

30^o CONGRESSO NAZIONALE S.I.S.A.

ROMA - 20/22 NOVEMBRE 2016



Study of miRNome to identify new molecular causes of Familial Hypercholesterolemia

Borsa di studio "Andrea Mezzetti" 2015

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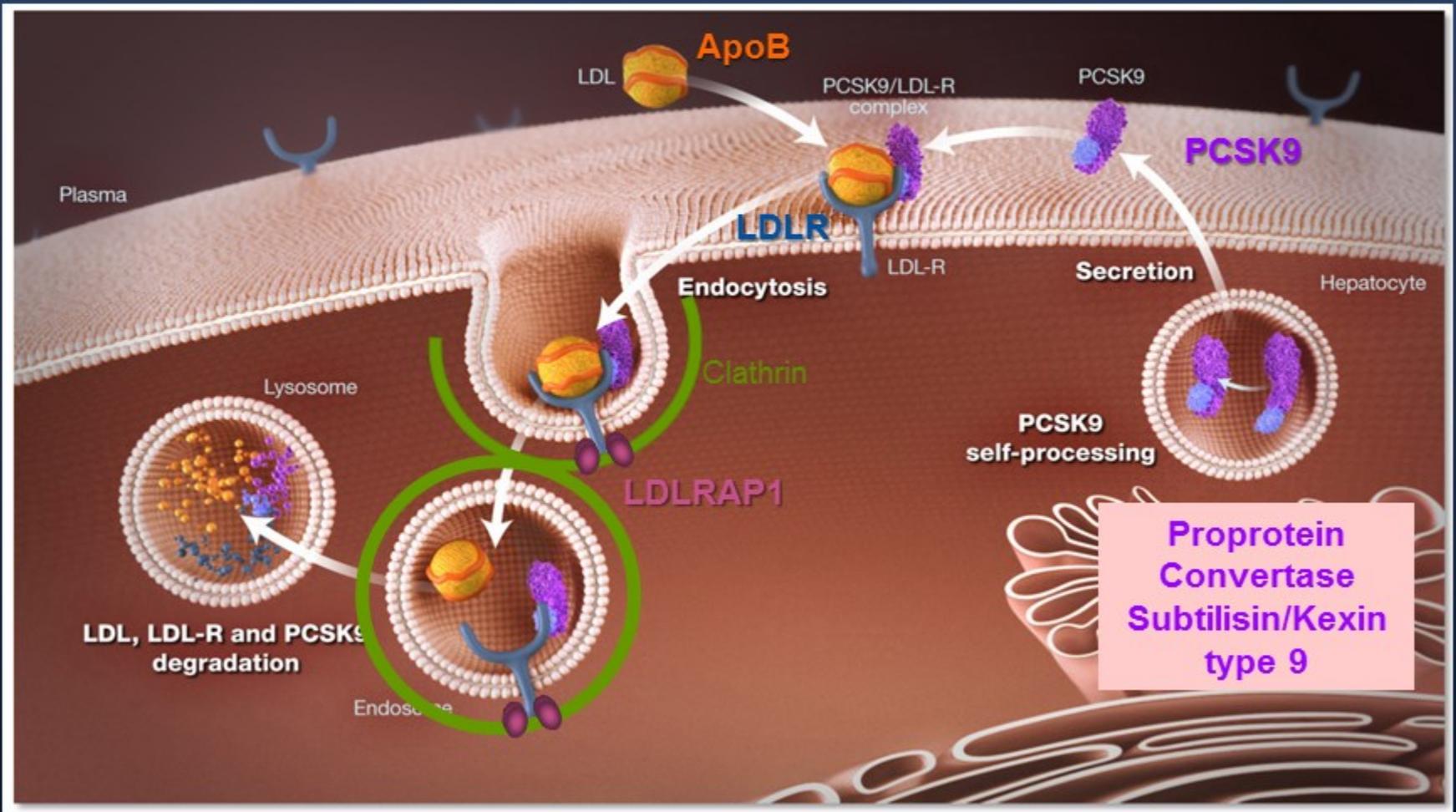


Università degli Studi
di Salerno

Familial Hypercholesterolemia (FH)

