08.09.2023

CIDEB pathobiology for NASH implications therapeutics

Highlighting NASH therapeutic targets with human genetics: protective germline mutations in CIDEB"

Niek Verweij, PhD
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Regeneron Genetics Center
on behalf of RGC team and collaborators

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Conflict of interest disclosure

This work was funded in part by the Regeneron Genetics Center, LLC, a fully-owned subsidiary of Regeneron Pharmaceuticals, Inc.

I, Niek Verweij, am an employee of Regeneron Pharmaceuticals, Inc. I receive salary, stock and stock options as compensation for my employment.

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Regeneron Genetics Center
- RGC Leadership Team
- Sequencing & Laboratory Operations
- Genome Informatics & Data Engineering
- Clinical Informatics
- Analytical Genomics & Data Sciences
- Therapeutic Area Genetics
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- RGC Biology
- Research, Business & Operations
- RGC Administrative team

Regeneron R&pD
- Therapeutic Focus Areas
- Early Clinical Development
- REGN Scientific Leadership

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- Sofia Enhörning

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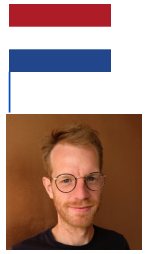
- Christopher Still
- Tooraj Mirshahi
- David Carey

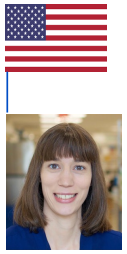
UK Biobank

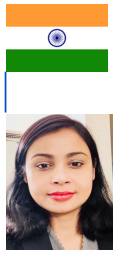
& **Many more colleagues, collaborators, participants**

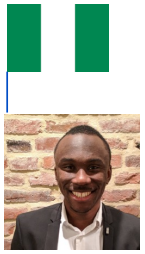
Meet the team: Translational genetics of cardiovascular-metabolic-skeletal disease at The Regeneron Genetics Center


Parsa Akbari

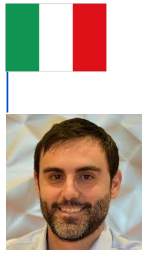

Niek Verweij

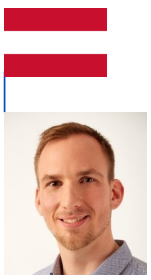

Mary Haas


Tanima De

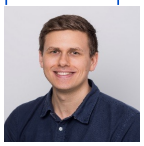

Olu Sosina

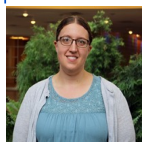

Jonas Nielsen


Luca Lotta


Ernst Mayerhofer


George Hindy


Jonas Bovijn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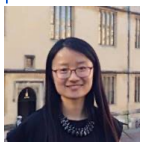
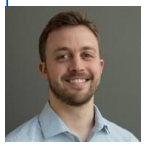

Luanluan Sun


Cristen Willer

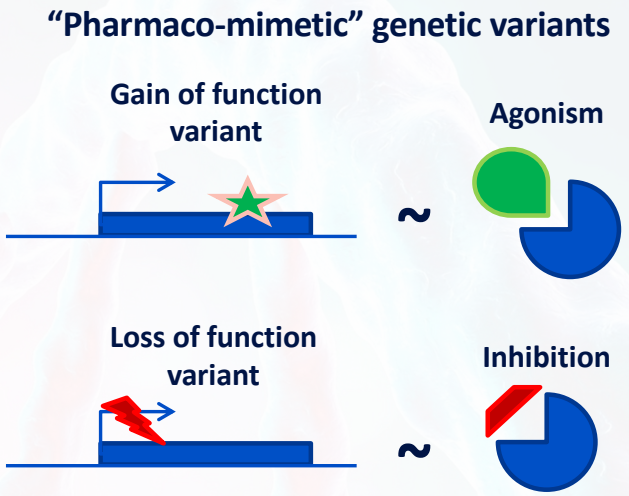

Peter Dornbos


Arthur Gilly

We're a diverse group of scientists with a shared interest in the application of human genetics to the development of new therapeutics

Human genetics may provide “blueprints” for therapeutic development

- Naturally-occurring human genetic variants that activate or inactivate a target gene may be helpful “proxies” for pharmacological modulation.
- Studying their phenotypic associations in large datasets may inform on therapeutic efficacy and safety in humans.



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Plenge et al. Nature Reviews Drug Discovery 2013; Cook et al. Nature Reviews Drug Discovery 2014; Nelson et al. Nat Genet 2015

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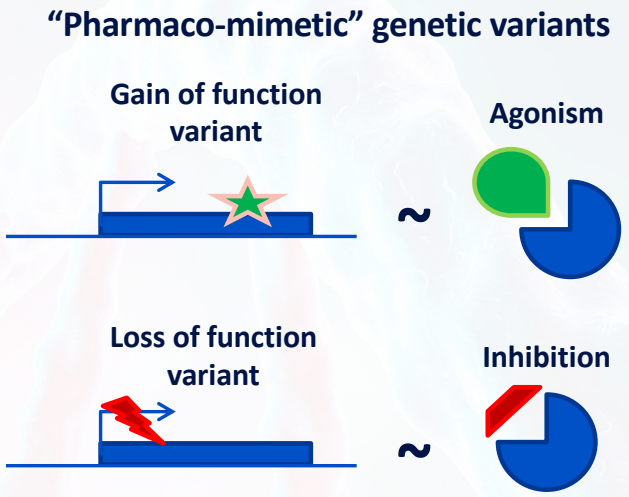

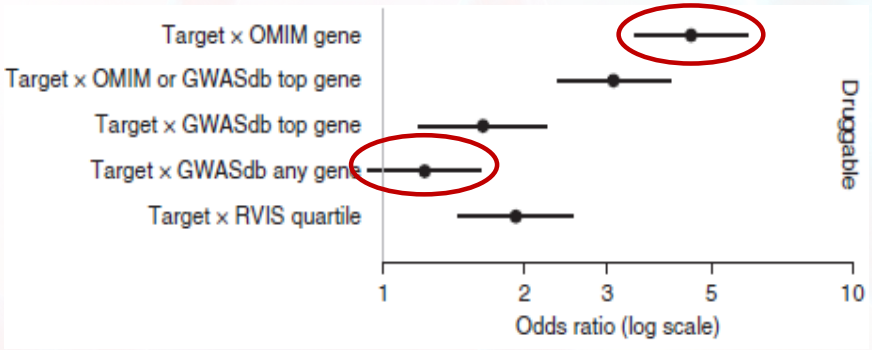

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  - Which gene/target?
  - Which direction of modulation?
  - Understanding mechanism/biology

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  - Understanding mechanism/biology

Solution: Exome sequencing!

Identification of rare coding variants:

1. Helps pinpoint causal genes with high confidence
2. Helps clarify directionality of association
3. Large-effect coding variant associations may accelerate translation to biological insight (i.e. PCSK9, ANGPTL3/4, APOC3 etc)
4. Can identify associations not easily found via other approaches

BUT: Needs HUGE sample size!

