Carboxyl-ester lipase: Beyond the beta cell in diabetes

16.02.2006

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What is diabetes?

Definition

- Chronic hyperglycemia
- Associated with complications from eyes, kidneys and neurological system

Prognosis

- Untreated leads to death
- High degree of morbidity and mortality

Diabetes is heterogeneous disease

I: Type 1 diabetes

Children, insulin dependent

II: Type 2 diabetes

Adults, associated with obesity

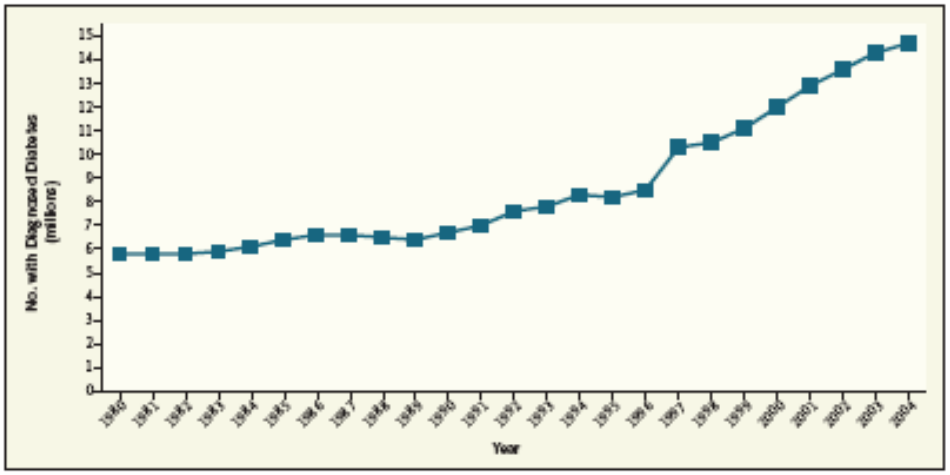
III: Specific forms

Monogenic types

Secondary types

IV: Pregnancy

The NEW ENGLAND JOURNAL of MEDICINE



Perspective

FEBRUARY 9, 2006

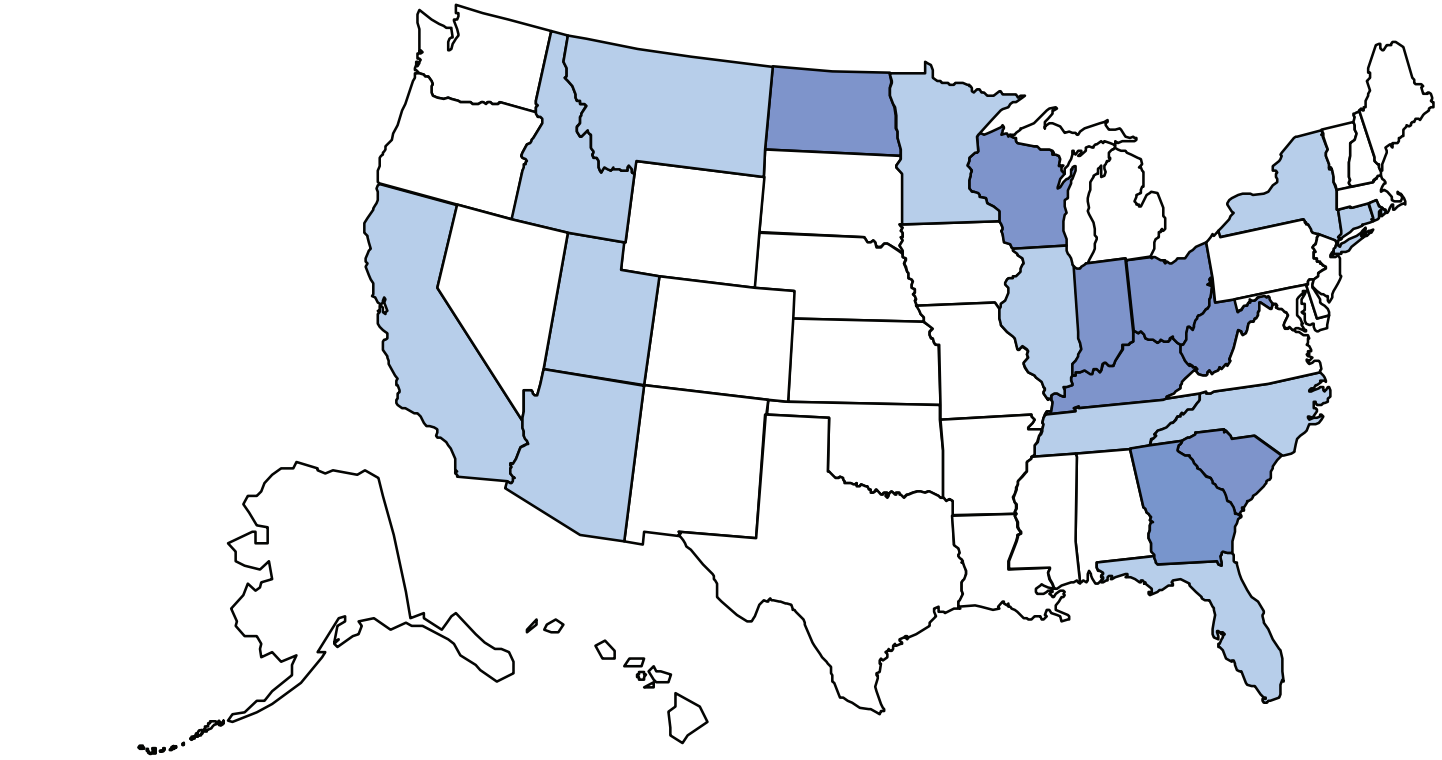
Facing the Diabetes Epidemic — Mandatory Reporting of Glycosylated Hemoglobin Values in New York City

Robert Steinbrook, M.D.

Obesity Trends* Among U.S. Adults

BRFSS, 1985

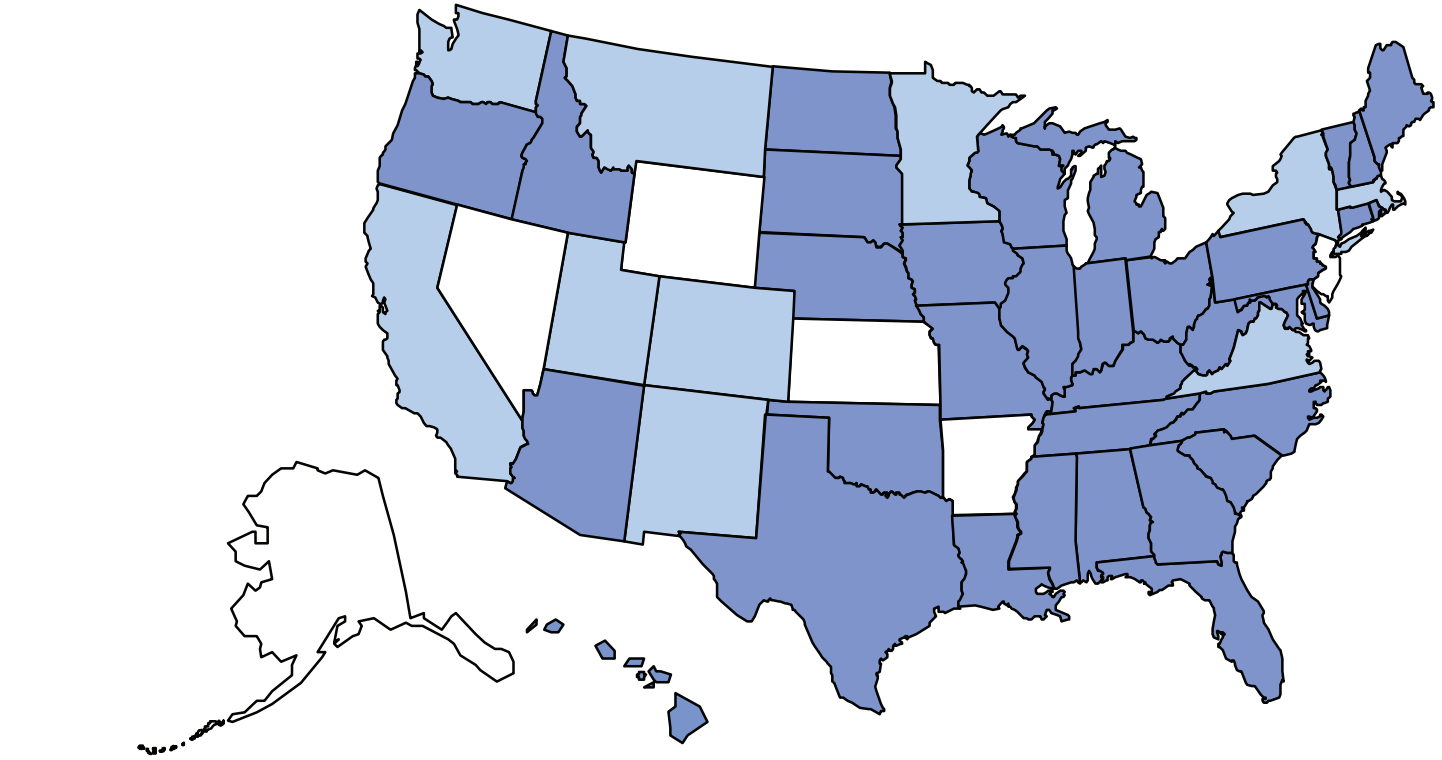
(*BMI ≥30, or ~ 30 lbs overweight for 5’ 4” person)



Obesity Trends* Among U.S. Adults

BRFSS, 1990

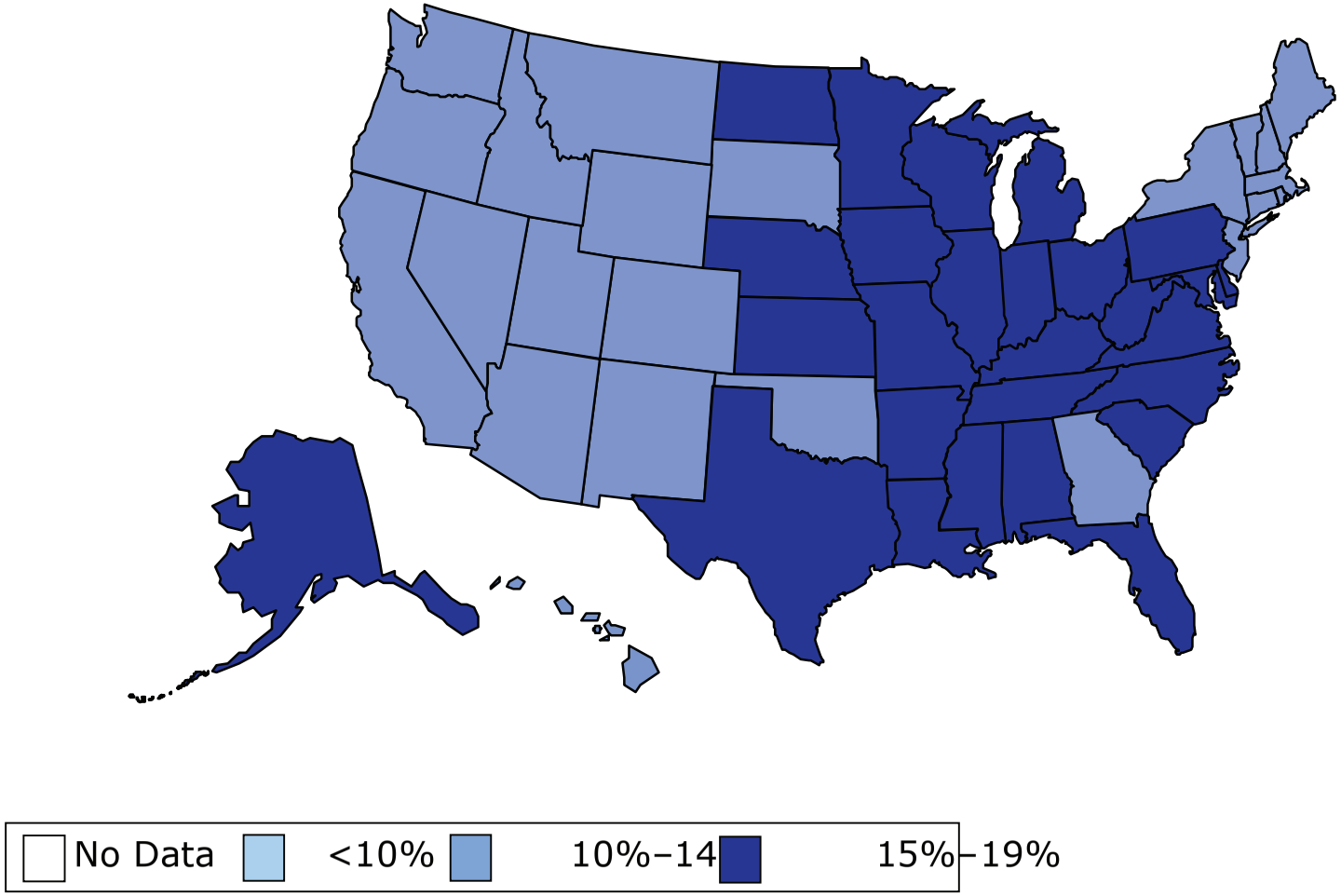
(*BMI ≥30, or ~ 30 lbs overweight for 5’ 4” person)



Obesity Trends* Among U.S. Adults

BRFSS, 1995

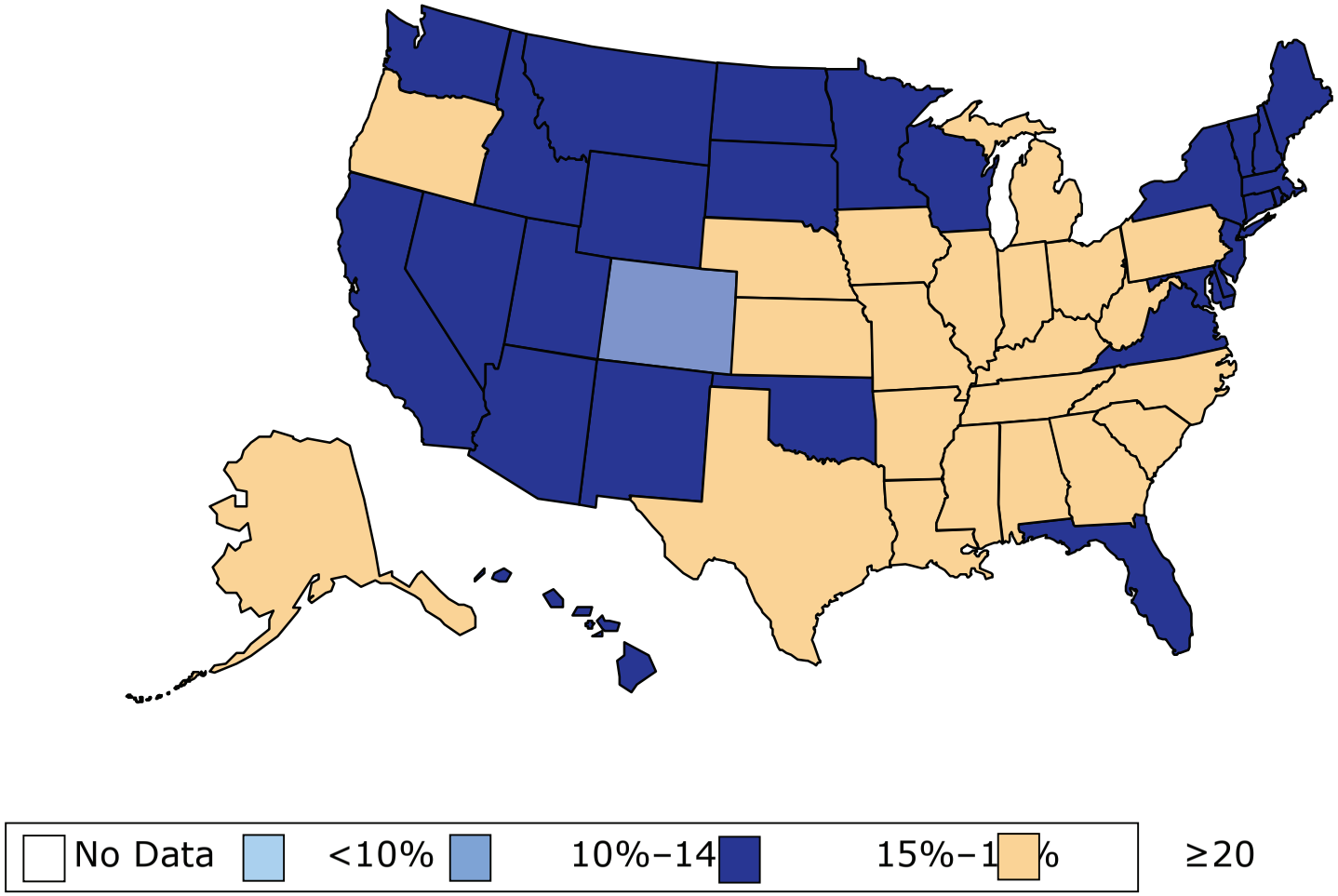
(*BMI ≥30, or ~ 30 lbs overweight for 5’ 4” person)



