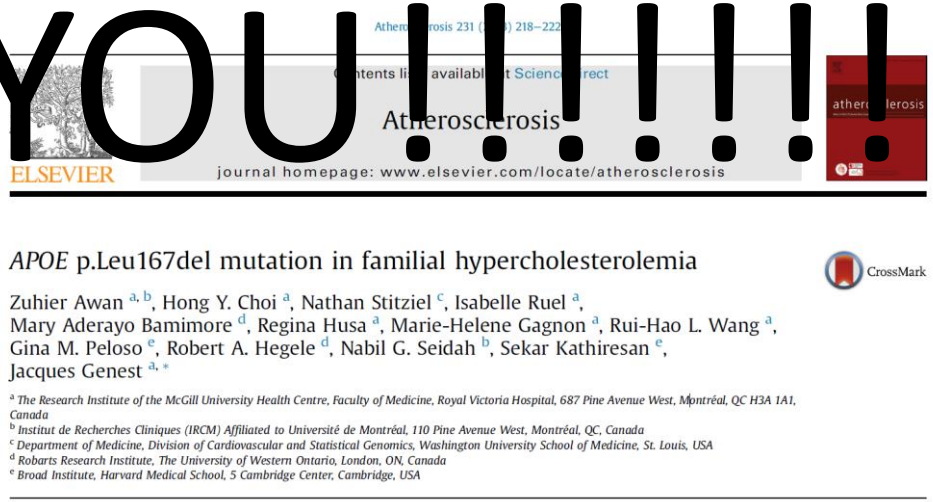
THANK YOU!!!!!!!!!!!



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