Technische Universität Dresden

# Old Challenges And New Opportunities

Ezio Bonifacio

# Disclosures

Patent screening for genetic risk and oral insulin therapy

Lectures for Sanofi

# Old Challenges

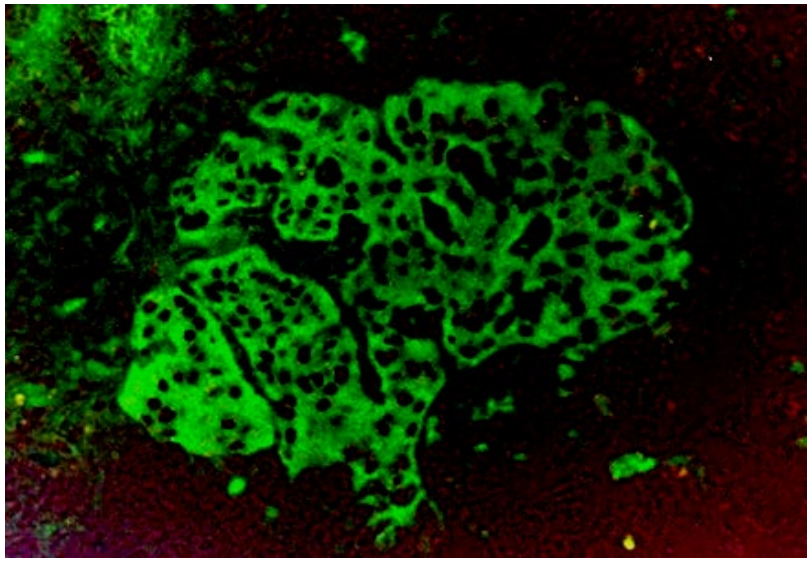
Biomarkers that predict type 1 diabetes

Who to Screen?

When to screen?

# 1985

Islet Cell Antibodies



Human pancreas
Subjective reading

Insulin Autoantibodies

600 μl radiobinding assay

Critical Questions in 1985

What are the best methods for AAb detection?

What are the antigens of ICA?

What are the genes contributing to risk?

Is Coxsackie B virus the cause of T1D?

The role of the beta cell (homocide vs suicide)