Obesity Trends* Among U.S. Adults

BRFSS, 2000

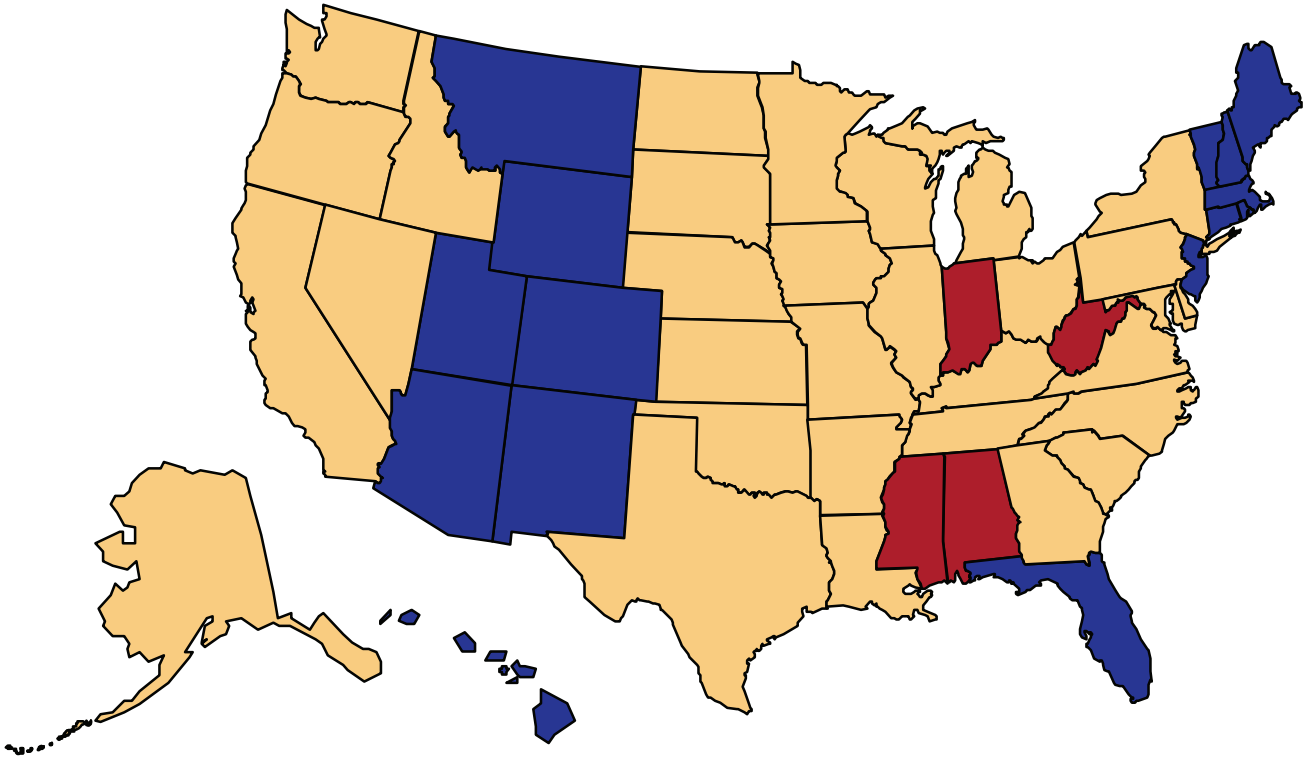
(*BMI ≥30, or ~ 30 lbs overweight for 5’ 4” person)



Obesity* Trends Among U.S. Adults

BRFSS, 2003

(*BMI ≥30, or ~ 30 lbs overweight for 5’ 4” person)



The diabetes epidemic - impact for Norway?

Prevalence today

- Not known but believed to be 5-7%
- Increasing (HUNT data)

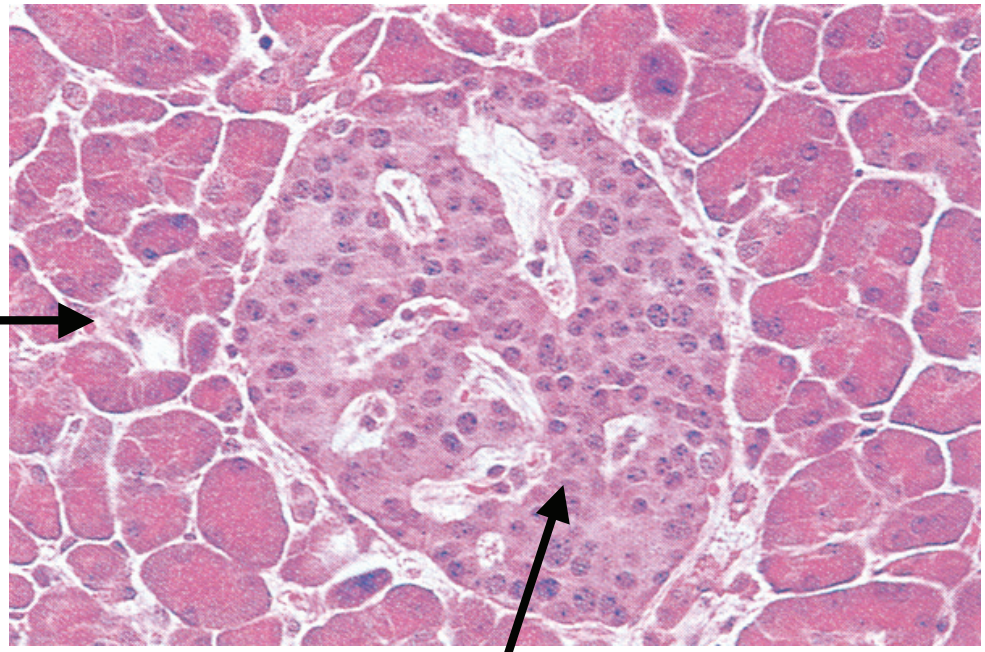
Obesity

- Norwegian males 40 years most overweight in Europe

Not only a common disease, but increasing rapidly!

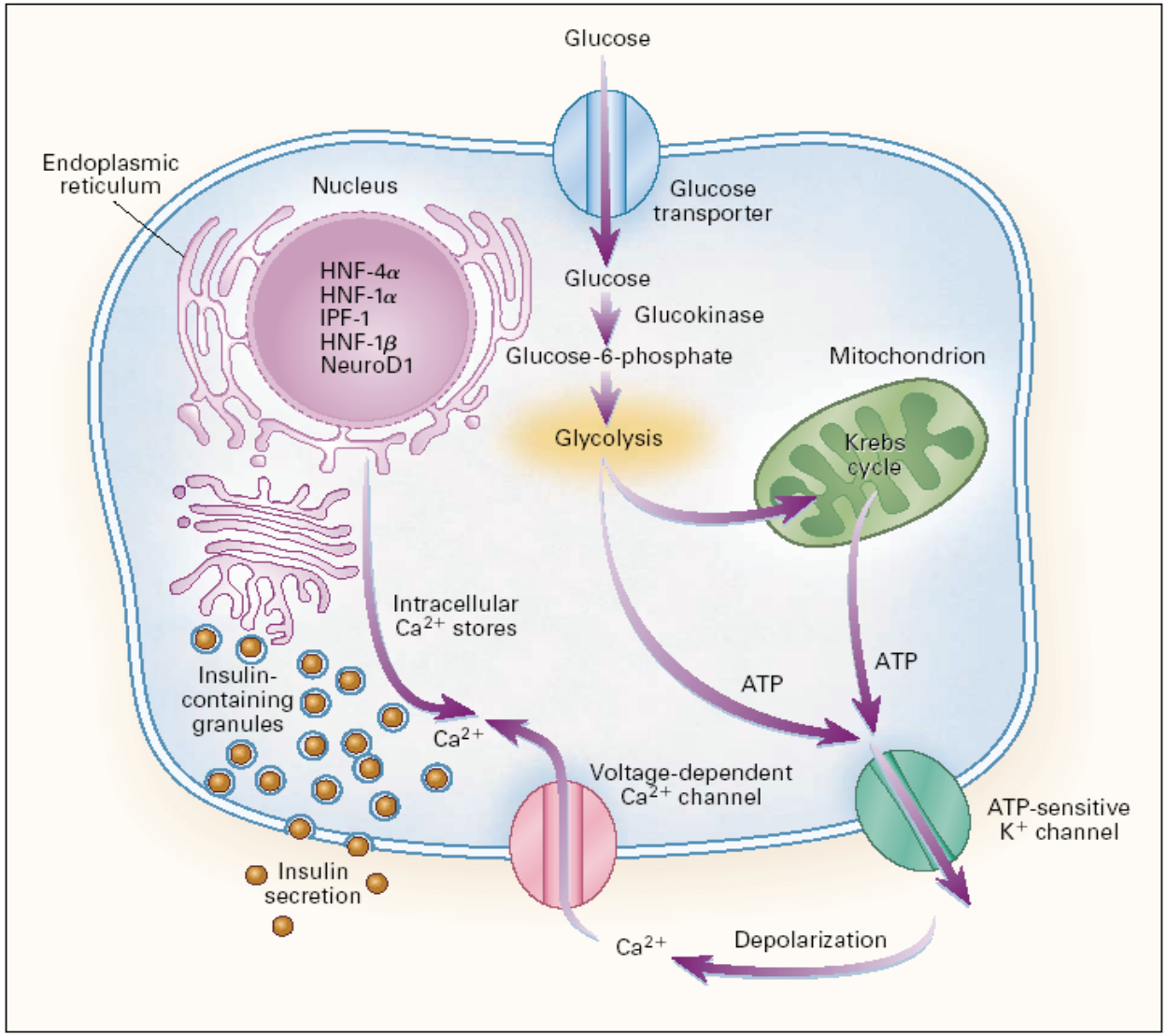
Insulin is produced in pancreas

Exocrine cells
The acinar cells
and duct cells



Endorine cells
The beta cells

The beta cell

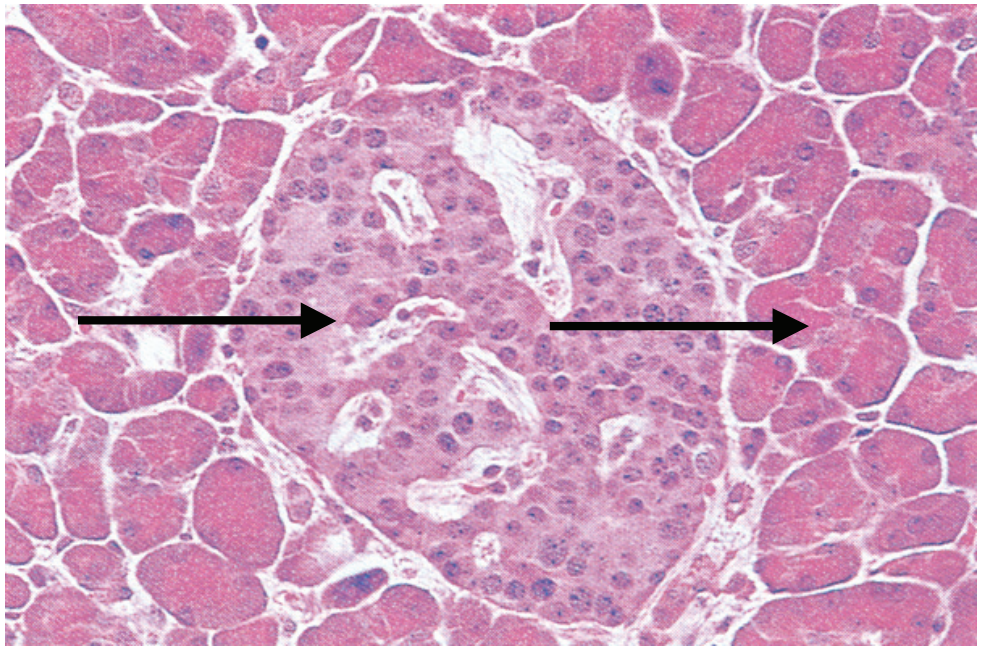


Endocrine vs exocrine disease

Interdependency and cross-talk

Exocrine disease primary event

Cystic fibrosis
Chronic pancreatitis



Endocrine disease primary event

Type 1 and type 2 diabetes exocrine deficiency

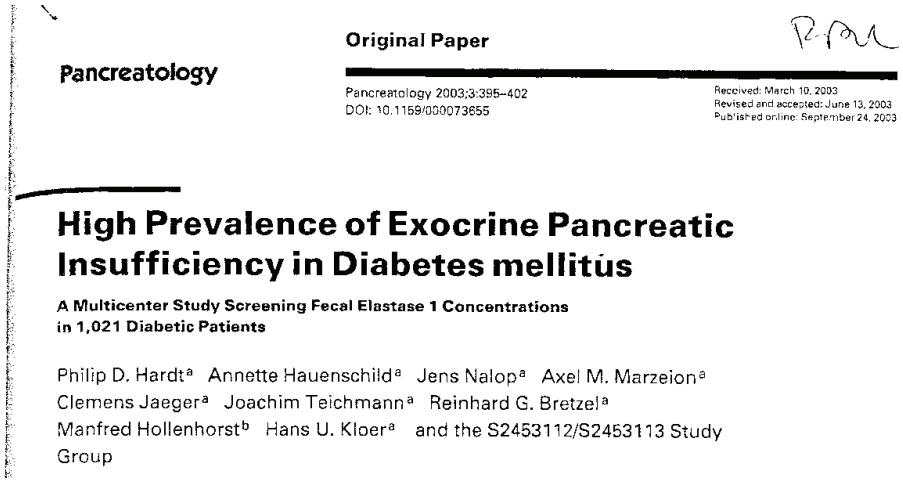
Common factor

IPF-1
HNF-1beta

Exocrine dysfunction in diabetes

Table 2. Prevalence of pancreatic exocrine dysfunction in 1,015 diabetic patients

Exocrine function	Fecal elastase 1	n	%
Normal	>200 µg/g	602	59.3
Mild insufficiency	100–200 µg/g	181	17.8
Severe insufficiency	<100 µg/g	232	22.9



Pancreatology

Original Paper

Pancreatology 2003;3:395–402
DOI: 10.1159/000073655

Received: March 10, 2003
Revised and accepted: June 13, 2003
Published online: September 24, 2003

High Prevalence of Exocrine Pancreatic Insufficiency in Diabetes mellitus

A Multicenter Study Screening Fecal Elastase 1 Concentrations in 1,021 Diabetic Patients

Philip D. Hardt[a] Annette Hauenschild[a] Jens Nalop[a] Axel M. Marzeion[a] Clemens Jaeger[a] Joachim Teichmann[a] Reinhard G. Bretzel[a] Manfred Hollenhorst[b] Hans U. Kloer[a] and the S2453112/S2453113 Study Group

What is fecal elastase-1?