RGC's commitment to exome sequencing

2013
1 staff, 0 exomes

John Overton
Head of Sequencing

2014
20 staff, 20K exomes

Aris Baras
Head of RGC

2017
60 staff, 250K exomes

2020
100 staff, 1M exomes

2021/22
130+ staff, 1.5M exomes

REGENERON

biobank uk
Improving the health of future generations

gsk
1st 50K Exomes

abbvie
Alnylam
AstraZeneca
Biogen
Bristol-Myers Squibb
Pfizer
Takeda
Remaining 450k

Through this Consortium, the UKB has now released 450,000 exomes to the scientific community for research

Largest exome-sequencing project in a single cohort

Article

Exome sequencing and characterization of 49,960 individuals in the UK Biobank

PERSPECTIVE
https://doi.org/10.1038/s41588-021-00885-0
Nature Genetics 2021
nature genetics

Advancing human genetics research and drug discovery through exome sequencing of the UK Biobank

Joseph D. Szustakowski[1], Suganthi Balasubramanian[2], Erika Kvikstad[1], Shareef Khalid[2], Paola G. Bronson[3], Ariella Sasson[1], Emily Wong[4], Daren Liu[2], J. Wade Davis[5], Carolina Haefliger[6], A. Katrina Loomis[7], Rajesh Mikkilineni[4], Hyun Ji Noh[5], Samir Wadhawan[1], Xiaodong Bai[2],

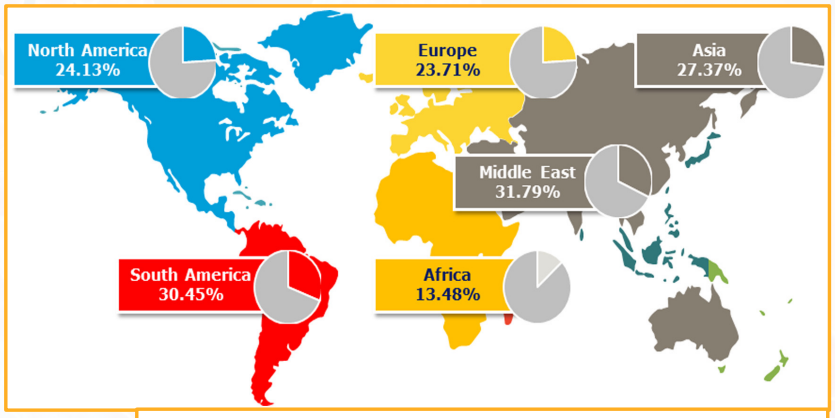
Article

Exome sequencing and analysis of 454,787 UK Biobank participants

To date:
The UKB Exome Sequencing Consortium has enabled >120 Publications from Researchers around the world; and this number is still growing!

Nonalcoholic liver disease is a common metabolic disease with high global prevalence

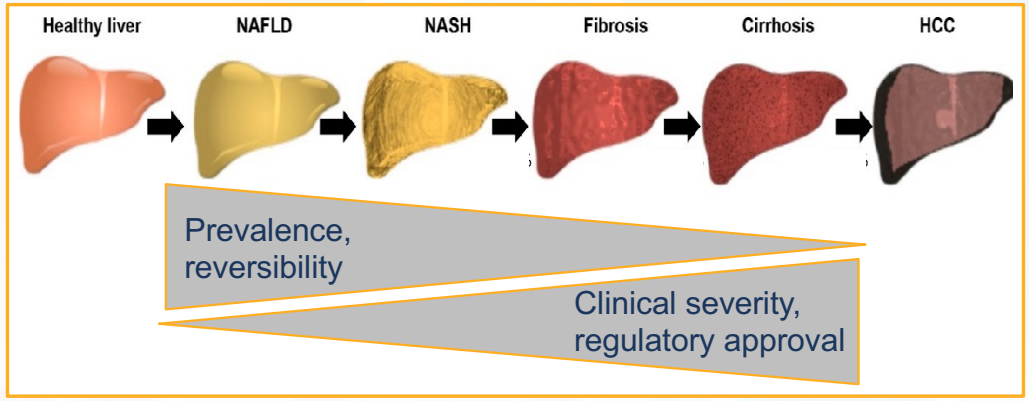
Nonalcoholic fatty liver disease (NAFLD)



1 in 4 people worldwide have NAFLD

Younossi et al, Hepatology, 2018
(NAFLD prevalence estimates from meta-analyses including 8.5M individuals)

- Common, chronic disease characterized by the accumulation of fat in the liver in the absence of alcohol abuse
- Higher risk of cirrhosis and hepatic failure, heart disease
- Strongly associated with obesity and fat distribution, insulin resistance and diabetes
- NO approved therapies as of end of 2022 in US/EU in spite of numerous therapeutic development efforts



NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma

NAFLD activity score		
Components	**Score**	**Extent**
Steatosis	0	<5%
	1	5-33%
	2	33-66%
	3	>66%
Hepatocyte Ballooning	0	None
	1	Few balloon cells
	2	Many balloon cells
Lobular Inflammation	0	No foci
	1	<2 foci/200x
	2	2-4 foci/200x
	3	>4 foci/200x

Fibrosis score

Stage 0
No fibrosis

Stage 1
Zone 3 perisinusoidal fibrosis
- Mild – 1a
- Moderate – 1b
- Portal/periportal – 1c

Stage 2
Perisinusoidal and portal/ periportal fibrosis

Stage 3
Bridging fibrosis

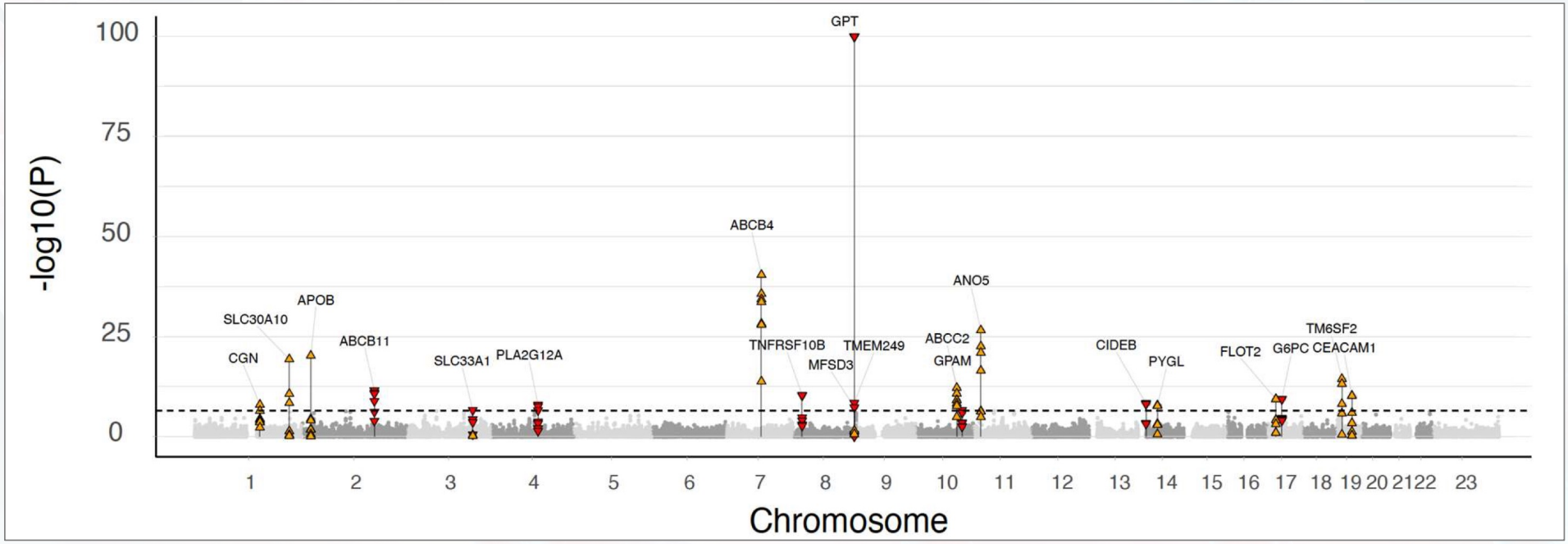
Stage 4
Cirrhosis

RGC

enter

Staged multiancestry exome analysis of liver phenotypes identifies associations at 5 liver-expressed genes including a novel protective association for *CIDEB* mutations