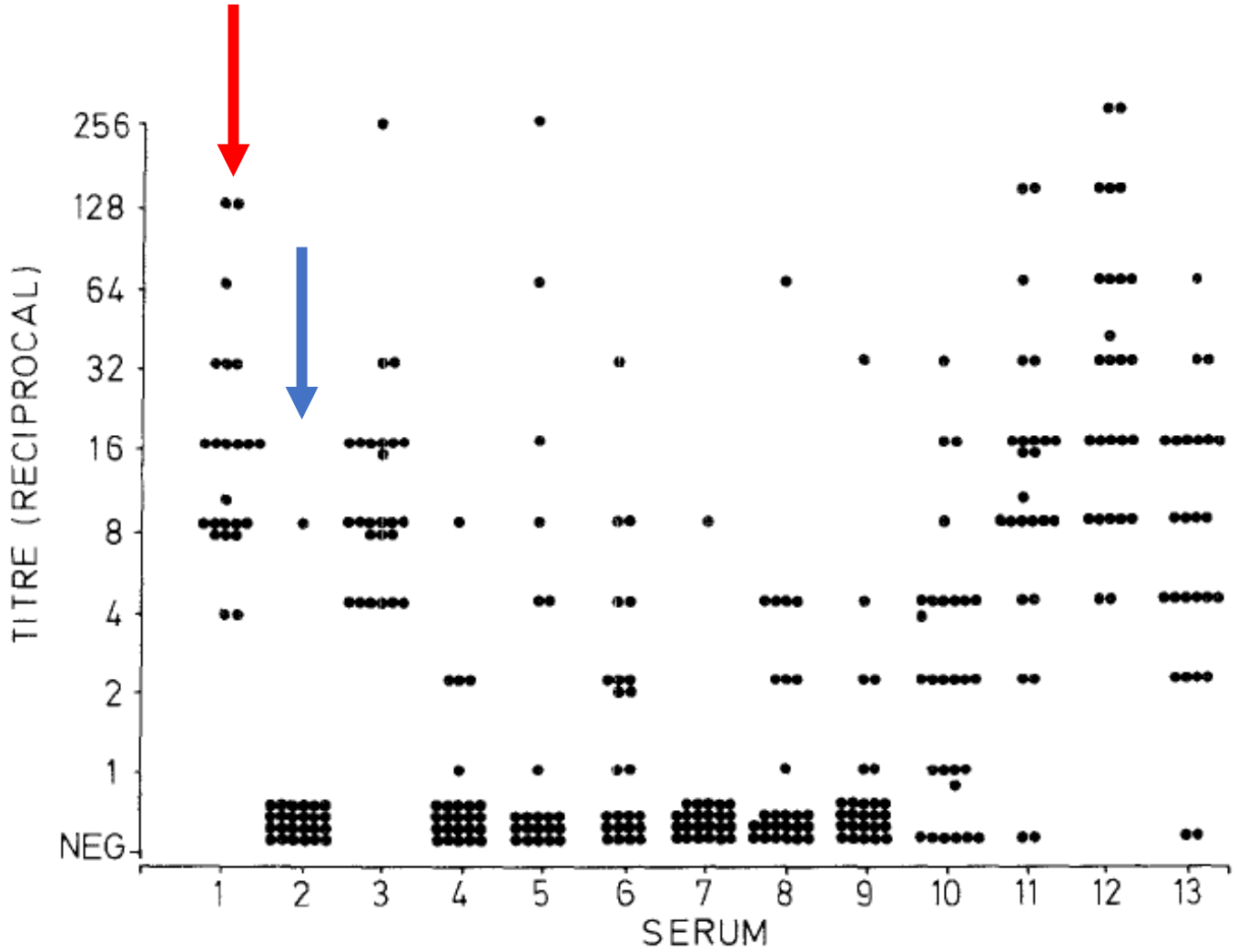
Hyperclass I expression on beta cells

Lucienne Chatenoud was treating NOD mice with monoclonal Abs

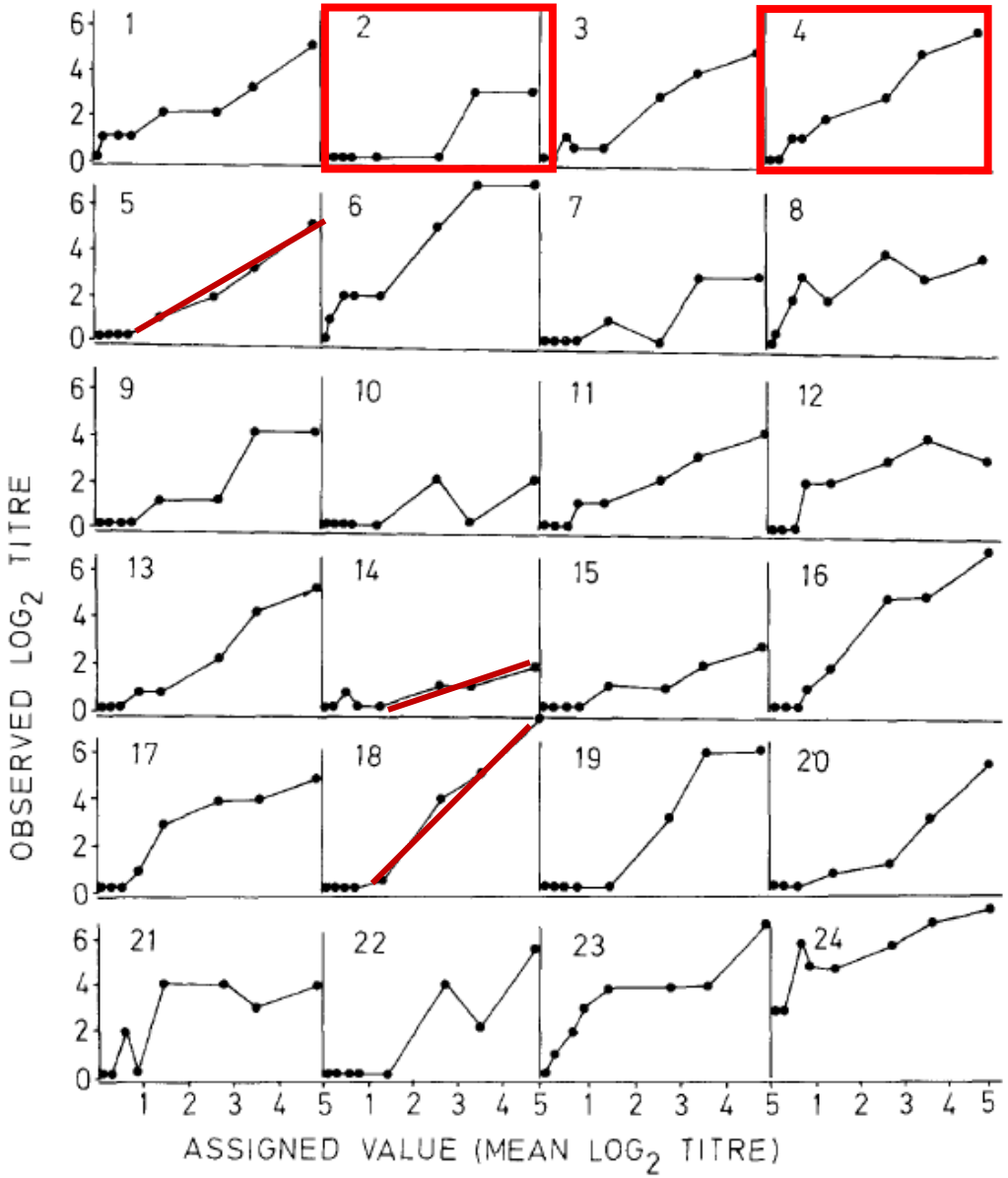
Cyclosporin A and other immune modulators