Clin Chem Lab Med 2002; 40(4):325–332 © 2002 by Walter de Gruyter · Berlin · New York

Review

Fecal Elastase-1 as a Test for Pancreatic Function: a Review

Roberto Dominici[1] and Carlo Franzini[1, 2]*

[1] Istituto di Scienze Biomediche,
[2] Università degli Studi di Milano,
Ospedale Luigi Sacco, Milano, Italy

matostatin (δ-cells) and pancreatic polypeptide (PP-cells) organized into islets scattered throughout the exocrine pancreas. The endocrine and exocrine compartments are structurally and functionally integrated through an islet-acinar portal blood system that facili-

Pancreatic enzyme
Stabile in the gut
Reflects exocrine dysfunction

ELISA
100-200 μg/g: moderate deficiency
100 μg/g: severe deficiency

A novel diabetes syndrome

Dagbladet.no Helse

Ny diabetes funnet

Forskerne i Bergen fant diabetes som kan skyldes fordøyelsesproblemer.

ELIN DAVIDSEN
Tirsdag 20.12.2005, 11:31
oppdatert 15:43

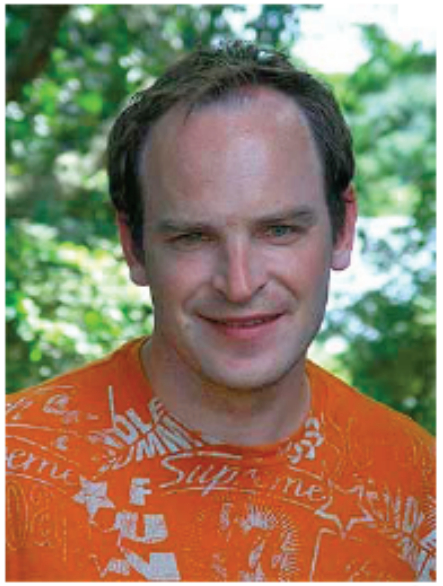
(Dagbladet.no): En gruppe forskere ved Haukeland universitetssykehus har studert en familie med maveproblemer i fem år. Nå har forskerne funnet ut at familien har en unik diabetes-variant som ikke tidligere er kjent.

Forskerteamet, som i lang tid forsket på arvelig diabetes, ble nysgjerrige da de fikk høre Arve Antonsen fra Askøy utenfor Bergen fortelle at mange i familien hans både led av diabetes og dårlig mave.

Det ble starten på en forskning som nå har resultert i at en ny type diabetes ble oppdaget.

Svikt

Forskere fant ut den unike diabetes-varianten skyldtes en svikt i den delen av bukspyttkjertelen som påvirker fordøyelsen. De som utvikler diabetes, sliter med maveproblemer i årevis før sykdommen bryter ut.



Helge Ræder: Torsdag kan du møte legen på nettmøte hos Dagbladet.no.
Foto: UNIVERSITETET I BERGEN

Mutations within a VNTR in *CEL* cause a novel syndrome of diabetes and pancreatic exocrine dysfunctio n

Helge Ræder[1], Stefan Johansson[2,11], Pål I Holm[3,4,10], Ingfrid S Haldorsen[5], Eric Mas[6], Véronique Sbarra[6], Ingrid Nermoen[7], Stig Å Eid e[2], Louise Grevle[2], Lise Bjørkhaug[2], Jørn V Sagen[1,4], Lage Aksnes[1], Oddmund Søvik[1], Dominique Lombardo[6], Anders Molven[8,9], Pål Rasmus Njølstad[1,2,10]

Nature Genetics, January 2006

What is MODY?

Maturity-onset diabetes of the young

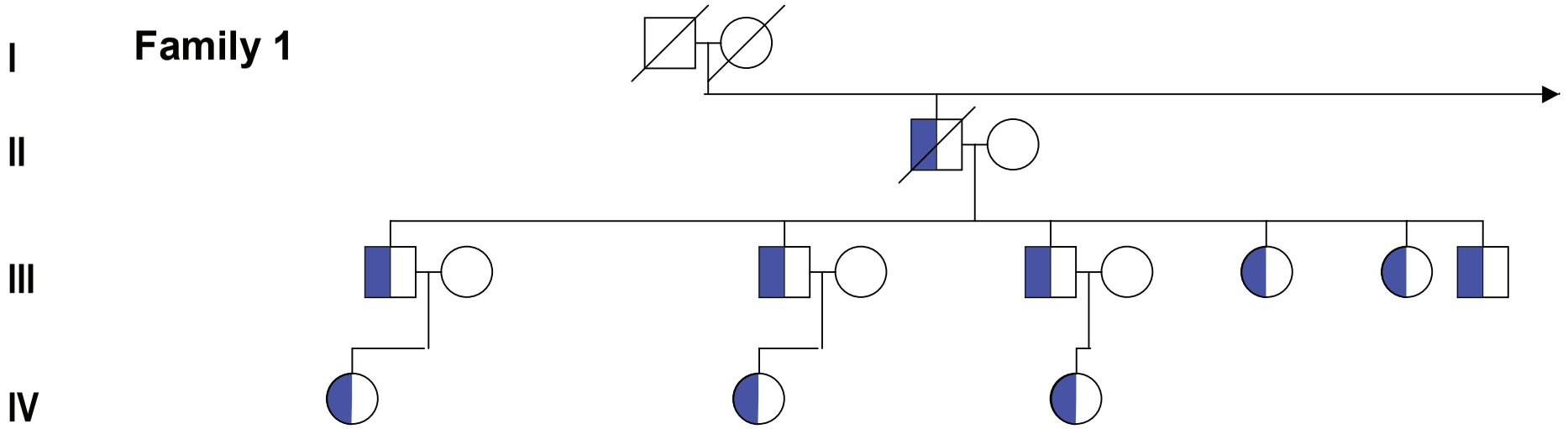
- Autosomal dominant
- Debut of diabetes before 25 years
- Beta cell dysfunction

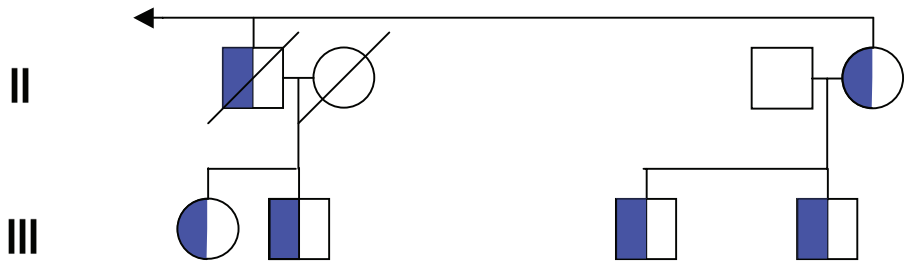
Glukocinase diabetes

- MODY2: GCK

Transcription factor diabetes

- MODY3: HNF-1α
- MODY1: HNF-4α
- MODY5: HNF-1β
- MODY4: IPF-1
- MODY6: NEUROD1





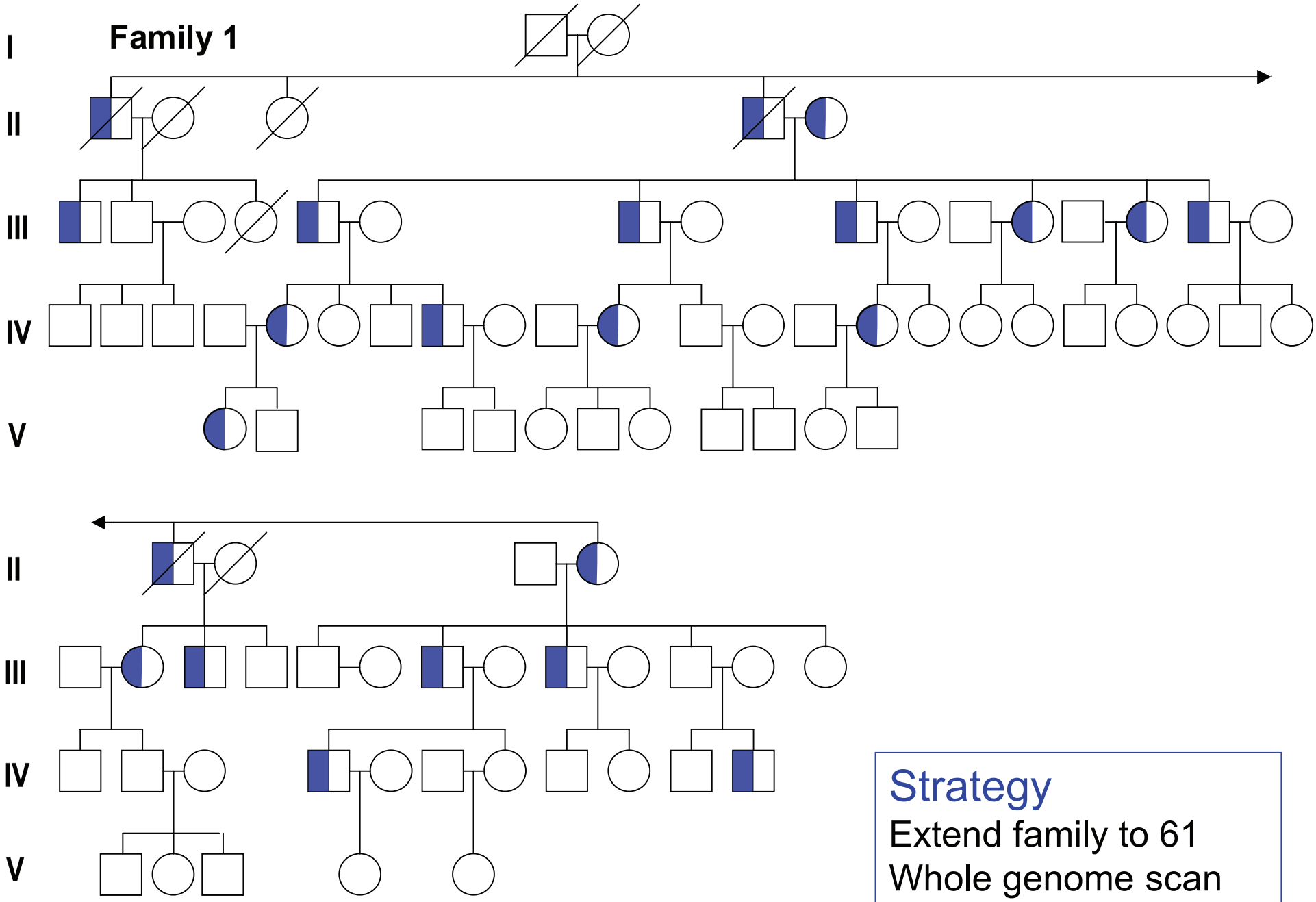
Holm

Phenotype

13 subjects with diabetes
Autosomal dominant (?)
Median age of diagnosis 38 years
7 insulin treatment
No signs of metabolic syndrome
No T1DM antibodies

Genes excluded by linkage analysis

TCF1, TCF2, HNF4A, IPF1, NEUROD1, NKX6A, ISL1, PAX4
GCK, INS, INSR, SLC2A2, GLP1R + 25 other genes/loci



Holm

Strategy

Extend family to 61

Whole genome scan

Linkage courses

Whole genome scan

Genotyping

- 374 markers
- ABI Mapping set MD10, 10 cM
- Custom markers for fine mapping
- ABI Prims 310
- ABI GeneMapper
- PedCheck
- All data checked manually

Linkage

- Autosomal dominant
- Diabetes: four liability classes
- Elastase deficiency: two liability classes
- Disease allele frequency 0.001
- Two-point LOD: MLINK
- Multipoint location: SimWalk 2.89

Two-point analysis: Diabetes linked to chromosome 9?

Diabetes as phenotype

Markers	Lodscores
D3S1601	1.39
D9S164	2.83
D9S1826	2.91
D12S310	1.07
D12S368	1.25
D13S285	1.05
D17S784	1.05

Additional abdominal disease?

Biocemical investigations

- Subnormal vitamin D spring samples
- Normal summer samples
- One hypocalcemia

Clinical symptoms and signs

- Vague, recurrent abdominal pain
- Frequent, loose stools
- Never hospitalized
- Many family members

Further strategy

- Feces from all affected
- Measured fecal elastase

Two-point analysis: Elastase deficiency linked to chromosome 9?