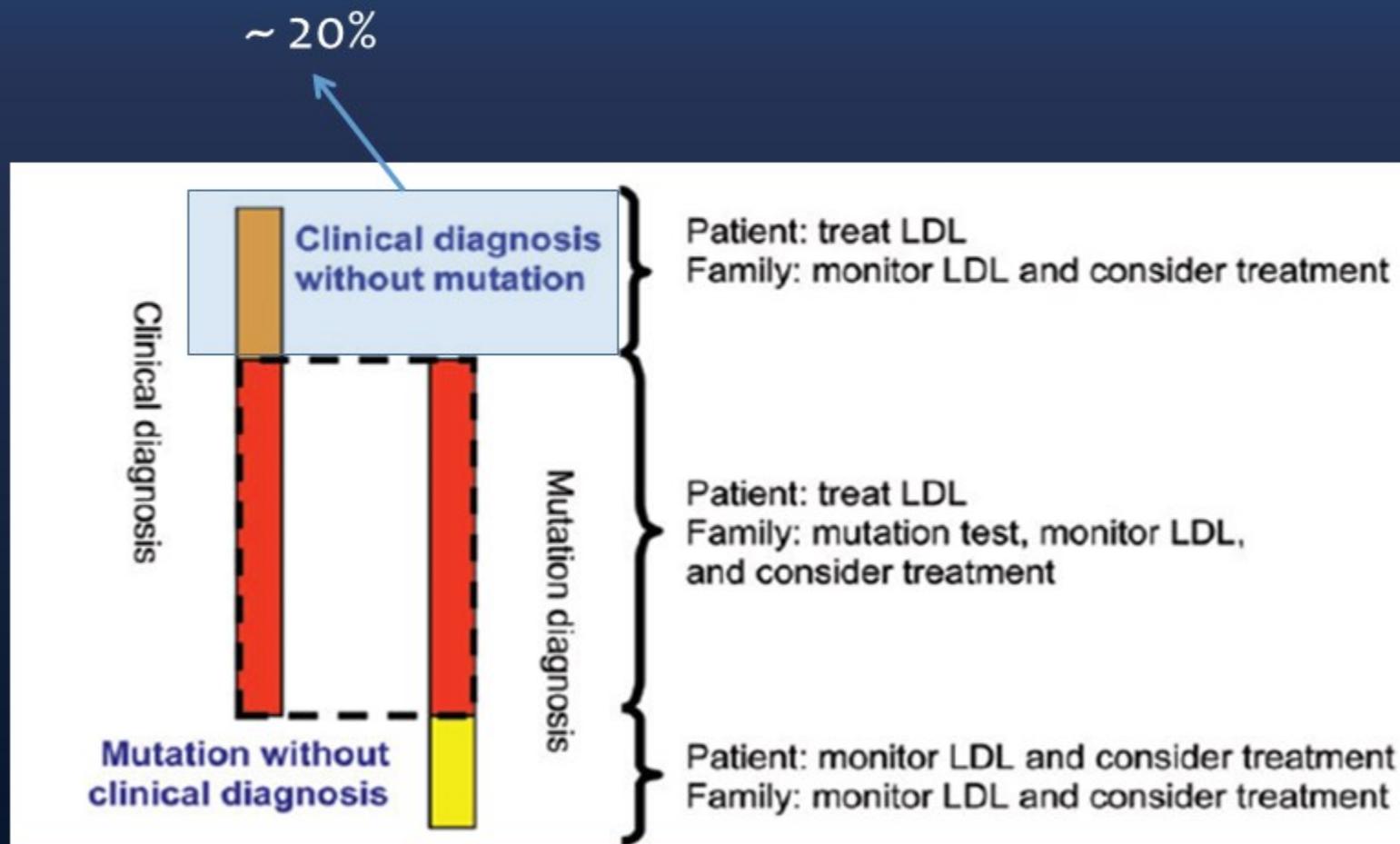
- increased levels of LDL-cholesterol
- premature atherosclerosis and increased cardiovascular risk
- tendon xanthomas, corneal arcus
- autosomal dominant and rarely autosomal recessive
- 2 forms
 - heterozygous
 - homozygous/compound heterozygous

Genetics of Familial Hypercholesterolemia



Adapted from Horton JD, et al. J Lipid Res. 2009;50 Suppl:S172-7

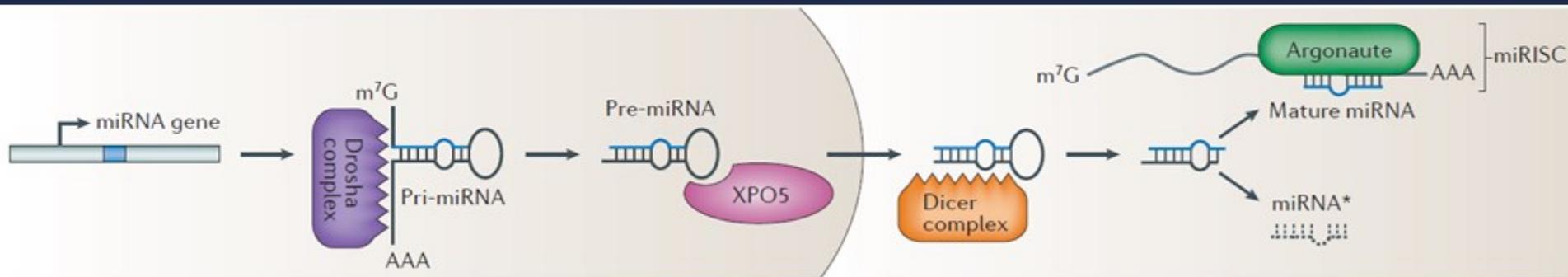
Molecular and clinical diagnosis



Pathogenic hypothesis in patients without mutations in causative genes

- Mutations in unknown genes
- Polygenic hypothesis
- Additional genetic mechanisms