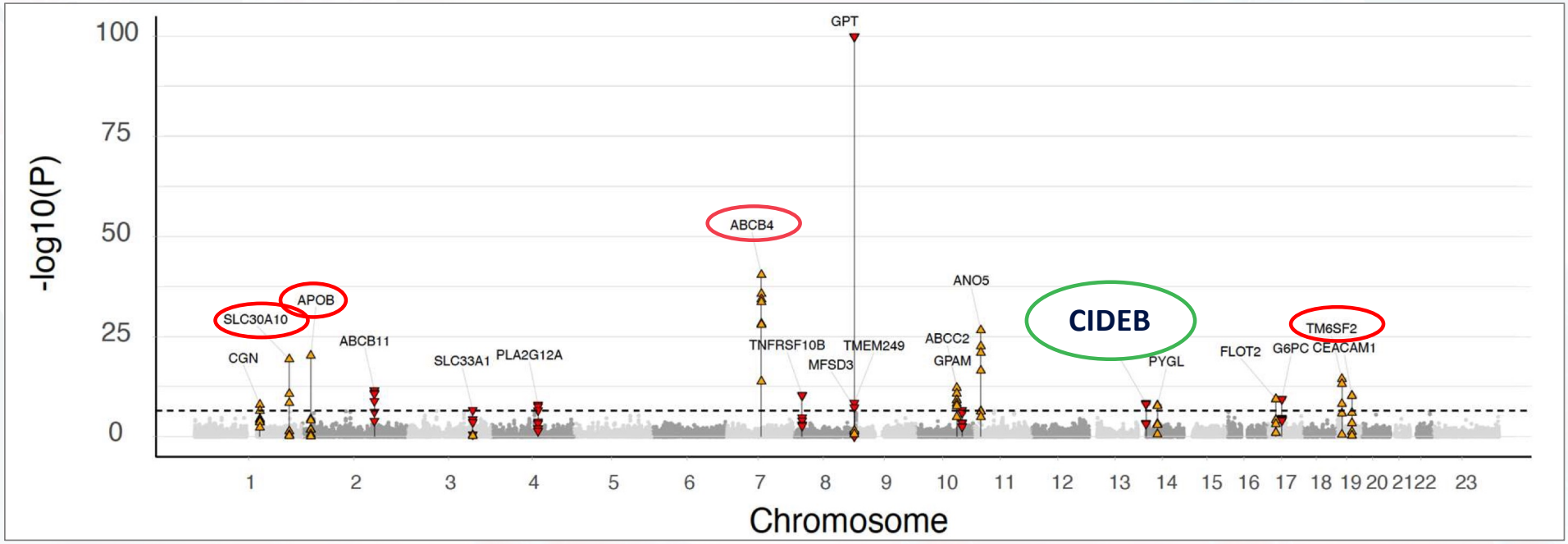
Exome wide analysis of the burden of rare coding variants with ALT levels



RGC & collaborators, N Engl J Med 2022; 387:332-344

Staged multiancestry exome analysis of liver phenotypes identifies associations at 5 liver-expressed genes including a novel protective association for *CIDEB* mutations

Exome wide analysis of the burden of rare coding variants with alanine transferase levels



Circled genes: robustly associated with ALT, AST and any liver disease

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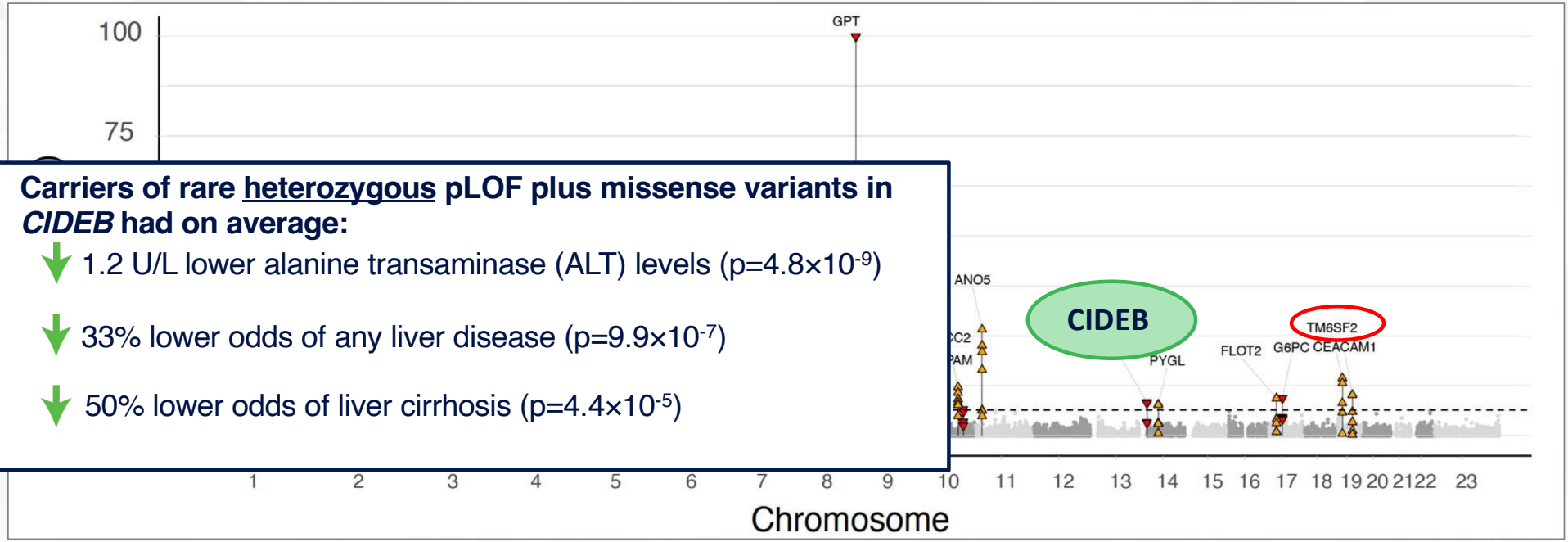


Carriers of rare <u>heterozygous</u> pLOF plus missense variants in *CIDEB* had on average:

- ↓ 1.2 U/L lower alanine transaminase (ALT) levels ($p=4.8\times10^{-9}$)
- ↓ 33% lower odds of any liver disease ($p=9.9\times10^{-7}$)
- ↓ 50% lower odds of liver cirrhosis ($p=4.4\times10^{-5}$)