Fecal elastase 1 <100 μg/g as phenotype

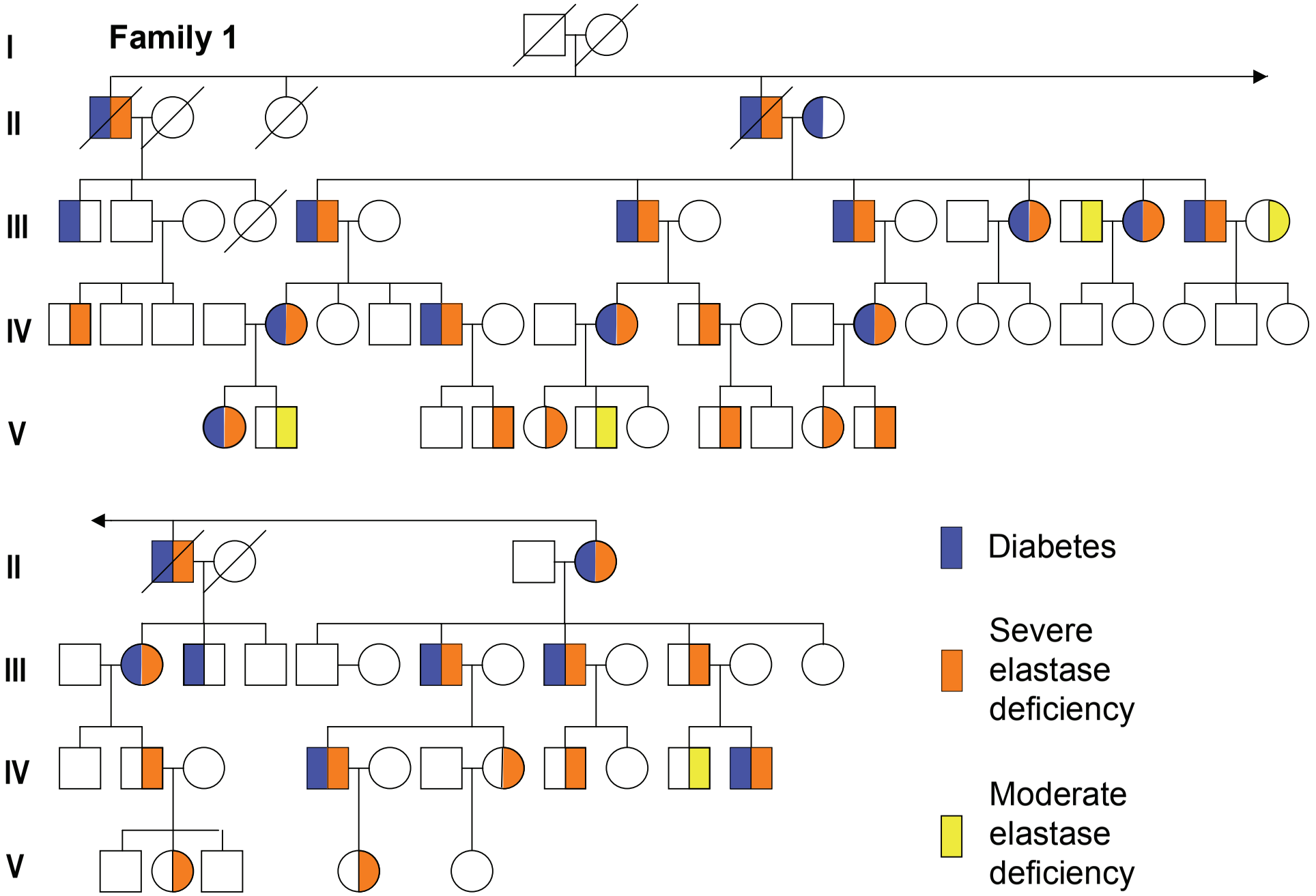
Markers	LOD scores
D6S462	3.6
D7S630	1.23
D9S164	5.65
D12S368	2.23

Johansson

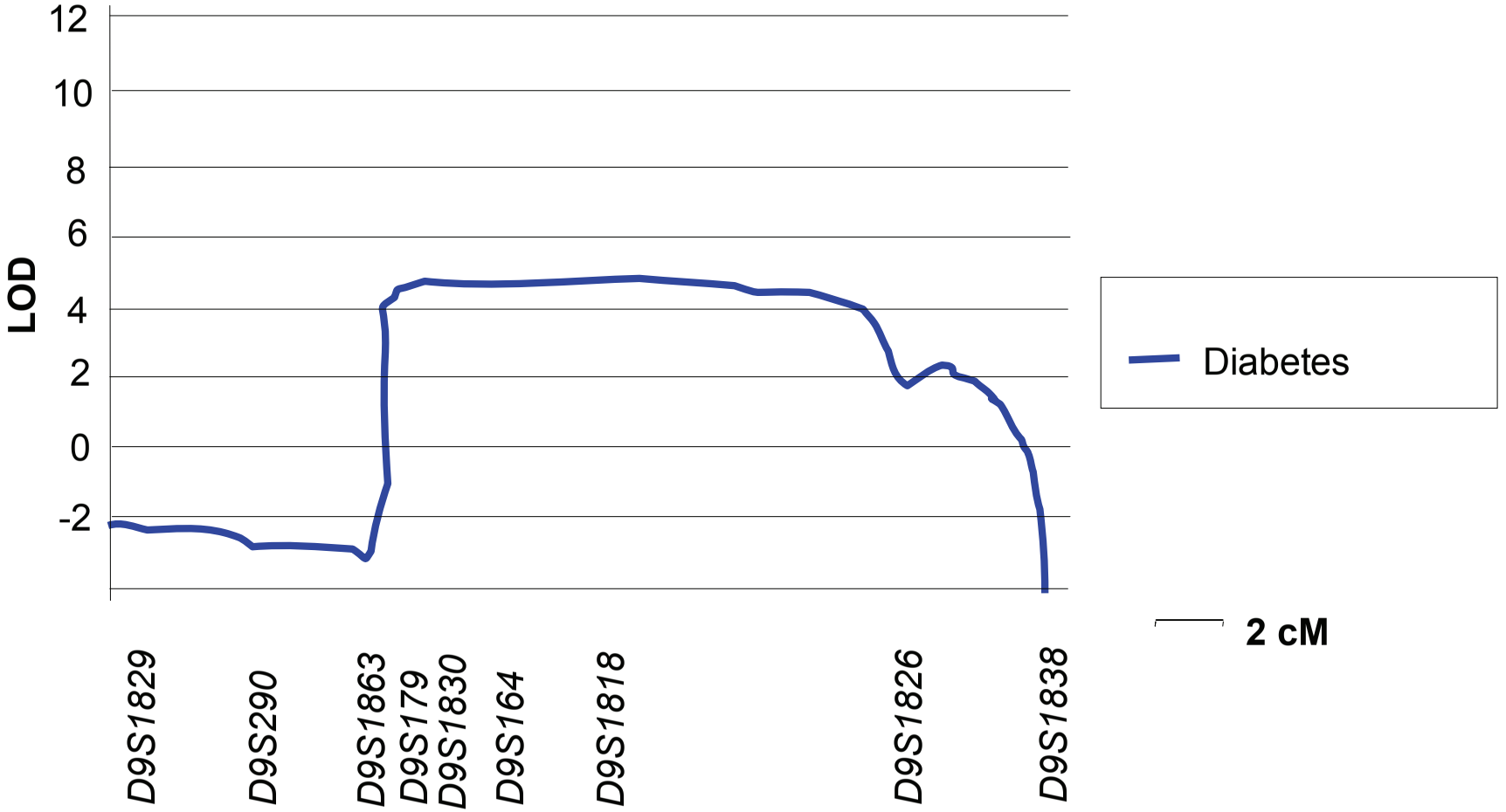
Abdominal disease most important phenotype?

Strategic choice

- Extended family to 184 members
- EDTA, serum, urine, feces from all available
- Oral glucose-tolerance tests in all possible
- Measured fecal elastase
- Custom markers for fine mapping chromsome 9

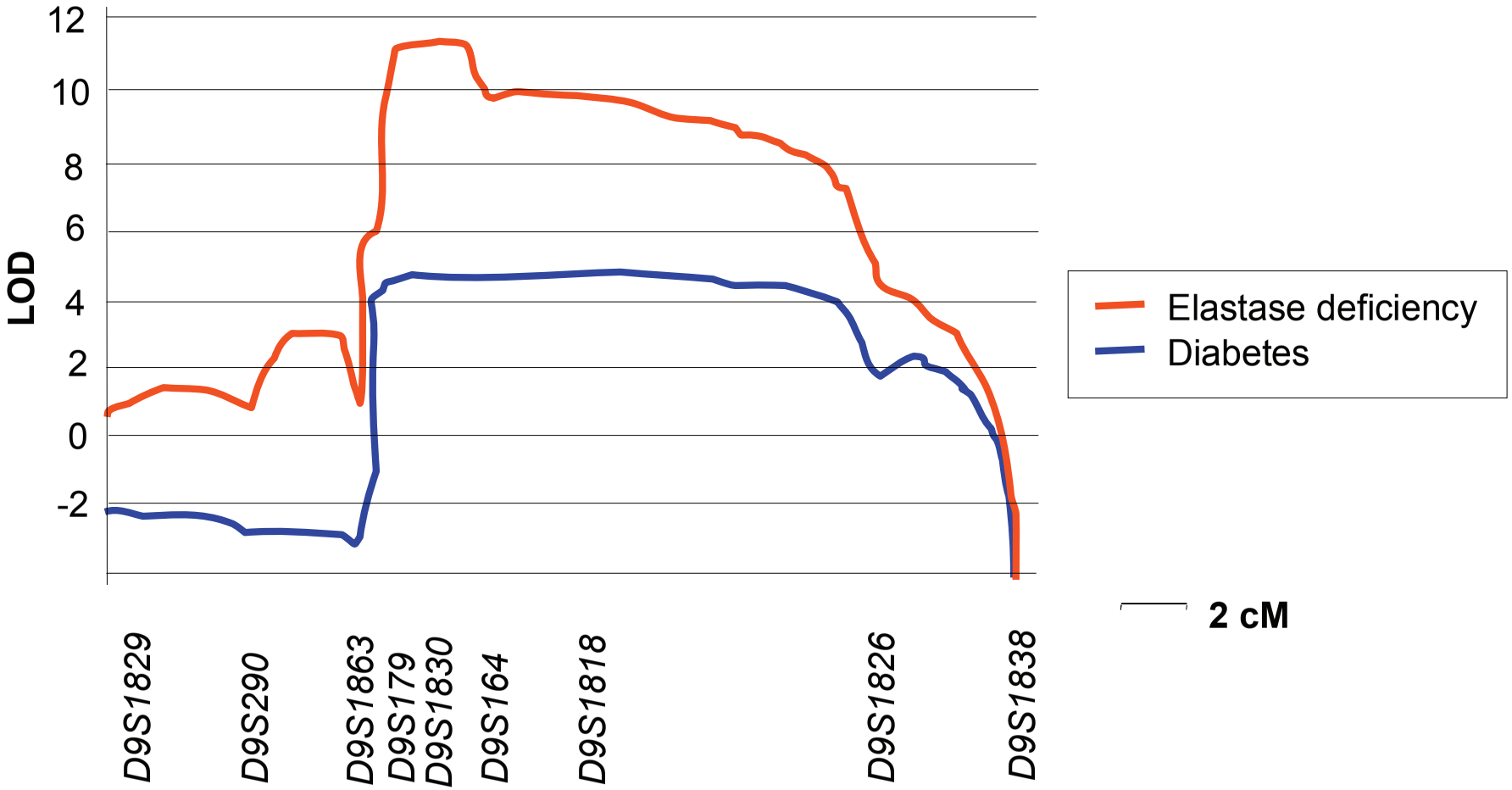

Family 1
I
II
III
IV
V
II
III
IV
V
Diabetes
Severe elastase deficiency
Moderate elastase deficiency

Multipoint analysis: Diabetes linked to chromosome 9 by fine mapping



Johansson

Multipoint analysis: Diabetes and elastase deficiency linked



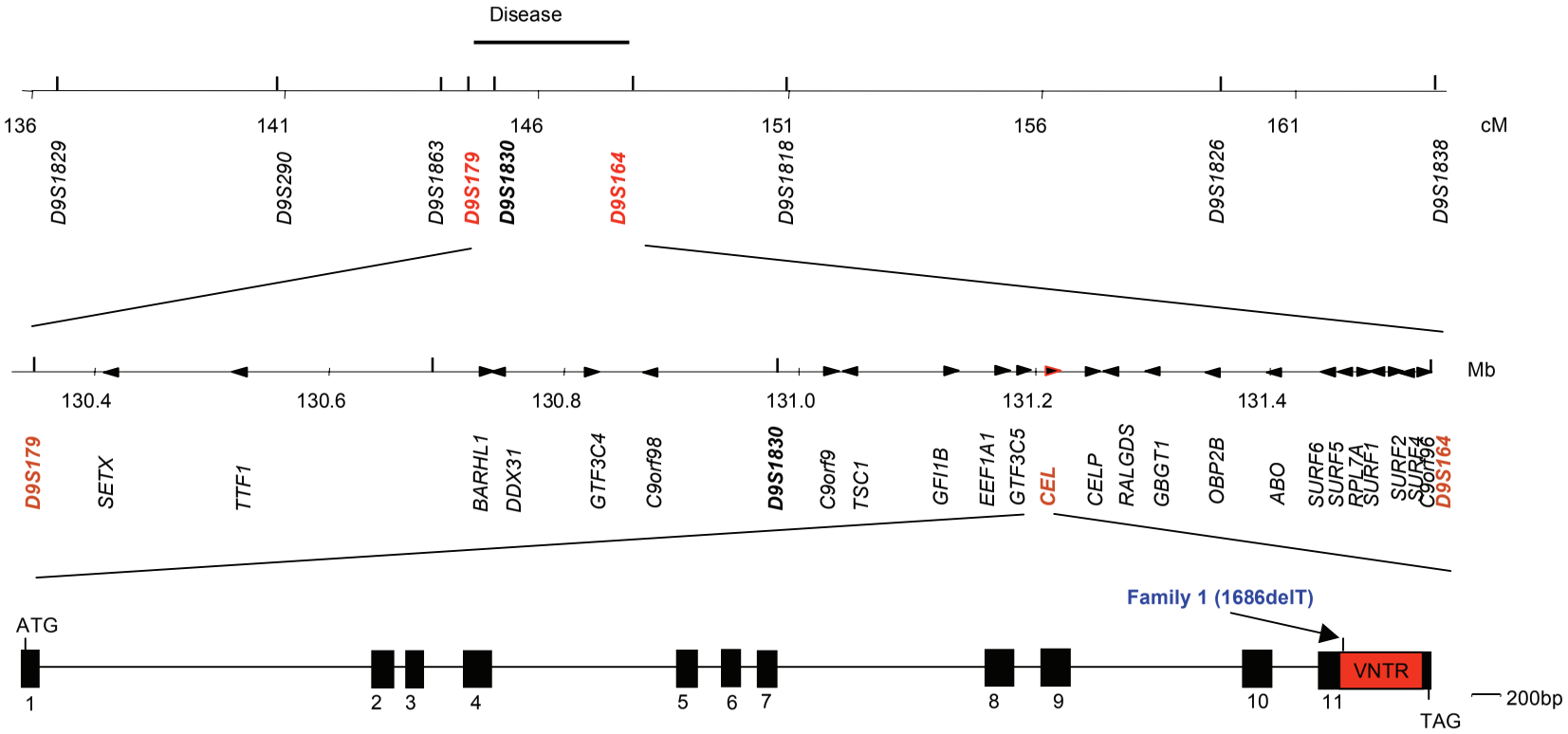
Johansson

Recombinant haplotypes define a critical region of 1.16 Mb

	Subject Affected	IV-27 Yes	III-309 Yes	III-15 Yes	IV-15 Yes	***IV-21 No***
Marker	Physical location(kb)					
D9S1829	124151	1	1	5	4	***1***
D9S290	126903	1	1	2	5	***6***
D9S1863	128776	1	1	4	3	***6***
D9S179	**130368**	**1**	**1**	**3**	**4**	***5***
D9S1830	**130992**	**2**	**2**	**2**	**2**	***4***
D9S164	**131532**	**2**	**2**	**2**	**2**	***2***
D9S1818	13257	2	2	2	2	***2***
D9S1826	133888	2	1	1	1	***1***
D9S1838	135855	4	2	2	2	***2***

Johansson

Genetic map chromosome 9q34



Johansson

What is carboxyl-ester lipase?