→ miRNA



Pasquinelli AE. Nat Rev Genet. 2012

mRNA degradation

mRNA translation inhibition

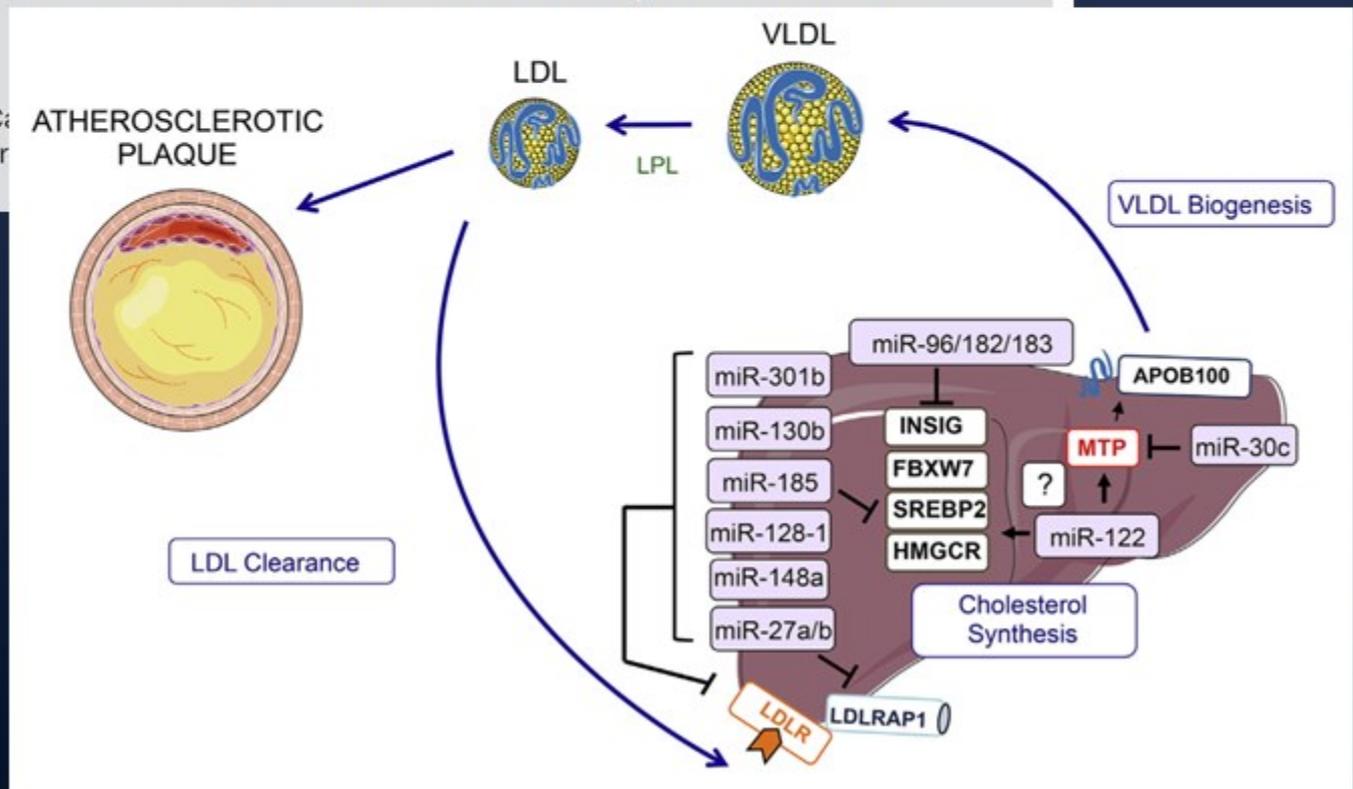
Decrease of protein encoded by the targeted gene

miRNA and FH

Clinical Science (2015) 129, 963–972 doi: 10.1042/CS20150235

Circulating *miR-33a* and *miR-33b* are up-regulated in familial hypercholesterolaemia in paediatric age

Francesco Martino*, Fabrizio C...
Francesco Barillà||, Marcello Ar...
Alessandra Magenta†



Rotllan N, et al.
Atherosclerosis.
2016;246:352-60.