Circled genes: associated with (1.) ALT levels <u>AND</u> (2.) AST levels <u>AND</u> (3.) any liver disease

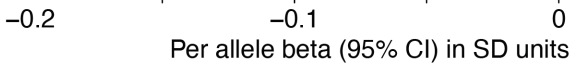
The protective associations for *CIDEB* mutations carriers reflect Loss of Function of CIDEB

pLOF = predicted Loss of Function (e.g. protein truncating variants)

Association of CIDEB pLOF compared to pLOF and missense variants

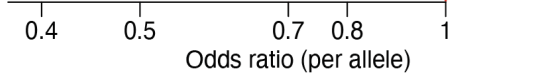
ALT levels

Exposure	Beta (95% CI), Clinical Units	Allele count	P
CIDEB pLOF	−1.53 (−2.46,−0.60) U/L	542,144 / 760 / 0	0.0012
CIDEB pLOF plus any missense	−1.28 (−1.71,−0.86) U/L	539,292 / 3,609 / 3	3.3e−9



Liver disease

Exposure	OR (95% CI)	Allele count (cases)	Allele count (controls)	P
CIDEB pLOF	0.54 (0.37,0.79)	24,754 / 23 / 0	486,538 / 758 / 0	0.0015
CIDEB pLOF plus any missense	0.67 (0.57,0.79)	24,800 / 144 / 0	487,005 / 3,628 / 3	9.9e−7



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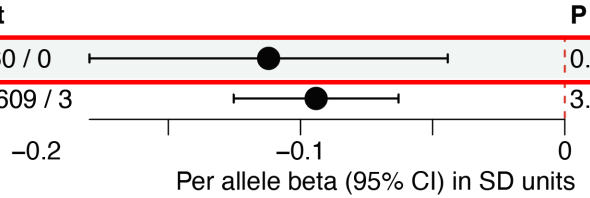

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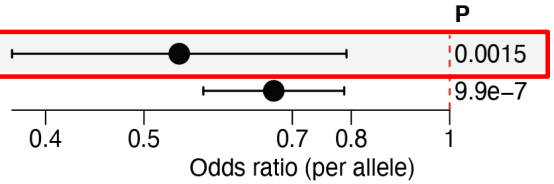

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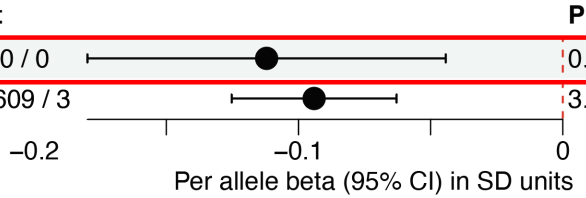

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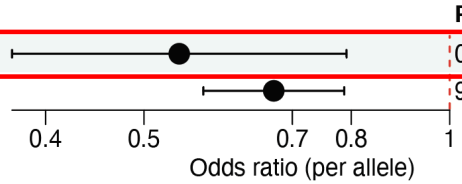


Important for therapeutic development!

Approximately 1 in 150 persons (0.7%) carried a rare predicted loss of function or missense variant.
99.3% individuals may benefit from CIDEB inhibition.

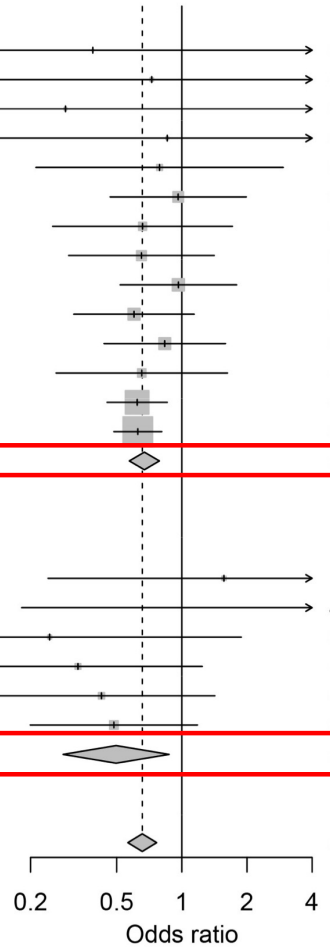
RGC & collaborators, N Engl J Med 2022; 387:332-344

The association between CIDEB and liver disease replicates in independent cohorts, and is consistent across cohorts and ancestry groups

Discovery

Replication

Cohort	Ancestry	Genotype Count (Cases) RR / RA /AA	Genotype Count (Controls) RR / RA /AA	AAF	OR	[95% CI]	P value
Stage 3 cohorts meta-analysis							
UKB	AMR	14 / 0 / 0	414 / 5 / 0	0.0058	0.39	[0.00; 260.49]	0.76
UKB	EAS	47 / 1 / 0	1,564 / 22 / 0	0.0070	0.73	[0.08; 6.88]	0.76
BioMe	SAS	52 / 0 / 0	694 / 18 / 0	0.0118	0.29	[0.01; 6.02]	0.42
BioMe	EAS	52 / 1 / 0	618 / 5 / 0	0.0044	0.86	[0.06; 11.89]	0.91
UKB	AFR	177 / 2 / 0	6,849 / 105 / 0	0.0075	0.79	[0.21; 2.94]	0.72
UKB	SAS	232 / 8 / 0	7,282 / 271 / 1	0.0180	0.96	[0.47; 1.99]	0.90
BioMe	AMR	452 / 4 / 0	2,732 / 31 / 0	0.0054	0.66	[0.25; 1.72]	0.39
UPENN−PMBB	EUR	678 / 6 / 0	4,992 / 55 / 0	0.0053	0.65	[0.30; 1.41]	0.26
BioMe	EUR	750 / 12 / 0	7,927 / 133 / 1	0.0083	0.96	[0.52; 1.79]	0.91
BioMe	AFR	885 / 7 / 0	9,333 / 142 / 0	0.0072	0.60	[0.32; 1.14]	0.10
UPENN−PMBB	AFR	944 / 11 / 0	6,640 / 92 / 0	0.0067	0.83	[0.44; 1.59]	0.58
MDCS	EUR	1,045 / 3 / 0	27,775 / 125 / 0	0.0022	0.65	[0.26; 1.63]	0.34
UKB	EUR	8,879 / 32 / 0	324,061 / 1,881 / 0	0.0029	0.62	[0.45; 0.86]	0.004
GHS	EUR	10,593 / 57 / 0	86,124 / 743 / 1	0.0041	0.63	[0.48; 0.81]	3.0×10^{-4}
Meta-analysis		**24,800 / 144 / 0**	**487,005 / 3,628 / 3**	**0.0013**	**0.67**	**[0.57; 0.79]**	**9.9×10^{-7}**
Heterogeneity: I^2 = 0%, p = 0.99							
Additional non-European cohorts meta-analysis							
UPENN−PMBB	SAS	48 / 3 / 0	409 / 16 / 0	0.0200	1.56	[0.24; 10.18]	0.62
UPENN−PMBB	EAS	71 / 1 / 0	523 / 7 / 0	0.0066	4.86	[0.18; 129.94]	0.33
UPENN−PMBB	AMR	101 / 0 / 0	498 / 9 / 0	0.0074	0.24	[0.03; 1.89]	0.16
GHS	AMR	312 / 1 / 0	1,230 / 16 / 0	0.0055	0.33	[0.09; 1.24]	0.10
Indiana-CLDB	AFR	337 / 2 / 0	1,061 / 14 / 0	0.0057	0.43	[0.13; 1.42]	0.15
GHS	AFR	496 / 3 / 0	3,451 / 56 / 0	0.0074	0.49	[0.20; 1.18]	0.11
Meta-analysis		**1,365 / 10 / 0**	**7,172 / 118 / 0**	**0.0074**	**0.50**	**[0.28; 0.87]**	**0.02**
Heterogeneity: I^2 = 0%, p = 0.52							
Overall meta-analysis		**26,165 / 154 / 0**	**494,177 / 3,746 / 3**	**0.0037**	**0.66**	**[0.56; 0.77]**	**7.9×10^{-8}**
Heterogeneity: I^2 = 0%, p = 0.97							