Known functions

- Pancreatic juice
- Hydrolysis of cholesterol esters in duodenum
- Digestion of milk fat in newborns
- Implicated in atheromas
- Intracellular effect

Expression

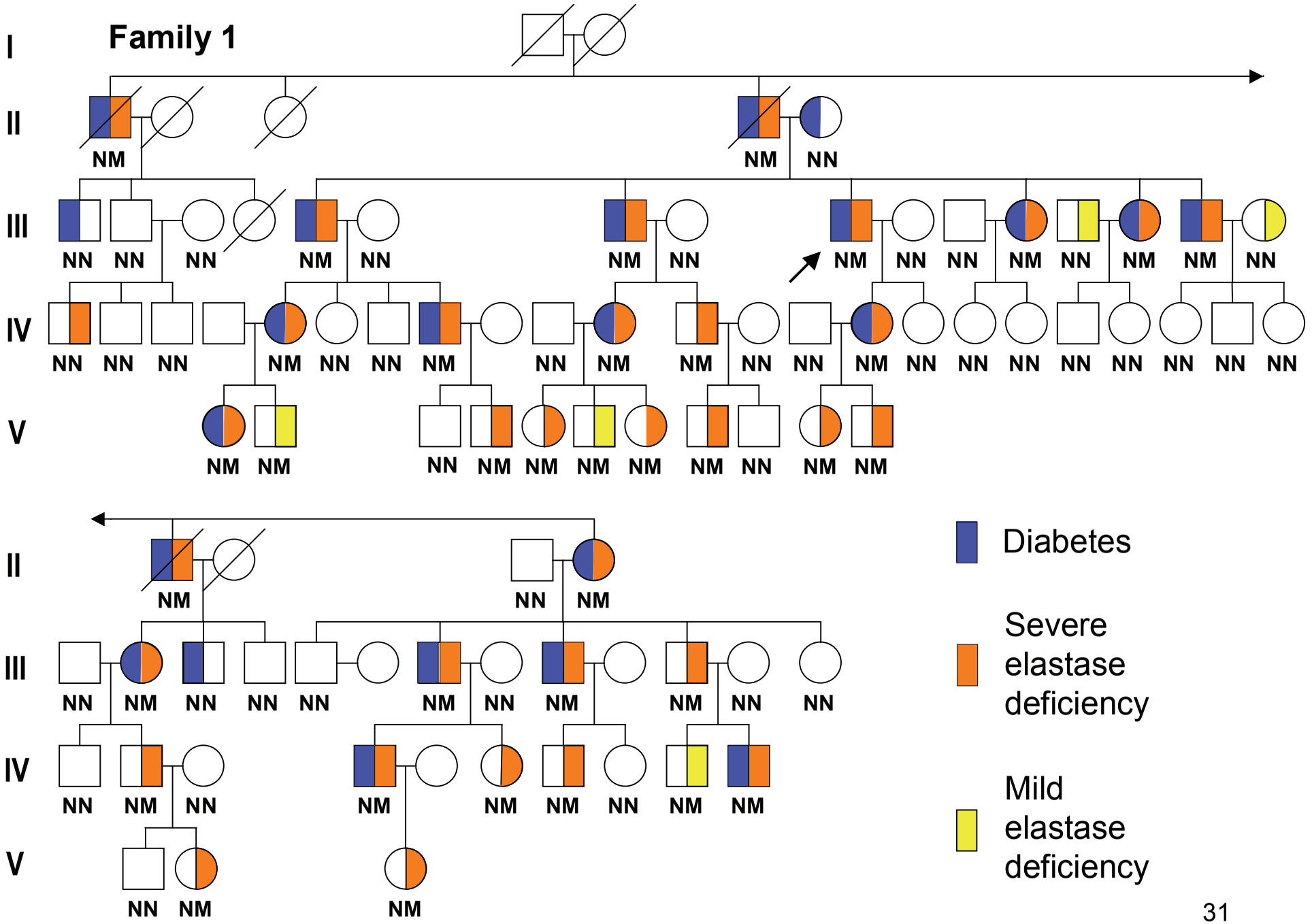
- Mainly exocrine pancreas
- Mammary glands
- Beta-cells negative

Molecular genetics

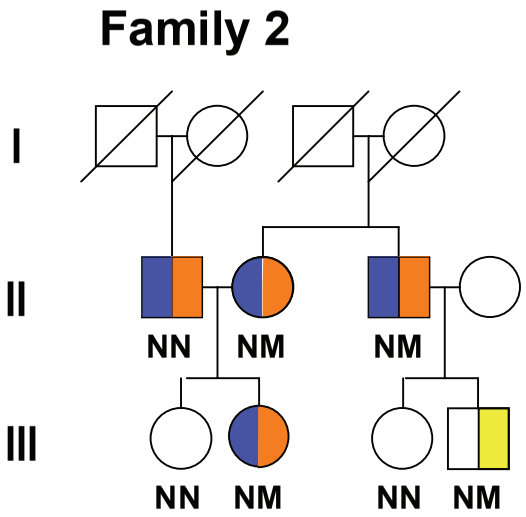
- VNTR in C-terminal

KO-mouse

- Reduced uptake of cholesteryl esters


Family 1
I
II
III
IV
V
NM
NN
Diabetes
Severe elastase deficiency
Mild elastase deficiency

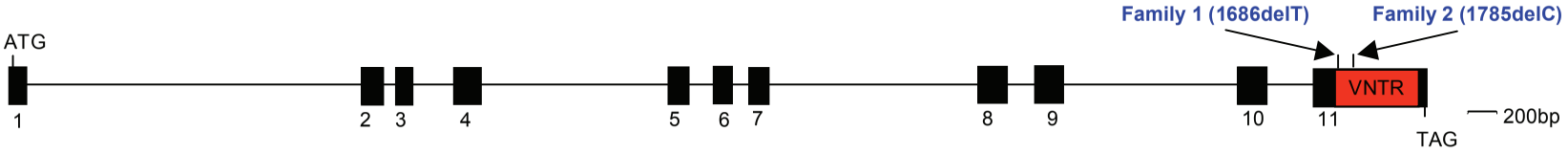
Confirmation in Family 2



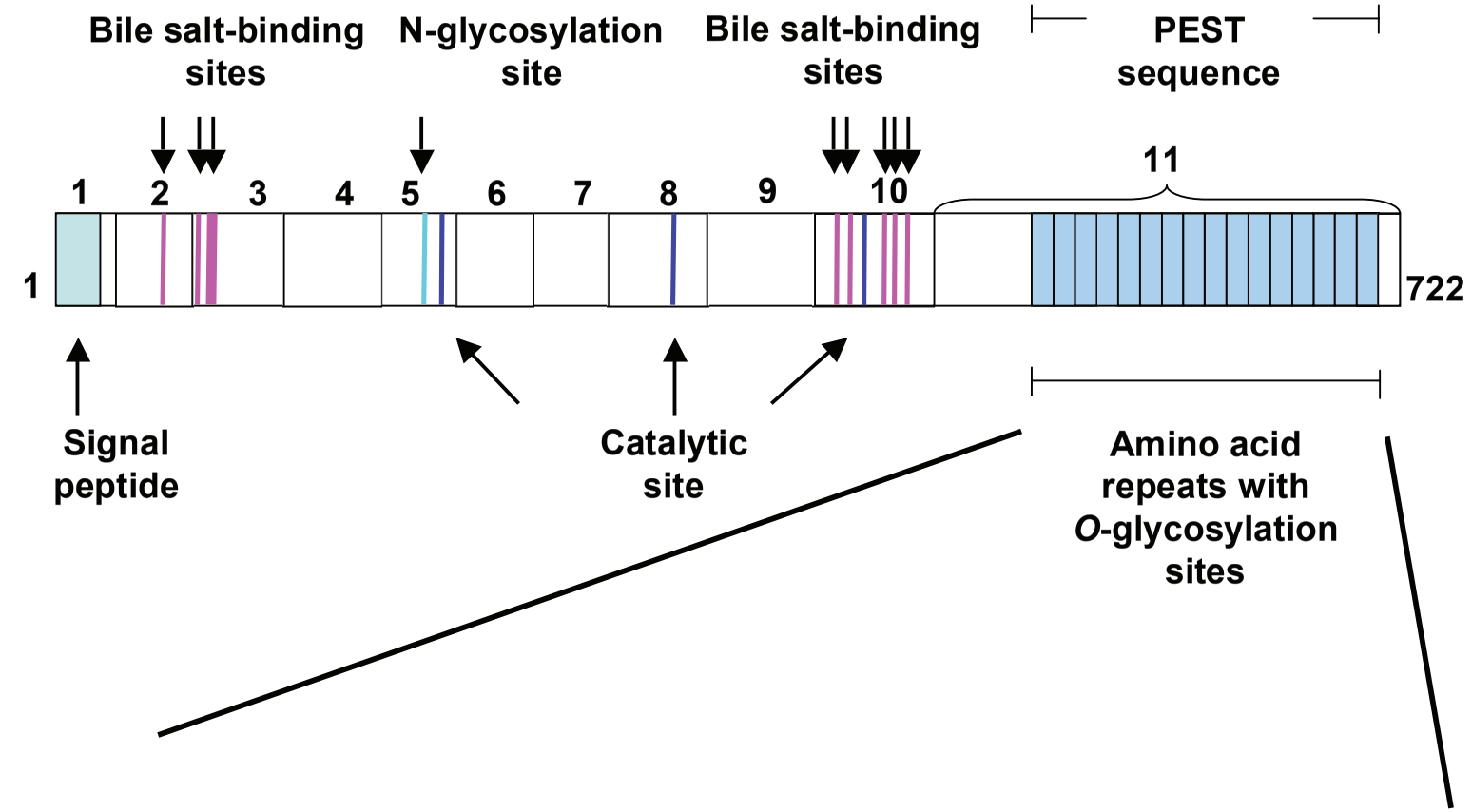
Diabetes

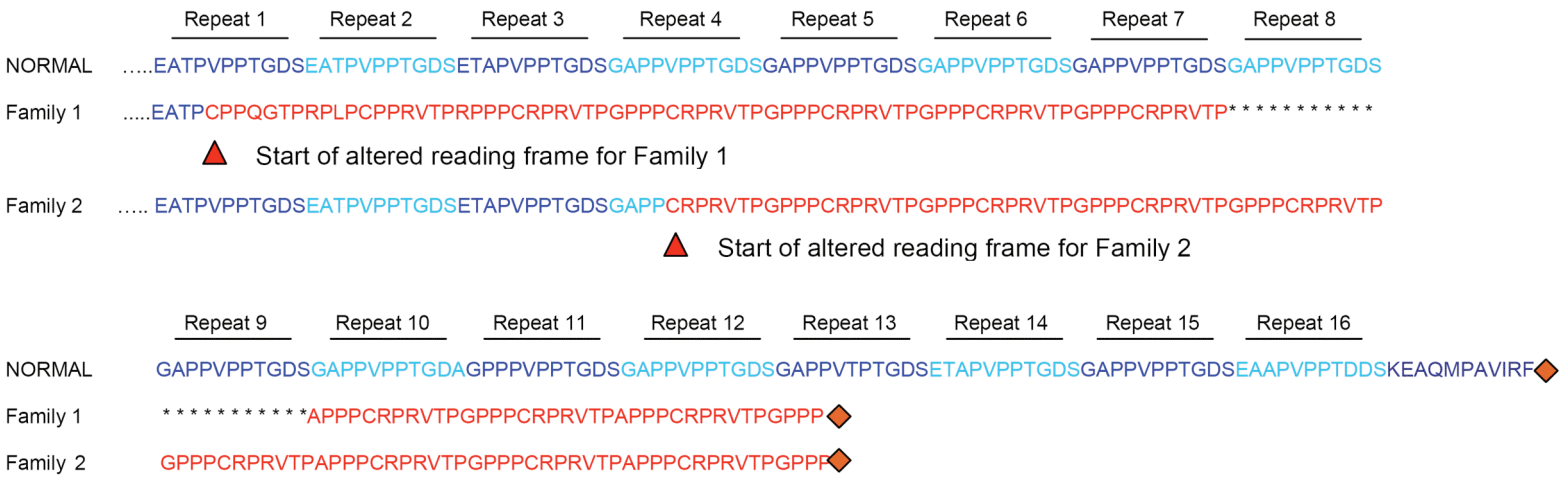
Severe elastase deficiency

Mild elastase deficiency



Nermoen


Bile salt-binding sites
N-glycosylation site
Bile salt-binding sites
PEST sequence
1
2
3
4
5
6
7
8
9
10
11
1
722
Signal peptide
Catalytic site
Amino acid repeats with O-glycosylation sites

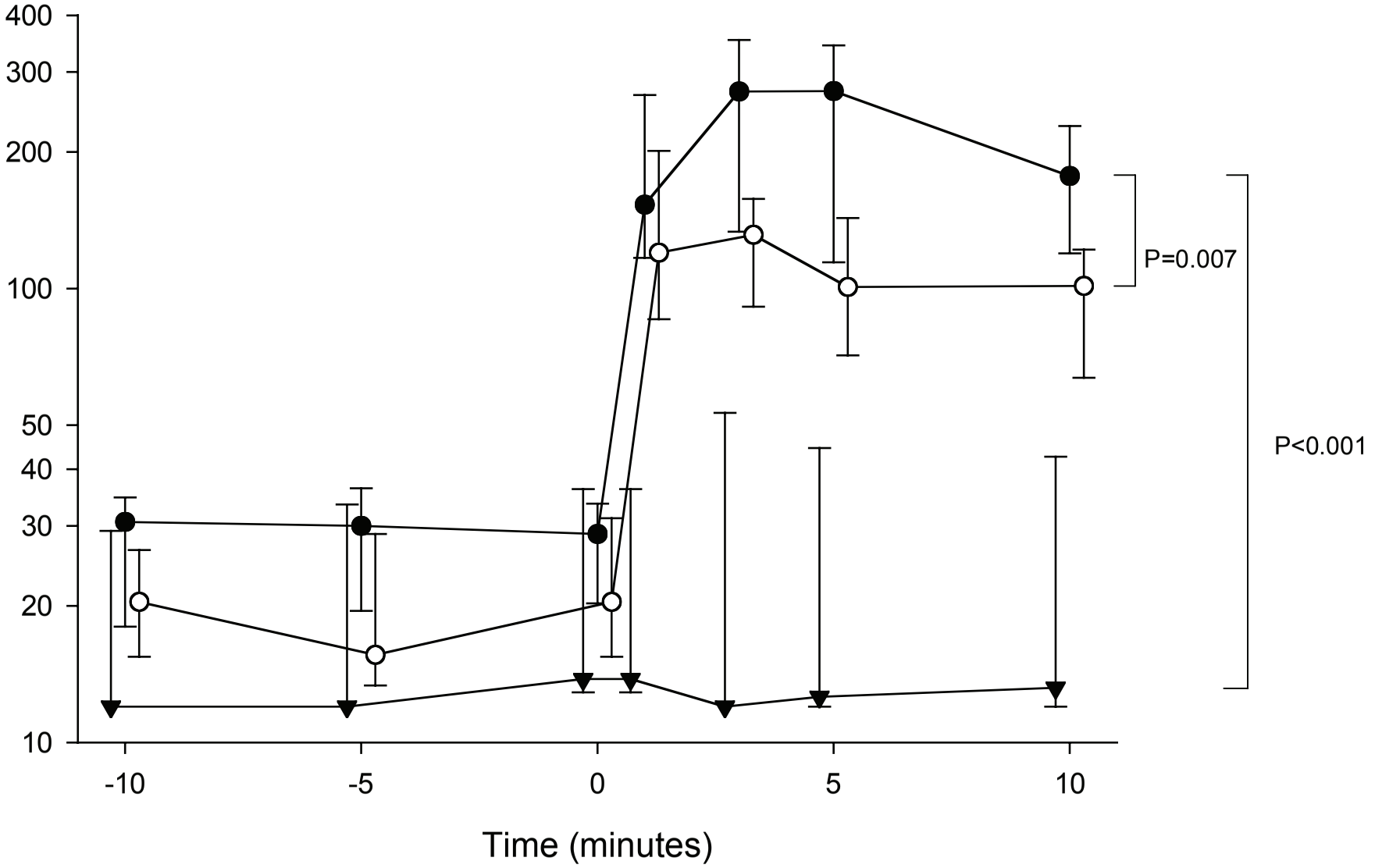

Repeat 1
Repeat 2
Repeat 3
Repeat 4
Repeat 5
Repeat 6
Repeat 7
Repeat 8
NORMAL .....EATPVPPTGDSEATPVPPTGDSETAPVPPTGDSGAPPVPPTGDSGAPPVPPTGDSGAPPVPPTGDSGAPPVPPTGDSGAPPVPPTGDS
Family 1 .....EATPCPPQGTPRPLPCPPRVTPRPPPCRPRVTPGPPPCRPRVTPGPPPCRPRVTPGPPPCRPRVTPGPPPCRPRVTPGPPPCRPRVTP**********
Start of altered reading frame for Family 1
Family 2 ..... EATPVPPTGDSEATPVPPTGDSETAPVPPTGDSGAPPCRPRVTPGPPPCRPRVTPGPPPCRPRVTPGPPPCRPRVTPGPPPCRPRVTP
Start of altered reading frame for Family 2
Repeat 9
Repeat 10
Repeat 11
Repeat 12
Repeat 13
Repeat 14
Repeat 15
Repeat 16
NORMAL GAPPVPPTGDSGAPPVPPTGDAGPPPVPPTGDSGAPPVPPTGDSGAPPVTPTGDSETAPVPPTGDSGAPPVPPTGDSEAAPVPPTDDSKEAQMPAVIRF
Family 1 **********APPPCRPRVTPGPPPCRPRVTPAPPPCRPRVTPGPPP
Family 2 GPPPCRPRVTPAPPPCRPRVTPGPPPCRPRVTPAPPPCRPRVTPGPPP