Aim of the project

Identify molecular alterations responsible of FH in patients without mutations by a combined approach of:

1. Functional characterization and expression quantification of LDLR
2. Identification of miRNA alterations

Peripheral Blood Mononuclear Cells

Patients and controls

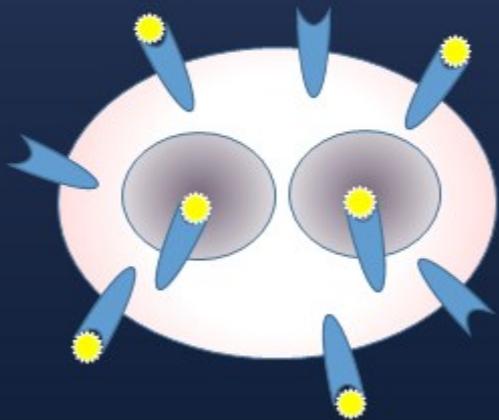
	FH patients n=20	Control subjects n=10	Difference significance
Age (years)			
Sex (male number and %)			
Total cholesterol (mg/dL)			
Triglycerides (mg/dL)			
HDL cholesterol (mg/dL)			
LDL cholesterol (mg/dL)			
Xanthomas (number and %)			
Corneal arcus (number and %)			

Dati non pubblicati

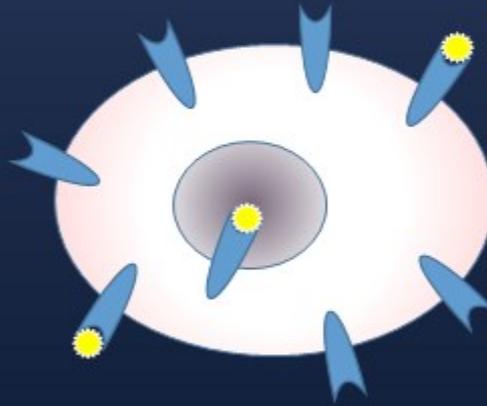


Functional assay of LDLR

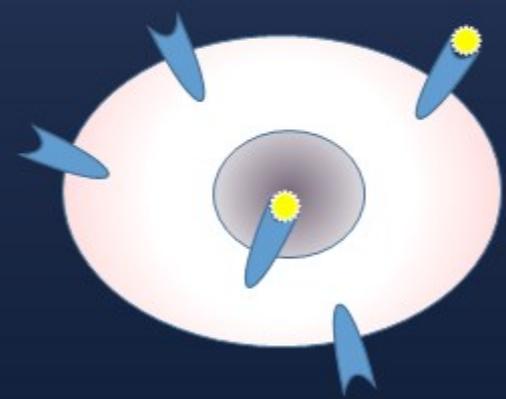
- Evaluation of:
1. LDLR activity (DiI-LDL)
 2. LDLR amounts on plasma membrane (antibody)



Controls and class 1.
normal LDLR activity
(normal LDLR
expression)



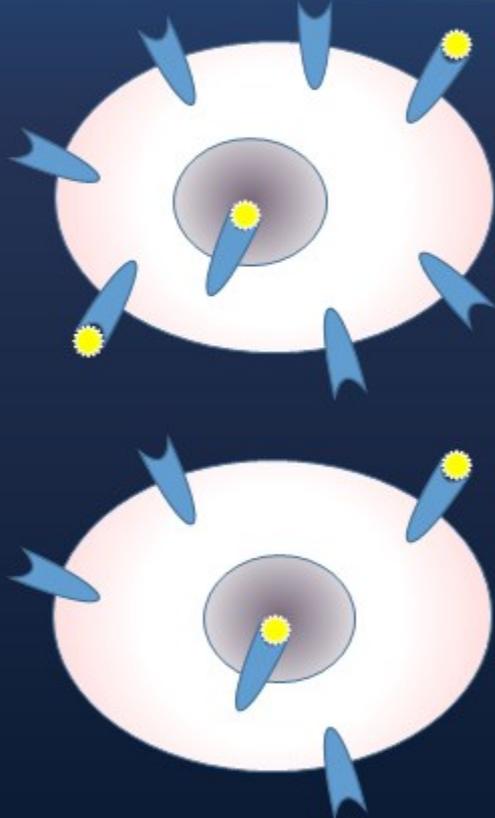
Class 2. decreased
activity with normal
expression of LDLR