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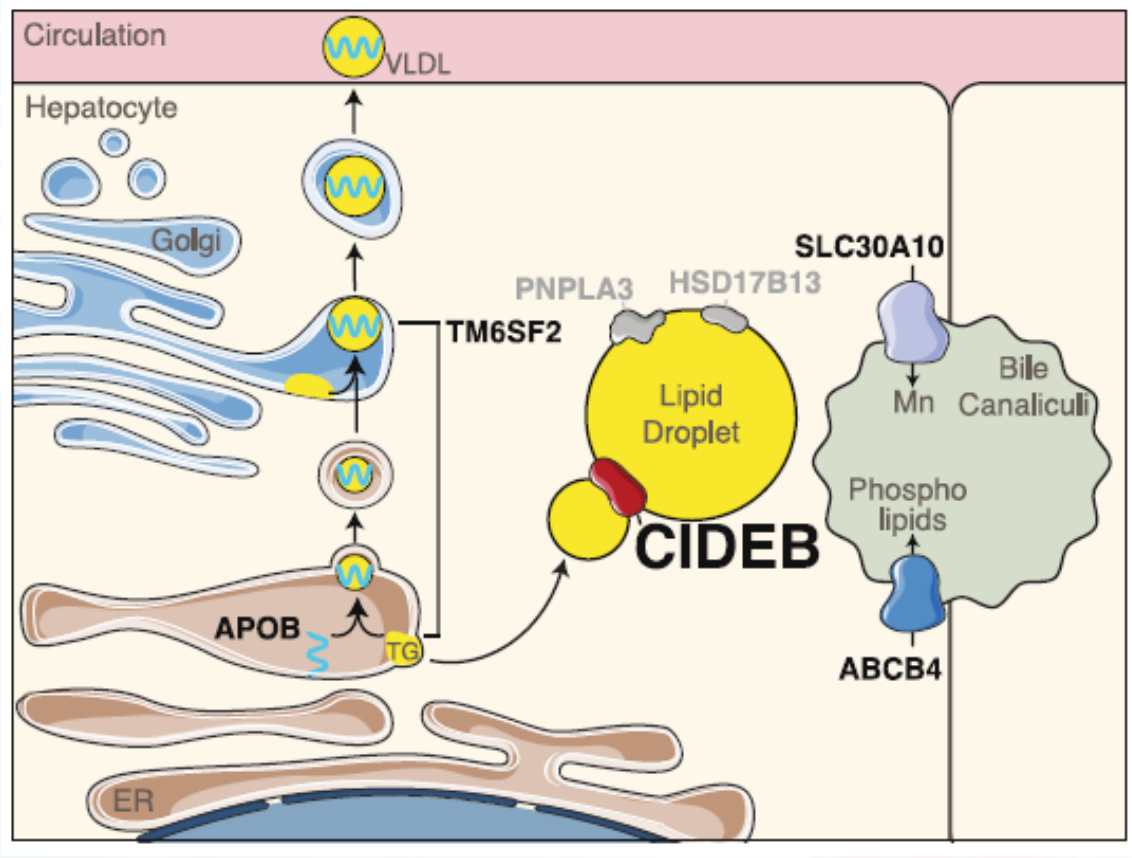
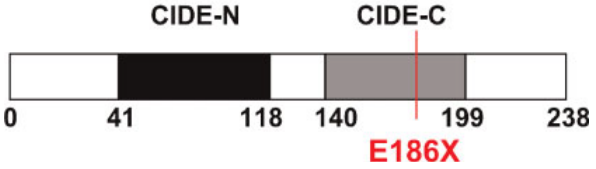
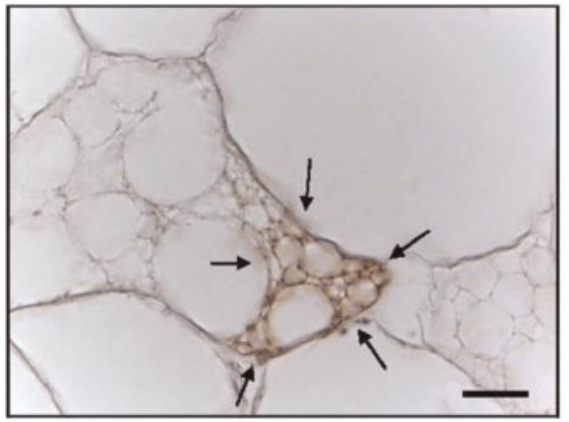
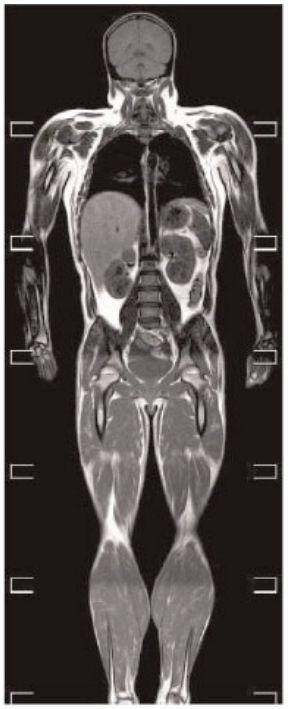
CIDEB is a structural protein of hepatic lipid droplets mediating droplet fusion and growth



A patient with homozygous LOF of *CIDEC,* a member of the same family with high adipose expression, results in familial partial lipodystrophy characterized by many adipocytes with multiple small lipid droplets