Endocrine pancreatic dysfunction

	Normal range	Diabetic carriers	P-value	Non-diabetic carriers	P-value	Family controls
Age at dx		36 ±10				
Complications	*n*	3/14				
Treatment	Ins/OHA	7/5				
HbA_{1c}	4-6.4 %	8.4 ±1.4	<0.001	5.5 ±0.3	<0.04	5.3 ±0.3
Fast glc, mM	3.9-6.0	10 ±4.1	<0.001	5.1 ±0.5	NS	5.2 ±0.5
2-h glc, mM	<7.8	12 ±6.6	NT	5.2 ±1.3	NT	4.9 ±1.0
Acute insulin resp., pmol/l		8 [0,14]	<0.001	99 [37,135]	0.007	242 [78,421]

Ræder

Intravenous glucose tolerance test



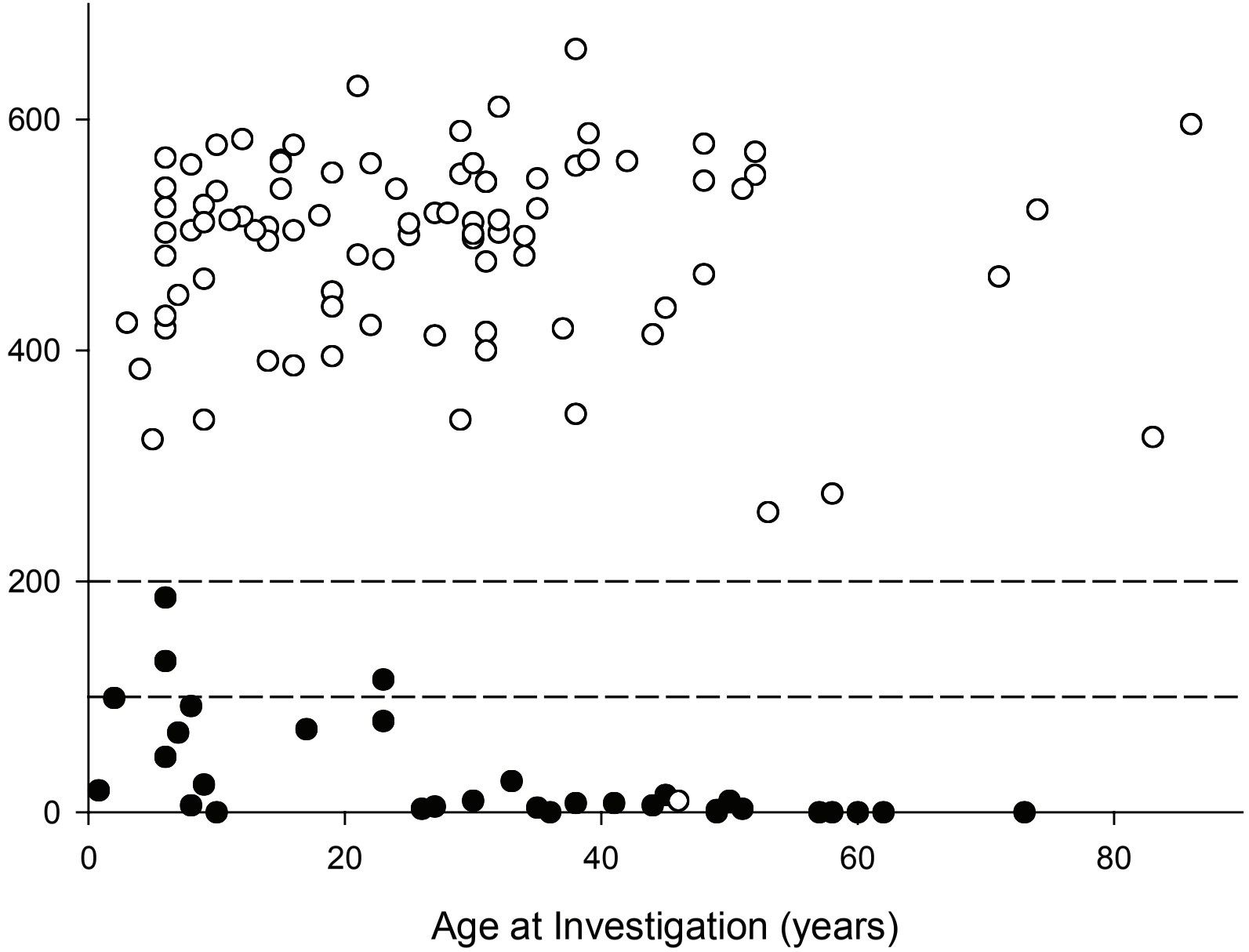
Ræder

Exocrine pancreatic dysfunction

	Normal range	Diabetic carriers	P-value	Non-diabetic carriers	P-value	Family controls
Fec elastase	<200 µg/g	4 ±5	<0.001	59 ±56	<0.001	490 ±91
Fec fat excr, g/24 h	<7	31 ±15		15 ±8		
Vit A, µM	>0.7	1.4 ±0.4	0.006	1.4 ±0.4	0.036	1.7 ±0.6
Vit D, mM	30-150	68 ±36	NS	61 ±30	NS	66 ±22
Tot Ca^{2+}, mM	2.2-2.6	2.3 ±0.2	0.013	2.4 ±0.1	NS	2.4 ±0.1
Vit E, µM	>11.6	8.2 ±5.4	<0.001	15 ±5.8	0.008	20 ±6.4
LDL chol, mM	1.8-5.7	2.4 ±0.7	<0.001	2.4 ±1.2	NS	2.9 ±0.8

Ræder

Exocrine pancreatic dysfunction



Pathogenesis

Patho-physiologic mechanism?

Different from chronic pancreatitis

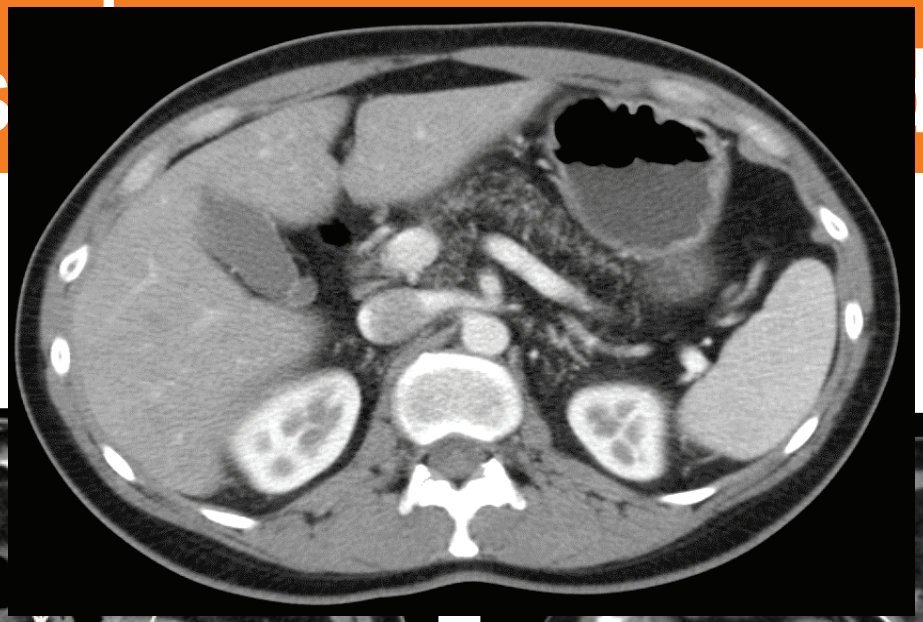
- Few hospitalized, only low-grade symptoms
- No clear signs of inflammation although fibrosis on autopsy

Degenerative process?

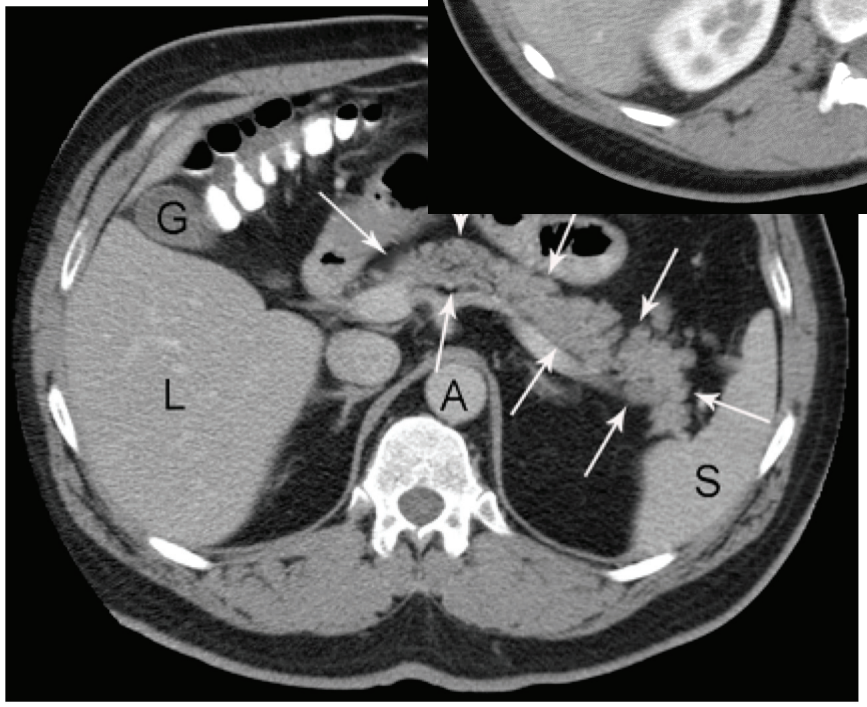
- Atrophy and fatty infiltration on CT scans primary and not secondary to diabetes

Initiated in the acinar cells?

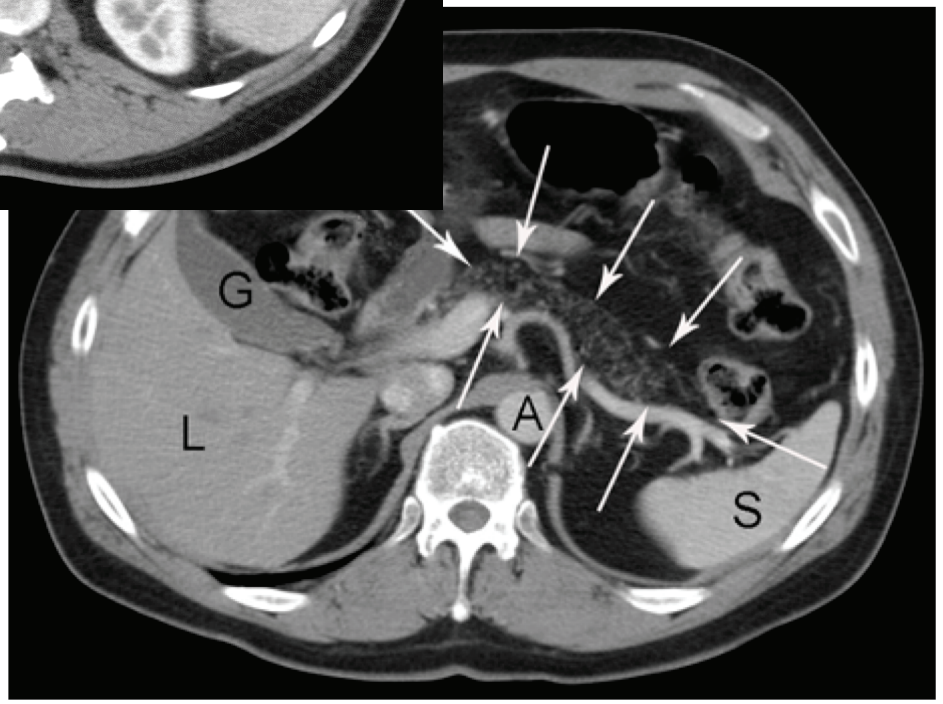
Reduced pancreas size and signal intens l by CT