Class 3. decreased
activity with decreased
expression of LDLR



Functional assay of LDLR



Class 2. decreased activity with normal expression of LDLR

Class 3. decreased activity with decreased expression of LDLR

Dati non
pubblicati

of FH patients without mutations has a defect in the LDLR



smallRNA-Seq

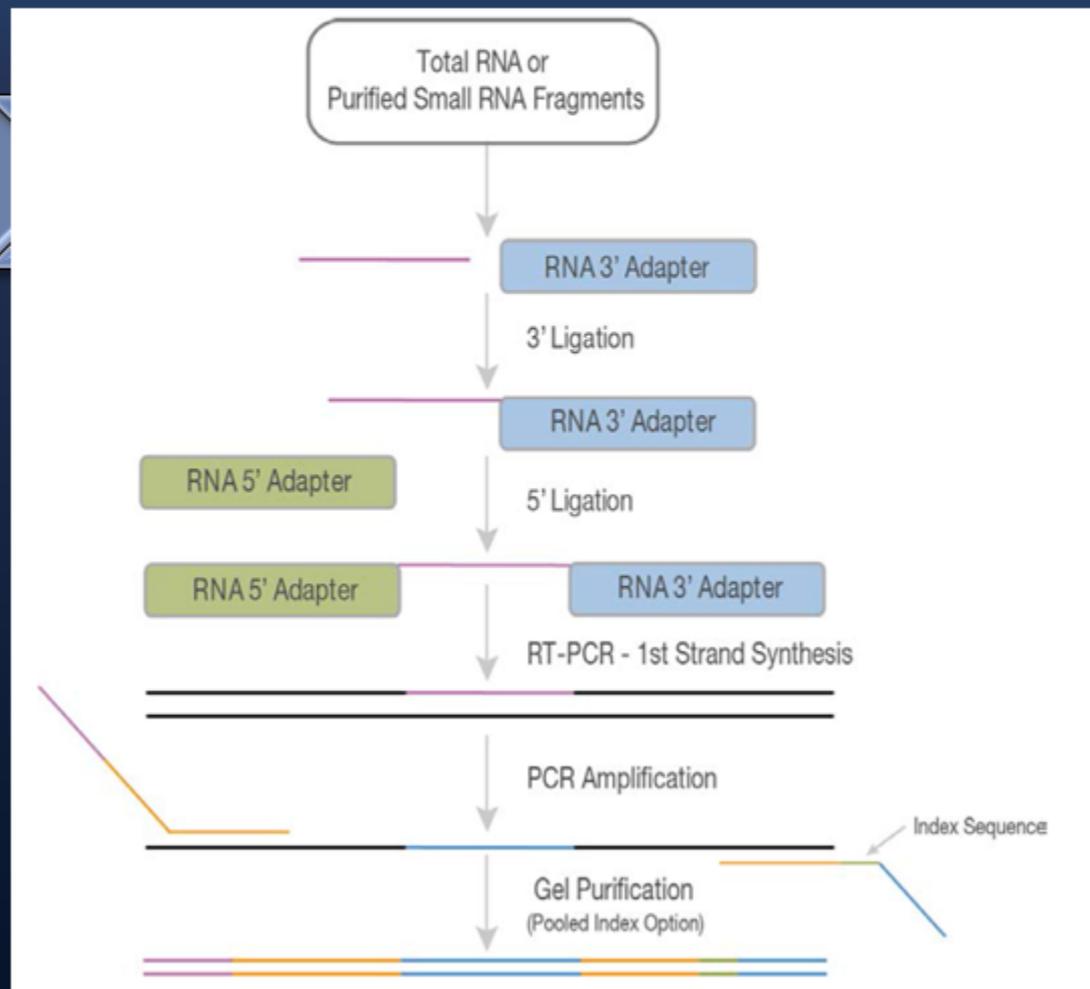
Small RNA analysis by massive parallel sequencing

Total RNA
isolation

Library
preparation

mirVana
miRNA
Isolation Kit

TruSeq Small
RNA Library
Prep Kit





smallRNA-Seq

Small RNA analysis by massive parallel sequencing



mirVana
miRNA
Isolation Kit

TruSeq Small
RNA Library
Prep Kit

HiSeq 2500
(Illumina)



miRNA data

- Number of reads for each samples:
15,136,656 - 57,626,151
→ accuracy of base calling >99%
- 1,784 different miRNA identified
- Raw data normalized for total counts: Normalized count of each miRNA > 0,5 → 897 miRNAs
- Frequency and mean comparisons (chi square, T-test and Mann-Whitney)
 - FH vs controls
 - FH class 1 vs class 2 vs class 3