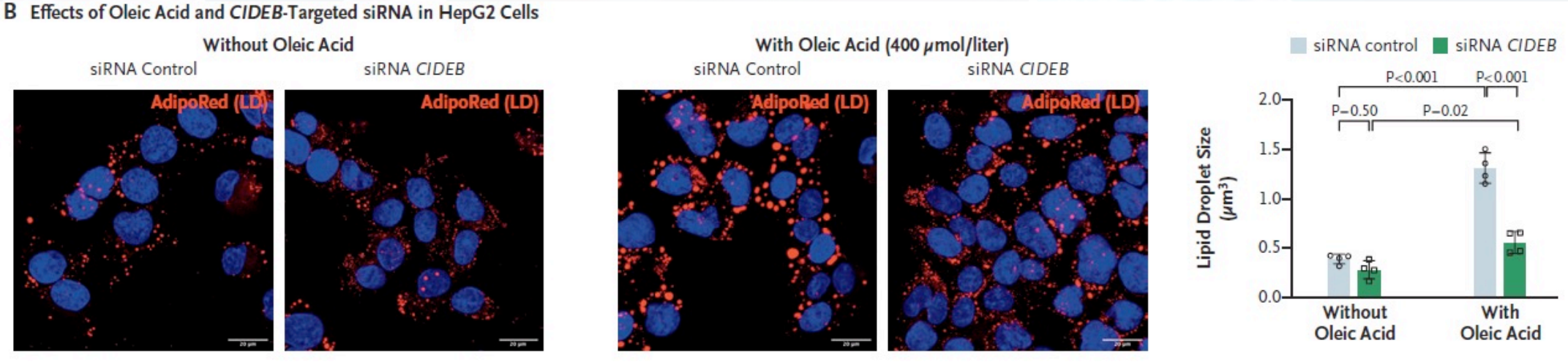




Rubio-Cabezas et al. EMBO MM 2009

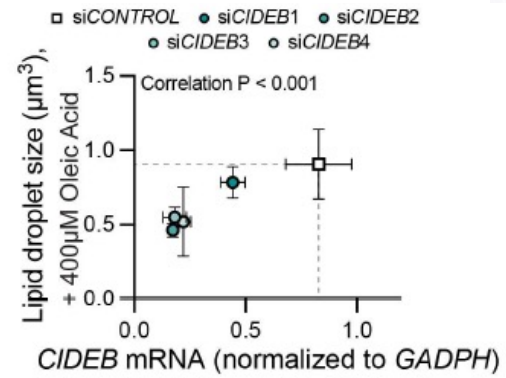
RGC & collaborators, N Engl J Med 2022; 387:332-344

Knockdown of *CIDEB* in oleate-treated human hepatoma cells results in smaller lipid droplets



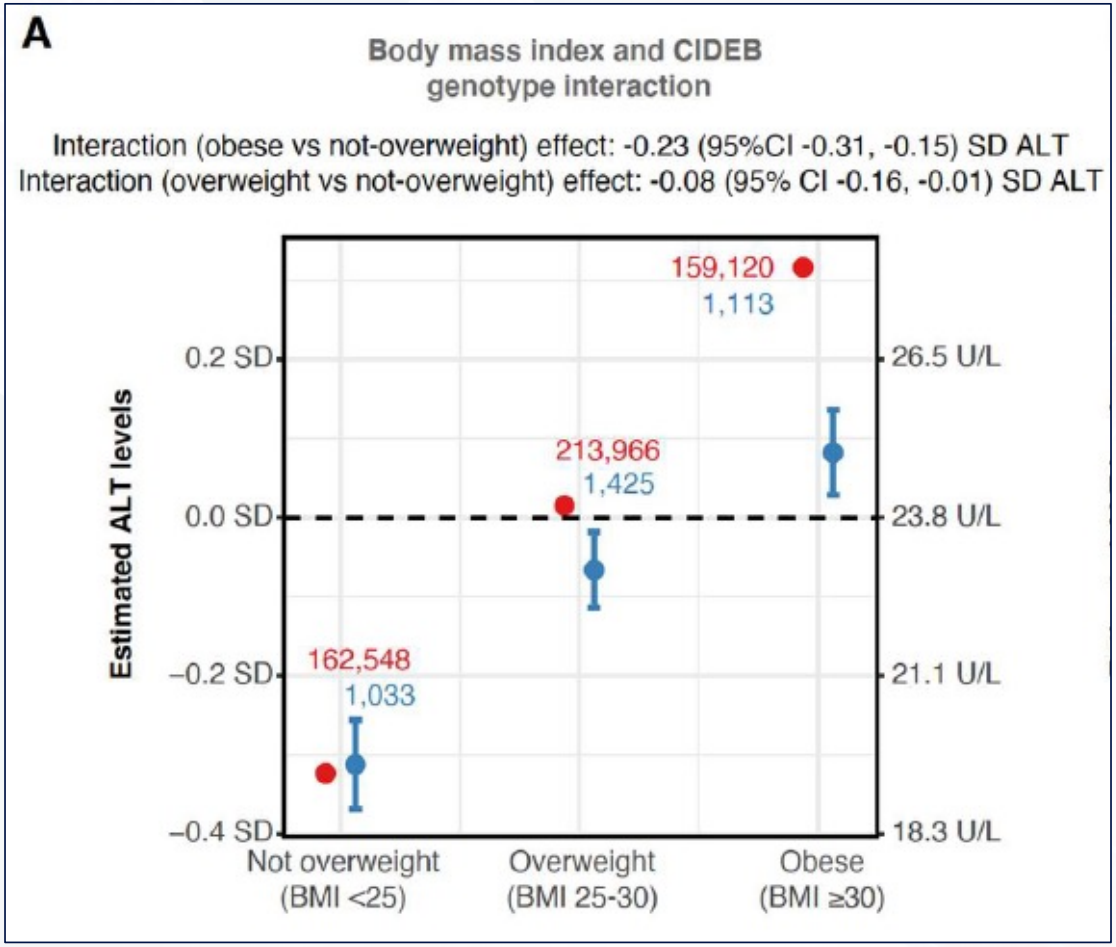
Experimental results are in line with literature on role of CIDE proteins in lipid droplet fusion and growth

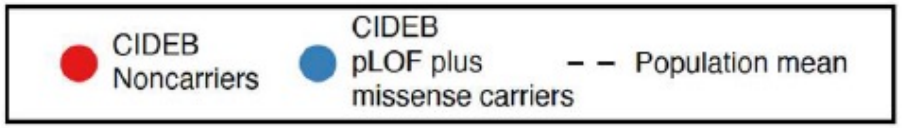
Correlation between knockdown level and lipid droplet size using different siRNA in a HUH-7 cell experiment



Experiments by Giusy Della Gatta and Minhee Kim (RGC Biology) from RGC & collaborators, N Engl J Med 2022; 387:332-344;
Other literature on CIDE proteins roles in lipid droplet size: Xu et al. JBC 2016; Chen et al. Traffic 2020; Li et al. Diabetes 2007; Nishino et al. JCI 2008; Singaravelu et al. BBRC 2013

The protective association for *CIDEB* mutation is amplified in individuals with higher BMI or carriers of the *PNPLA3* Ile148Met allele