Non-Diabetic
Mutation
Carrier

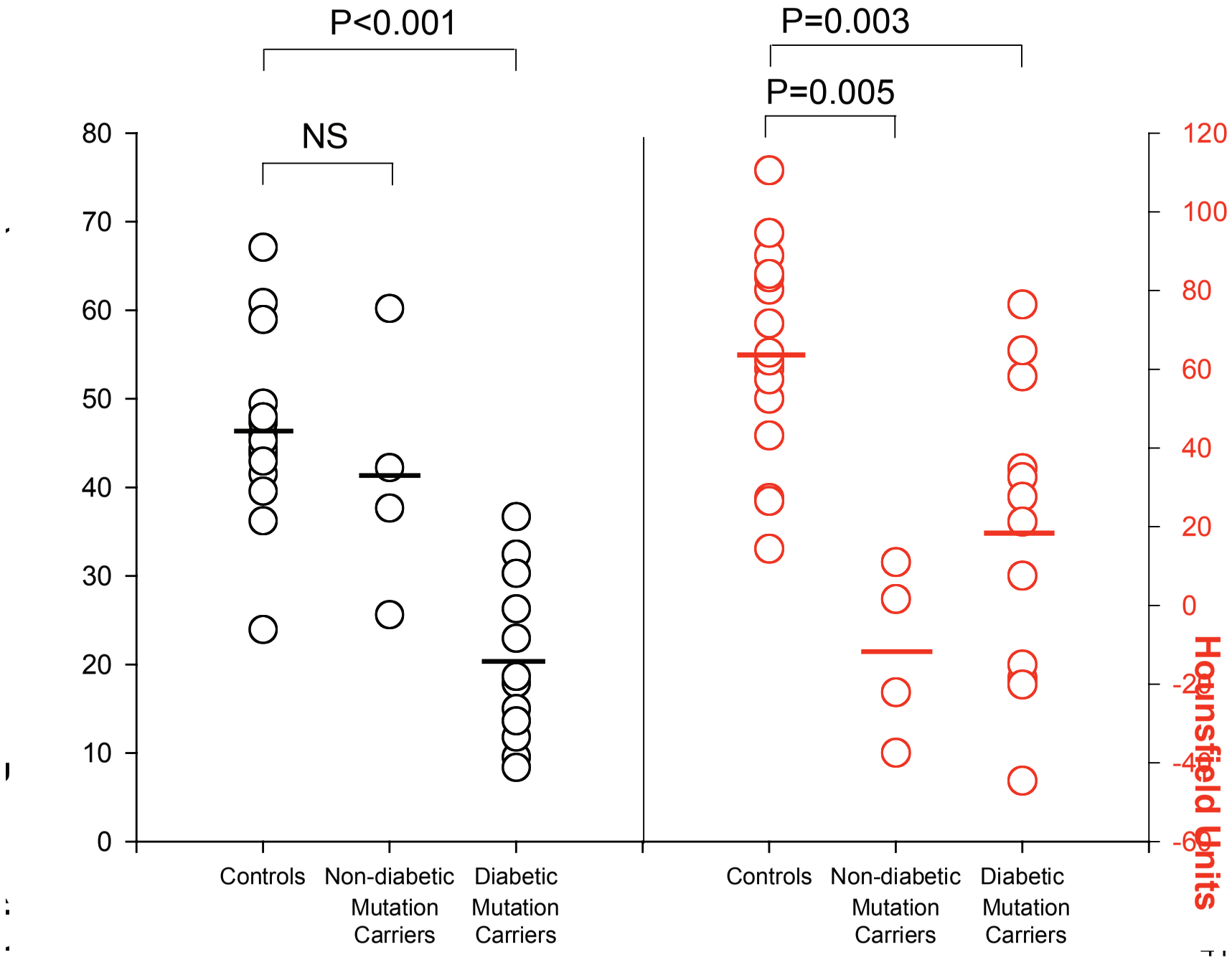


Control



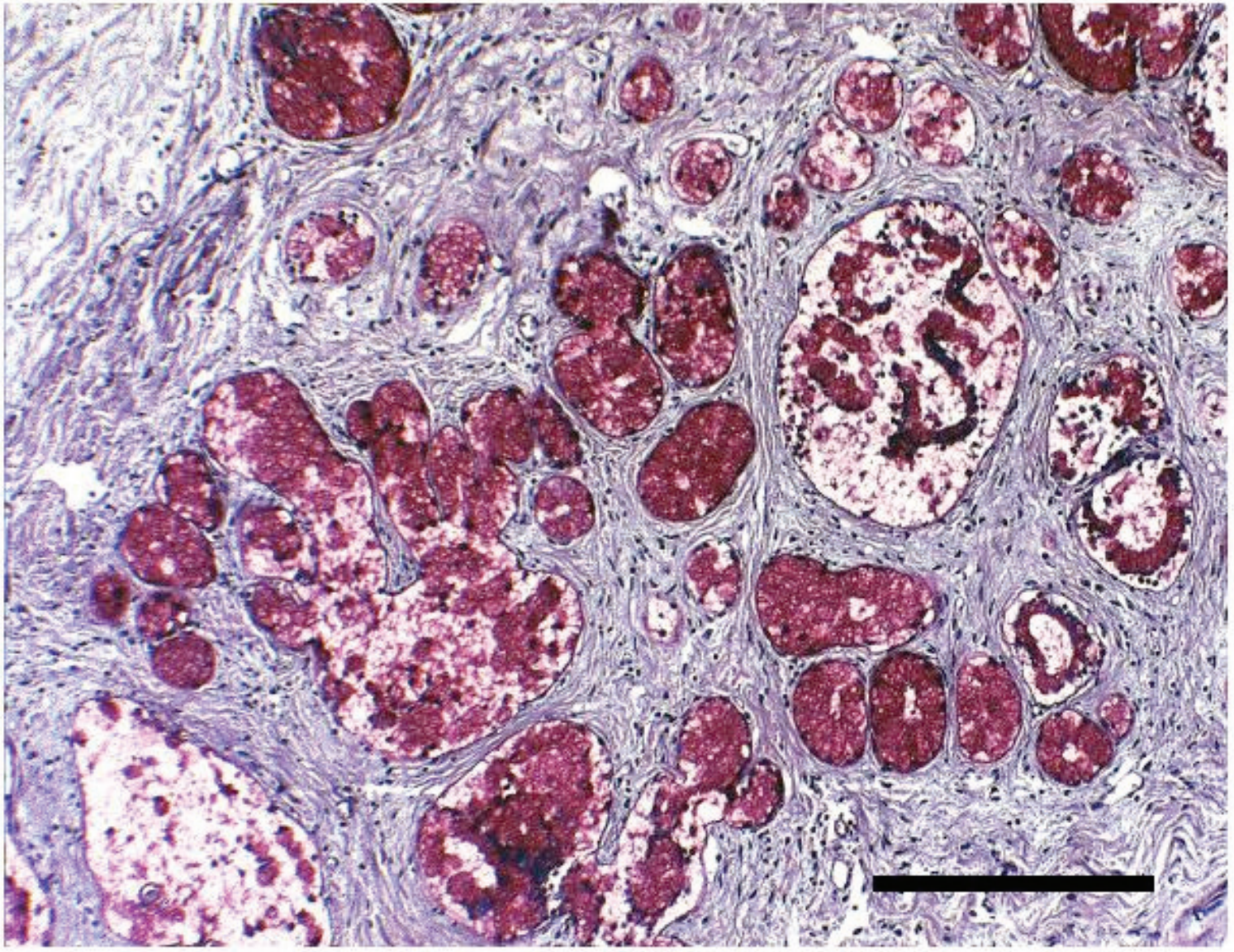
Diabetic mutation carrier

Haldorsen


P<0.001
NS
P=0.003
P=0.005
80
70
60
50
40
30
20
10
0
120
100
80
60
40
20
0
-2
-4
-6
Hounsfield Units
Controls
Non-diabetic Mutation Carriers
Diabetic Mutation Carriers
Controls
Non-diabetic Mutation Carriers
Diabetic Mutation Carriers

Haldorsen

Autopsy affected † 1957



Molven

Molecular mechanism

Catalytic properties normal

V_{max} and K_m

4-nitrophenyl hexanoate and cholesteryl oleate as substrates

Wt vs mutant CEL

Catalytic efficiencies (ratio V_{max}/K_m) similar

4-nitrophenyl hexanoate

Wt: 1.5 10^{-2} min^{-1}

Mutant 1.1 10^{-2} min^{-1}

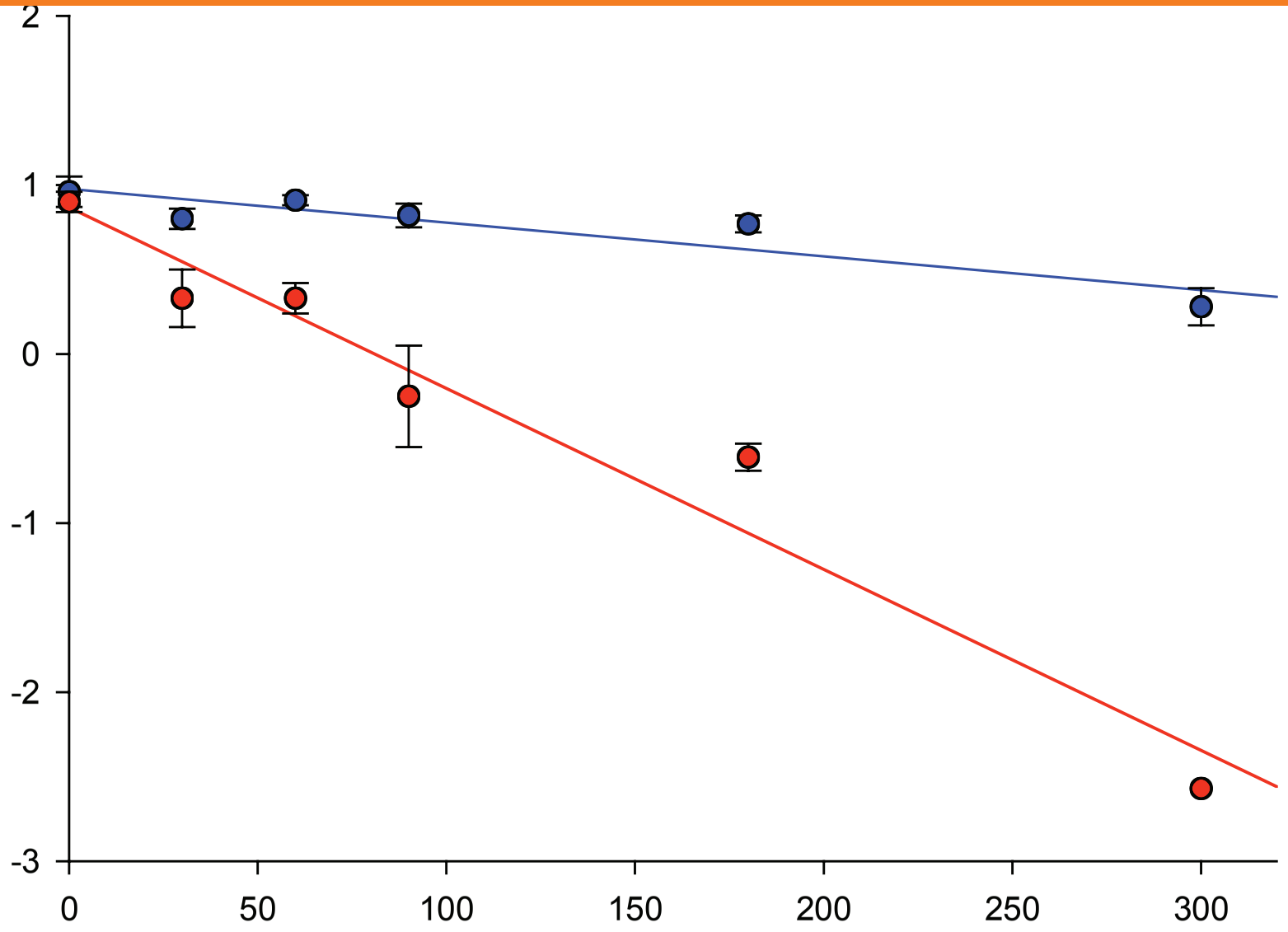
Micellar cholesteryl oleate

Wt: 2.1 10^{-2} min^{-1}

Mutant: 0.9 10^{-2} min^{-1}

Lombardo

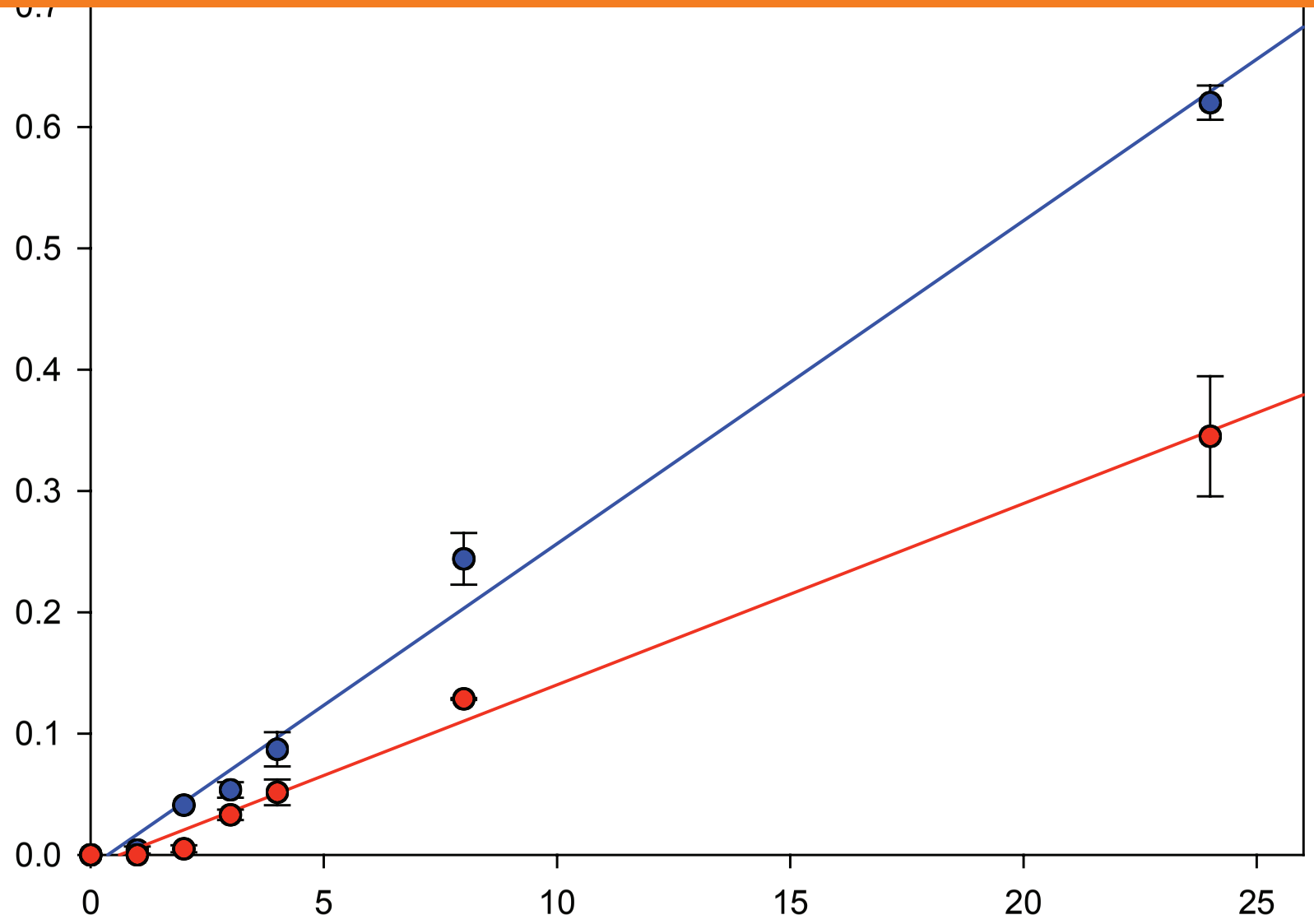
Stability at 37 °C



Lombardo

P<0.001

Secretion



Lombardo

P<0.001

And Boston?

Make cell model

1. Apoptosis of beta cells

No clear effect

2. Apotosis of acinar cells

No clear effect

And Boston?