Data analysis: miRNA differently expressed

- FH vs controls
 - 198 miRNAs $\left\{ \begin{array}{l} 30 \text{ up-expressed} \\ 168 \text{ down-expressed} \end{array} \right.$
- FH class 3 vs controls + class 1 and 2
 - 69 miRNAs $\left\{ \begin{array}{l} 54 \text{ up-expressed} \\ 15 \text{ down-expressed} \end{array} \right.$
- FH class 2 vs controls + class 1
 - 146 miRNAs $\left\{ \begin{array}{l} 20 \text{ up-expressed} \\ 126 \text{ down-expressed} \end{array} \right.$
- FH class 1 vs controls
 - 100 miRNAs $\left\{ \begin{array}{l} 12 \text{ up-expressed} \\ 88 \text{ down-expressed} \end{array} \right.$

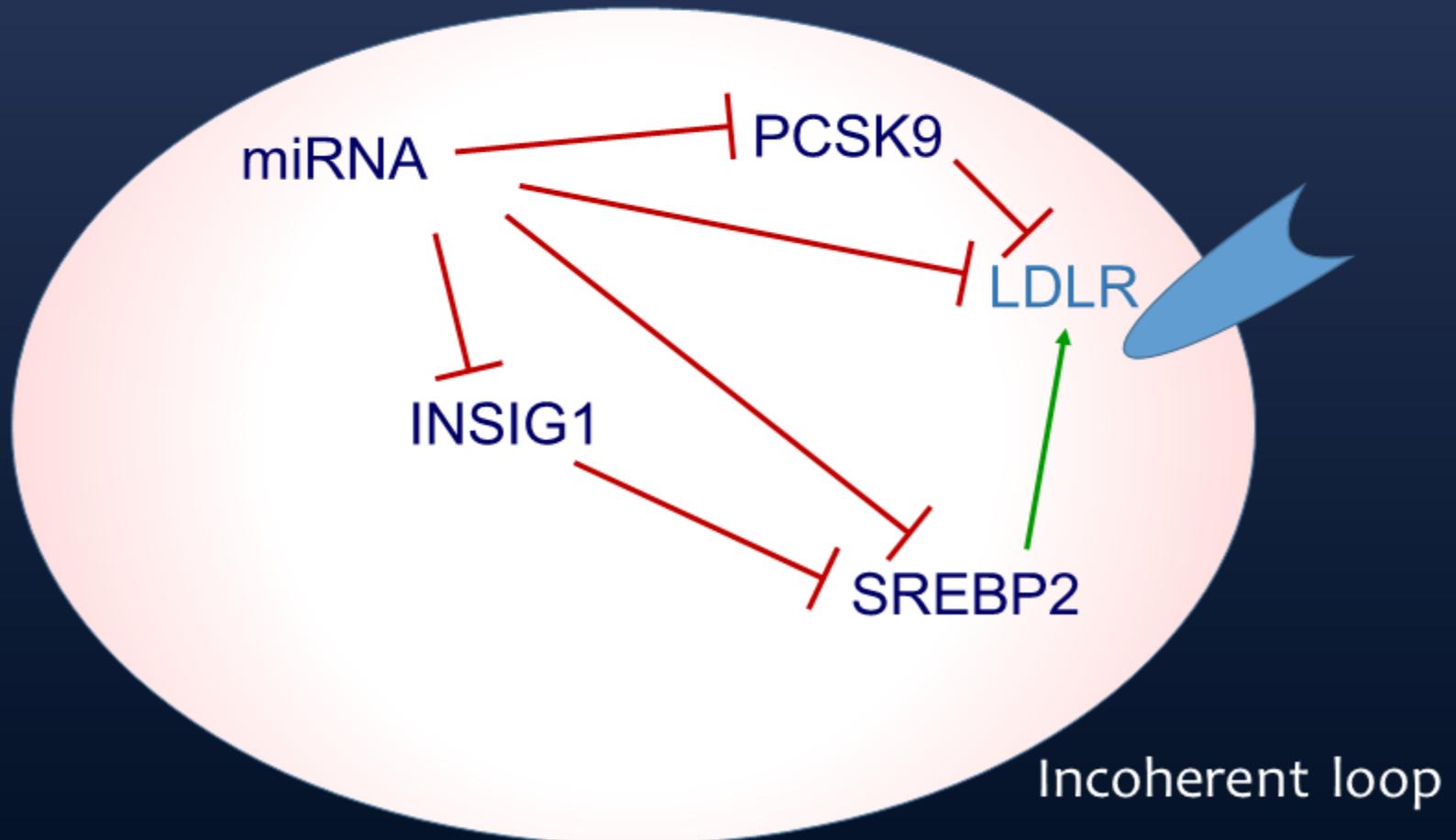
Pathway analysis

- Ingenuity Pathway Analysis (IPA) platform
- mirPath v.3

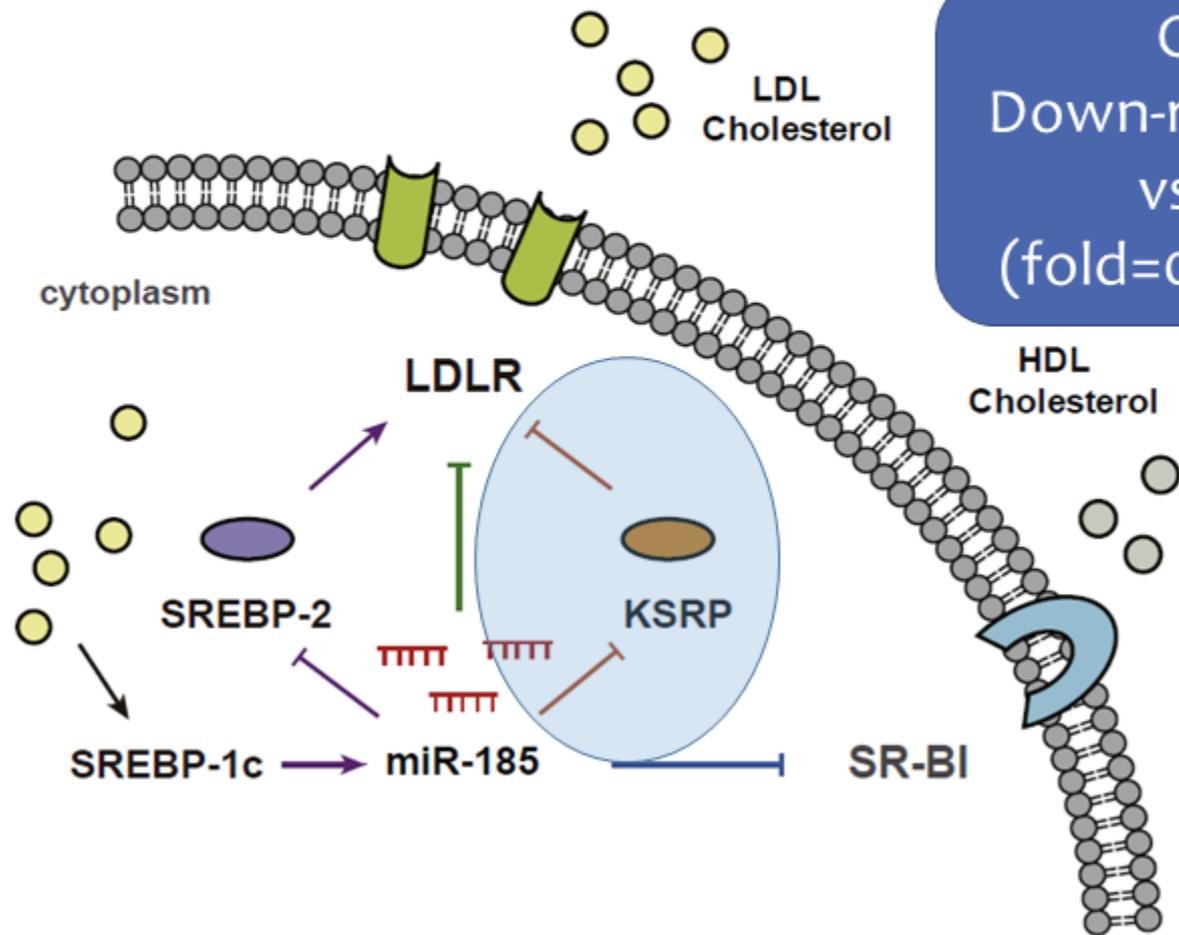
#	KEGG pathway	p-value	#genes	#miRNAs	download results
1.	MicroRNAs in cancer (rno05206)	9.70546106795e-57	133 see genes	68	details
2.	Adherens junction (mmu04520)	1.41159347402e-13	69 see genes	63	details
3.	Spliceosome (hsa03040)	1.2953510403e-12	111 see genes	64	details
4.	Protein processing in endoplasmic reticulum (hsa04141)	1.35418547626e-12	140 see genes	67	details
5.	Viral carcinogenesis (hsa05203)	2.04688270089e-10	161 see genes	68	details
6.	Renal cell carcinoma (mmu05211)	7.34776361442e-10	60 see genes	65	details
7.	Cell cycle (hsa04110)	2.47326290452e-09	102 see genes	68	details
8.	Transcriptional misregulation in cancer (rno05202)	3.62482121351e-09	135 see genes	66	details
9.	Ubiquitin mediated proteolysis (hsa04120)	6.6165531077e-09	113 see genes	64	details
10.	Pathways in cancer (mmu05200)	2.26405264691e-08	285 see genes	68	details