RGC & collaborators, N Engl J Med 2022; 387:332-344

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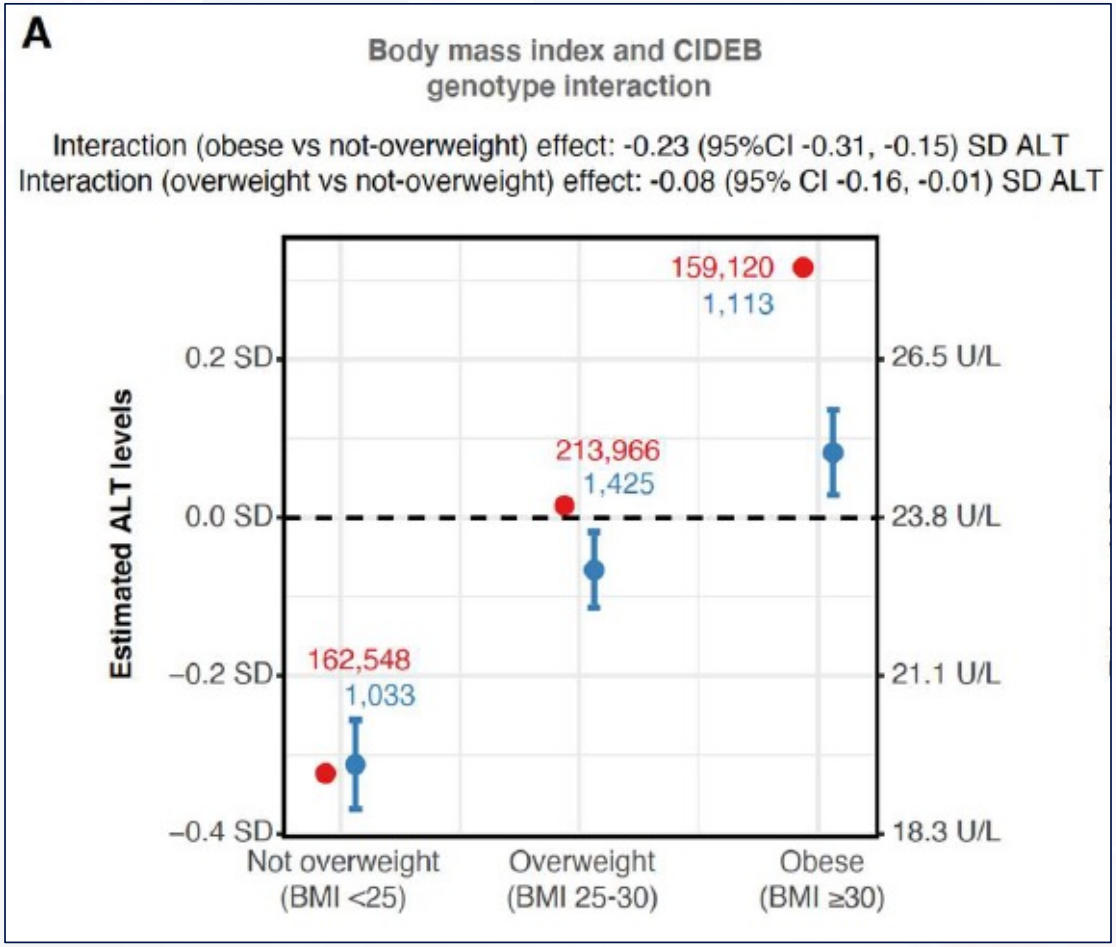
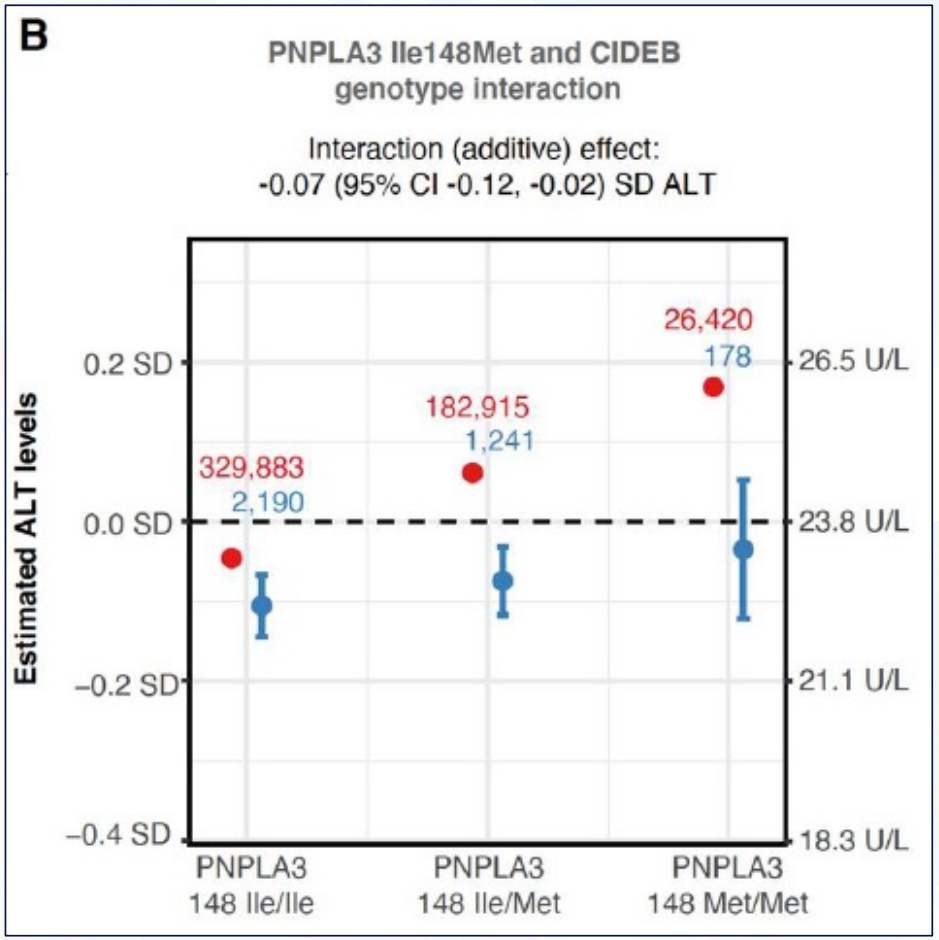