Make mouse models

1. KO mouse

No gene

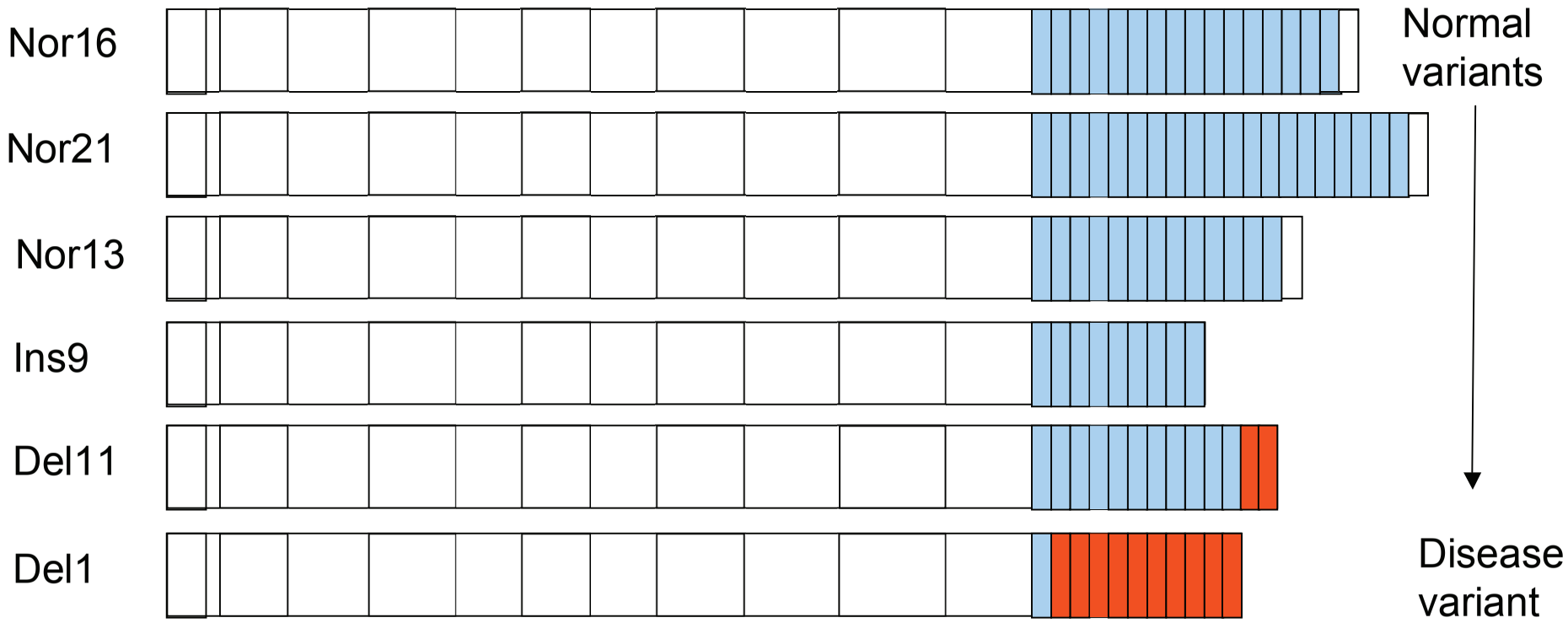
1. Transgenic mouse

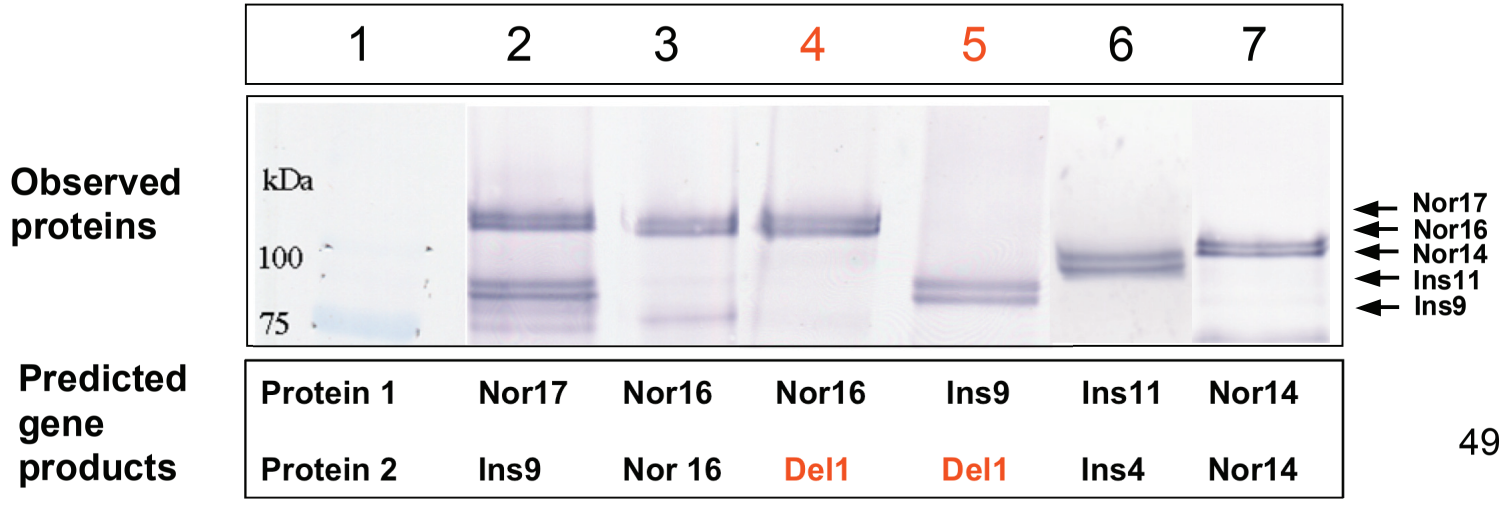
Too much of the mutant gene

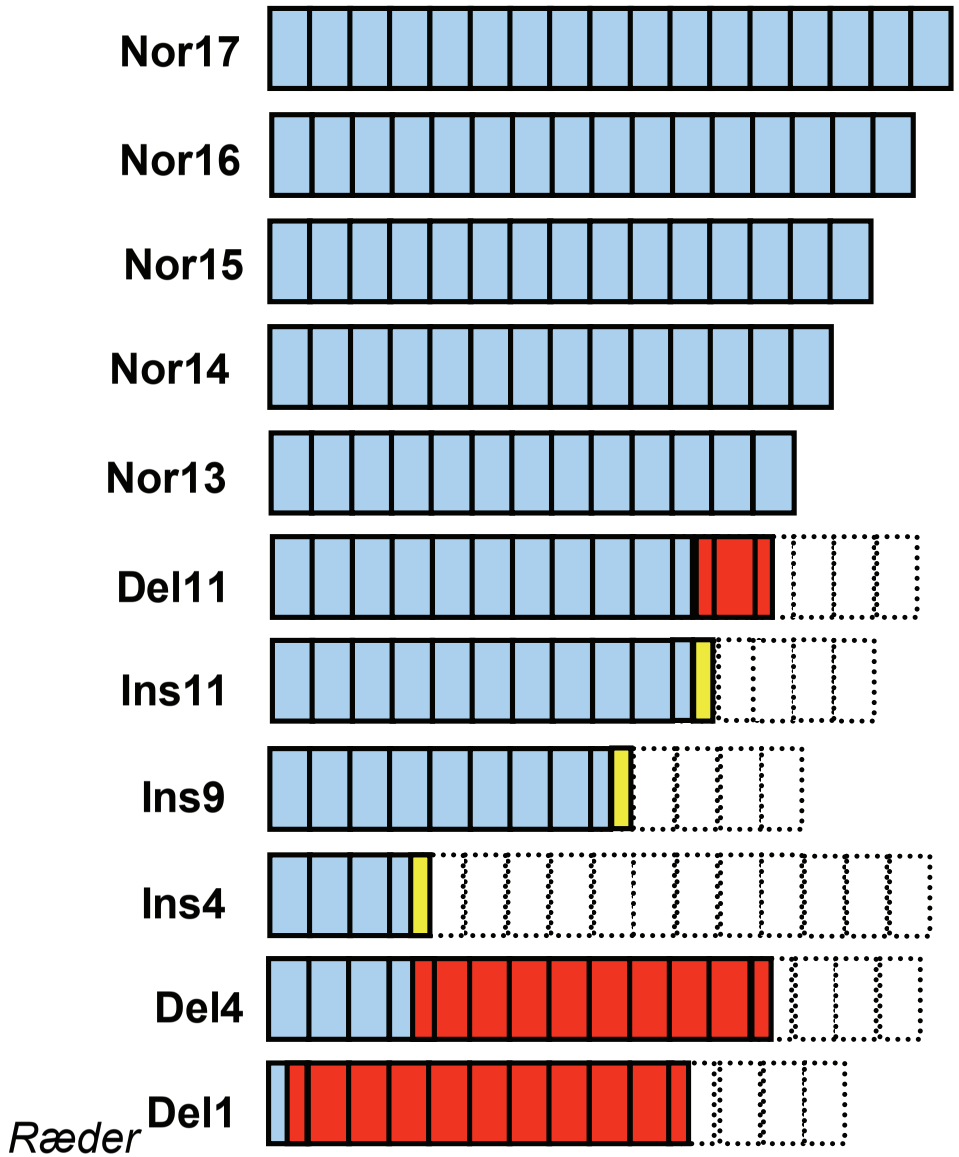
After one year's work:

Mice are born!

Helge Ræder and Mette Vesterhus to finish


Nor16
Nor21
Nor13
Ins9
Del11
Del1
Normal variants
Disease variant


1 2 3 4 5 6 7
Observed proteins
kDa
100
75
Nor17
Nor16
Nor14
Ins11
Ins9
Predicted gene products
Protein 1 Nor17 Nor16 Nor16 Ins9 Ins11 Nor14
Protein 2 Ins9 Nor 16 Del1 Del1 Ins4 Nor14



Detected by antibody in urine

Not detected by antibody in urine

DM	Exo	Freq
No	No	0.05
No	No	0.04
No	No	0.64
No	No	0.14
No	No	0.07
No	No	0.06
No	No	0.00
No	No	0.01
No	No	0.06
No	Yes?	0.00
Yes	Yes	0.00
Yes	Yes	0.00

Polygenic role for *CEL*?

182 unrelated diabetic subjects (90% T1DM):

	No insertion	**Insertion**
No FED	140 (86 %)	22 (14 %)
FED	12 (60 %)	8 (40 %)

P=0.007

Common insertions in the VNTR associated with exocrine dysfunction in subjects with diabetes (OR 4.2 [1.6, 11.5]).

Relevance for type 1 diabetes?

Relevance for type 1 diabetes?

- Lipase is highly polymorphic
- Common sequence variations confer
  - increased risk for T1D?
  - or exocrine dysfunction in T1D?

Study in Bergen, possibly in Hvidøre Study Group

Summary

DNA and disease

- Two families, two mutations, co-segregation
- Pattern of normal and disease-causing variants
- Several differences in phenotype
- Polygenic role?

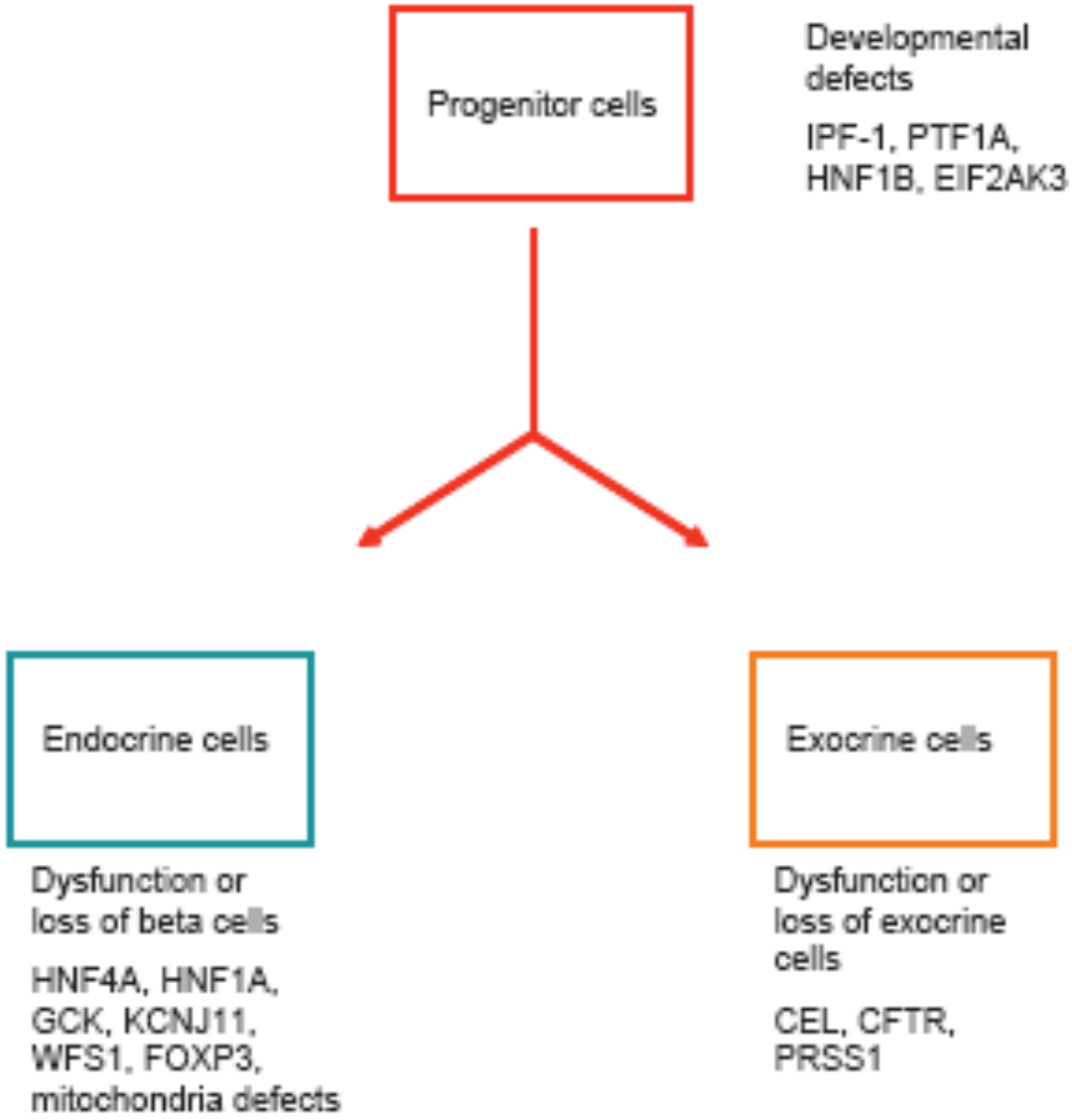
Molecular mechanism

- Mutant protein less stable and less secreted
- Loss-of-function mutation?

Pathogenesis

- Fat infiltration in pancreas
- Abnormalities in fatty acids in mutation carriers

Ræder et al, Nature Genetics, 2006


Progenitor cells
Developmental defects
IPF-1, PTF1A, HNF1B, EIF2AK3
Endocrine cells
Dysfunction or loss of beta cells
HNF4A, HNF1A, GCK, KCNJ11, WFS1, FOXP3, mitochondria defects
Exocrine cells
Dysfunction or loss of exocrine cells
CEL, CFTR, PRSS1

A great THANK YOU to……

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