Identification of relevant miRNAs

- match between dys-regulated miRNA and known players of LDL metabolism: class 3



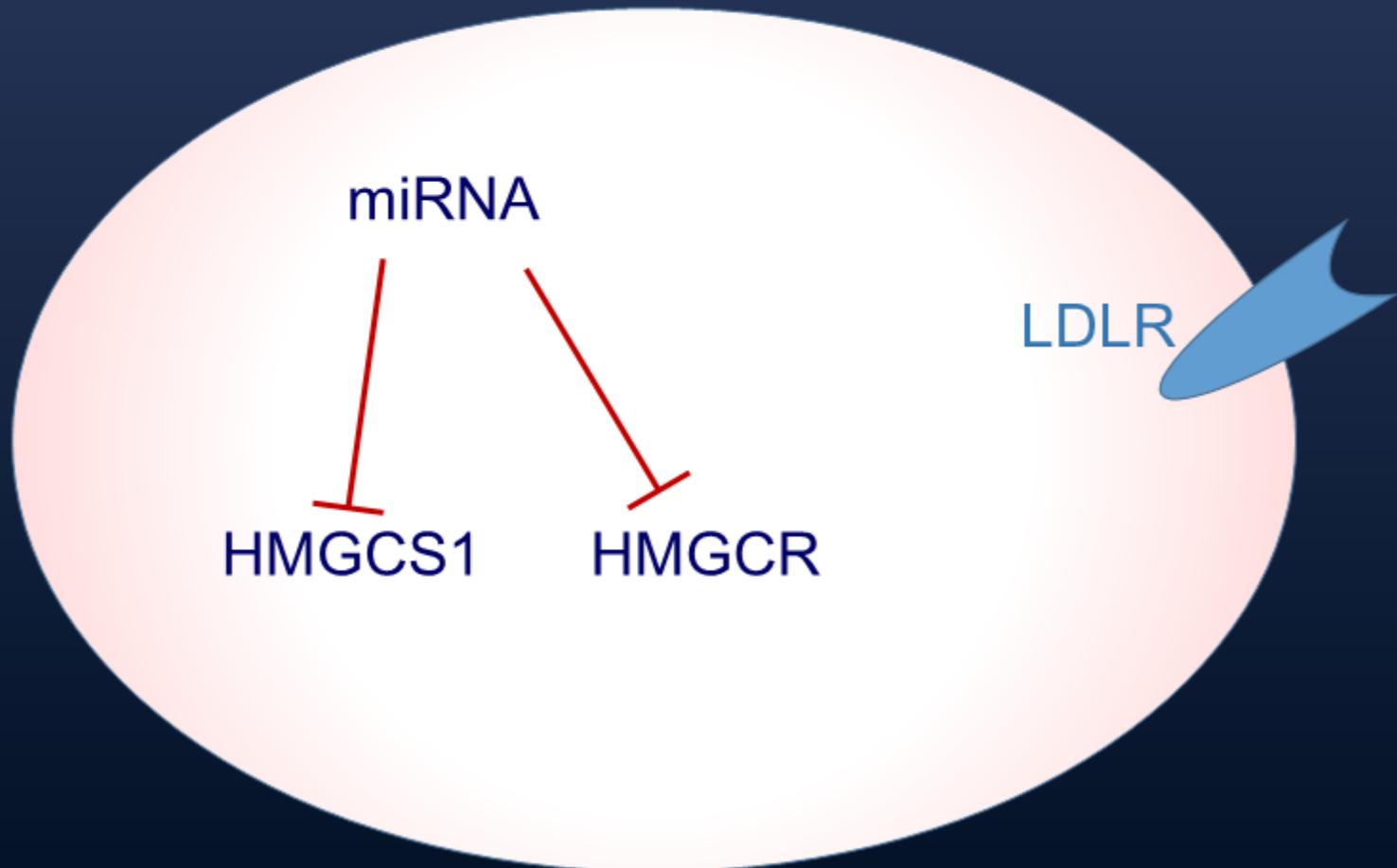
miR-185: to target or not to target



Our data:
Down-regulated in FH
vs controls
(fold=0.706; p=0.011)

Identification of relevant miRNAs

- match between dys-regulated miRNA and known players of LDL metabolism: class 1



Work in progress

- Verification of miRNA expression in human liver samples
- Evaluation of miRNA effects on an hepatic cell line (HepG2)
 - miRNA transfection
 - miRNA silencing

} Quantification of protein encoded by potential target genes
- Quantification of miRNA in additional samples

Conclusions

- A large number of miRNAs is differently expressed between patients and controls
- Pathway analysis is inadequate to our analysis
- Several miRNAs have been identified and need a functional validation

Acknowledgements

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