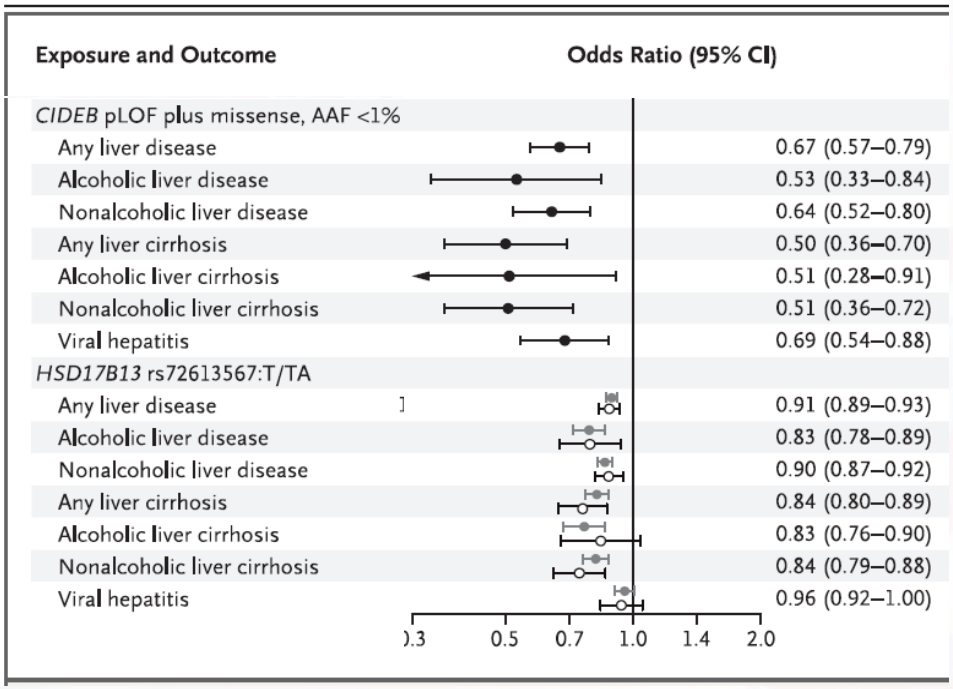


RGC & collaborators, N Engl J Med 2022; 387:332-344

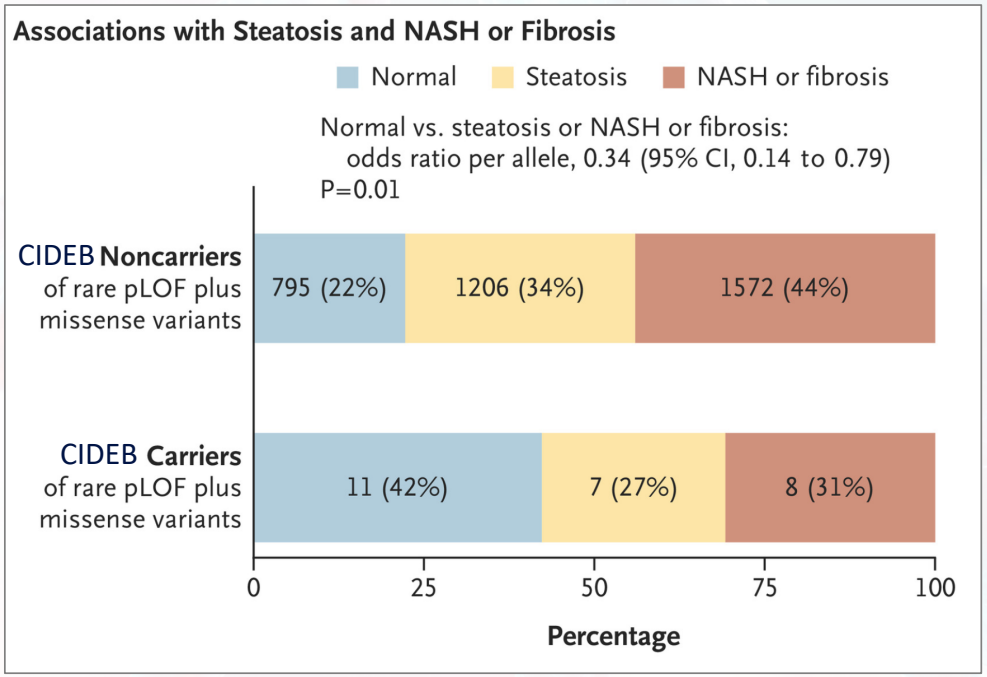
In comparison to the HSD17B13's splice allele, CIDEB's protective effect is much larger and protects against both steatosis and NASH/fibrosis (histology data).

CIDEB

HSD17B13



Association with lower NAFLD activity score in bariatric surgery patients, driven by lower steatosis *and* NASH odds



RGC & collaborators, N Engl J Med 2022; 387:332-344

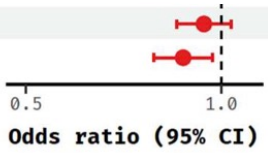
Loss of CIDEB does not associate with other metabolic traits in humans, but does in mouse.

No associations with lipids or anthropometric traits for human (mostly-heterozygous) carriers

Outcome	P	Allele count (RR|RA|AA)	Clinical Units Beta (95% CI)
Apolipoprotein B	0.94	428,376|2,741|2	0.03[-0.79,0.85] mg/dL
HDL-cholesterol	0.25	485,789|3,263|3	-0.27[-0.73,0.19] mg/dL
LDL-cholesterol	0.27	475,084|3,175|3	0.62[-0.49,1.74] mg/dL
Triglycerides	0.43	521,763|3,472|3	1.09[-1.61,3.78] mg/dL
Glucose	0.72	479,640|3,223|3	-0.13[-0.84,0.58] mg/dL
HbA1c	0.43	497,009|3,286|3	-0.01[-0.04,0.02] mg/dL
BMI	0.33	569,229|3,845|3	0.07[-0.07,0.21] kg/m2
Waist hip ratio	0.09	477,173|3,012|2	-0.00[-0.01,0.00] ratio
Body fat mass	0.38	441,584|2,832|2	0.15[-0.18,0.48] Kg
Body fat %	0.47	442,065|2,836|2	0.11[-0.18,0.40] %
Body lean mass	0.95	442,285|2,835|2	0.01[-0.37,0.39] Kg
Body lean %	0.49	442,283|2,835|2	-0.10[-0.40,0.19] %
DBP	0.92	570,591|3,862|3	-0.03[-0.57,0.52] mmHg
SBP	0.95	570,884|3,868|3	0.01[-0.28,0.30] mmHg

Nominal association with lower T2D risk

Description	P	Allele count cases (RR|RA|AA)	Allele count controls (RR|RA|AA)	Odds ratio (95% CI)
Coronary Artery Disease	0.22	92,131|644|3	358,153|2,729|1	0.94 (0.85 to 1.04)
Type 2 Diabetes	0.01	65,551|510|1	526,953|3,583|2	0.87 (0.79 to 0.97)



CIDEB pLOF — CIDEB pLOF and missense

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RGC & collaborators, N Engl J Med 2022; 387:332-344

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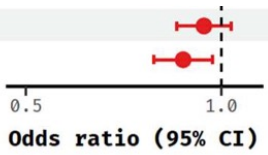
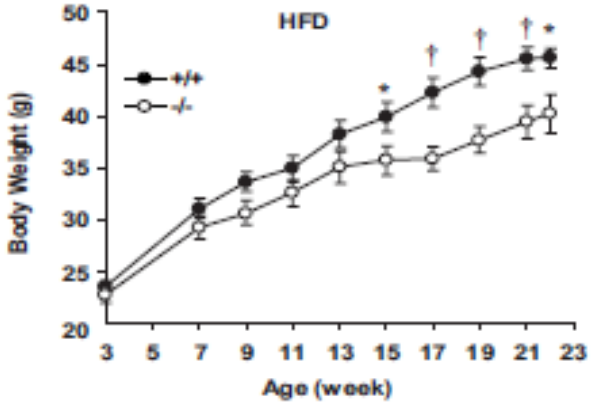
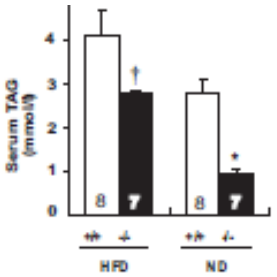


CIDEB pLOF CIDEB pLOF and missense

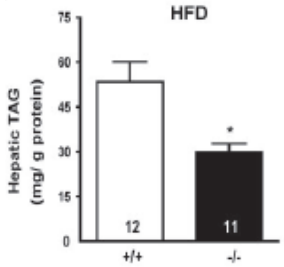
Protection from weight gain in high-fat diet (HFD) in CIDEB KO mouse



Lower circulating triglycerides



Lower liver triglycerides



Li et al. Diabetes 2007

RGC & collaborators, N Engl J Med 2022; 387:332-344

Conclusions

- We performed target identification in a high-unmet need therapeutic area where preclinical models have not been predictive
- Rare germline mutations in *CIDEB* confer substantial protection from liver damage and liver disease
- Build-up of lipid droplets mediated by CIDEB is a driver of human liver disease.
- The majority of individuals do not carry CIDEB mutations and might benefit most from inhibition
- We are pursuing siRNA approaches to CIDEB inhibition with our partner Alnylam