# Immunology and Diabetes Workshops: report of the first international workshop on the standardisation of cytoplasmic islet cell antibodies

## Summary of a workshop organised by the Juvenile Diabetes Foundation International held in Monte Carlo on 31 October and 1 November 1985



# Calibration curves and the birth of JDF units




No single best method

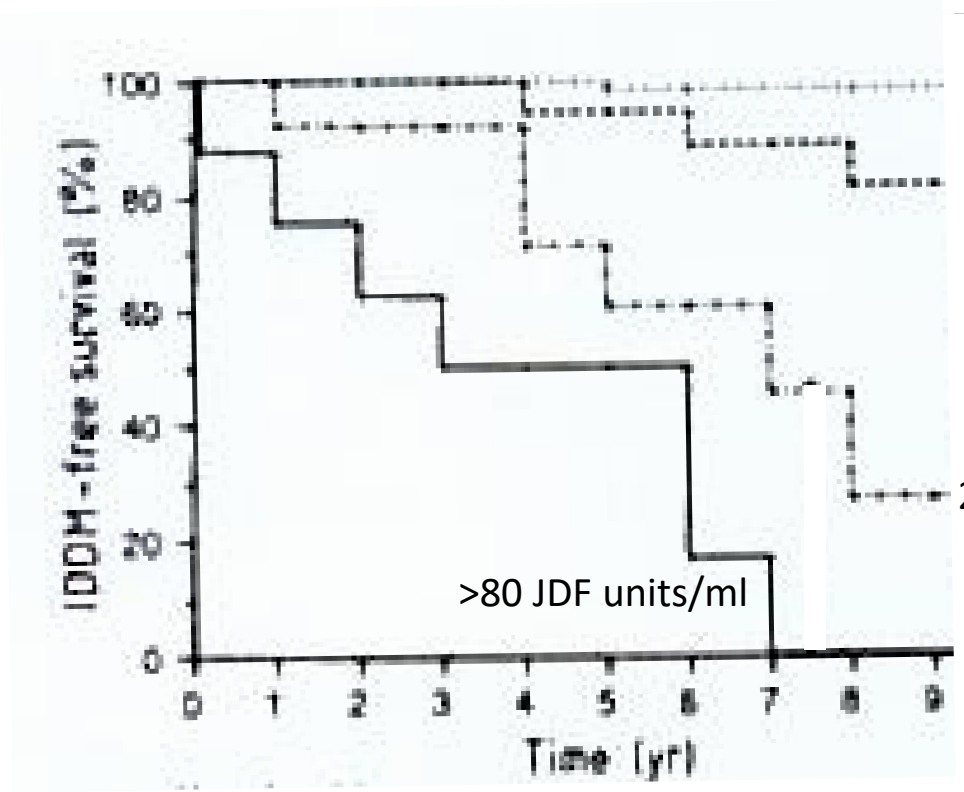
Best methods had a steep and wide range of measurement

Calibrators allowed harmonization across methods

VOL 335 THE LANCET 147

# Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes

EZIO BONIFACIO POLLY J. BINGLEY MARION SHATTOCK
BETTY M. DEAN DAVID DUNGER EDWIN A. M. GALE
GIAN FRANCO BOTTAZZO




<5 JDF units/ml

5-20 JDF units/ml

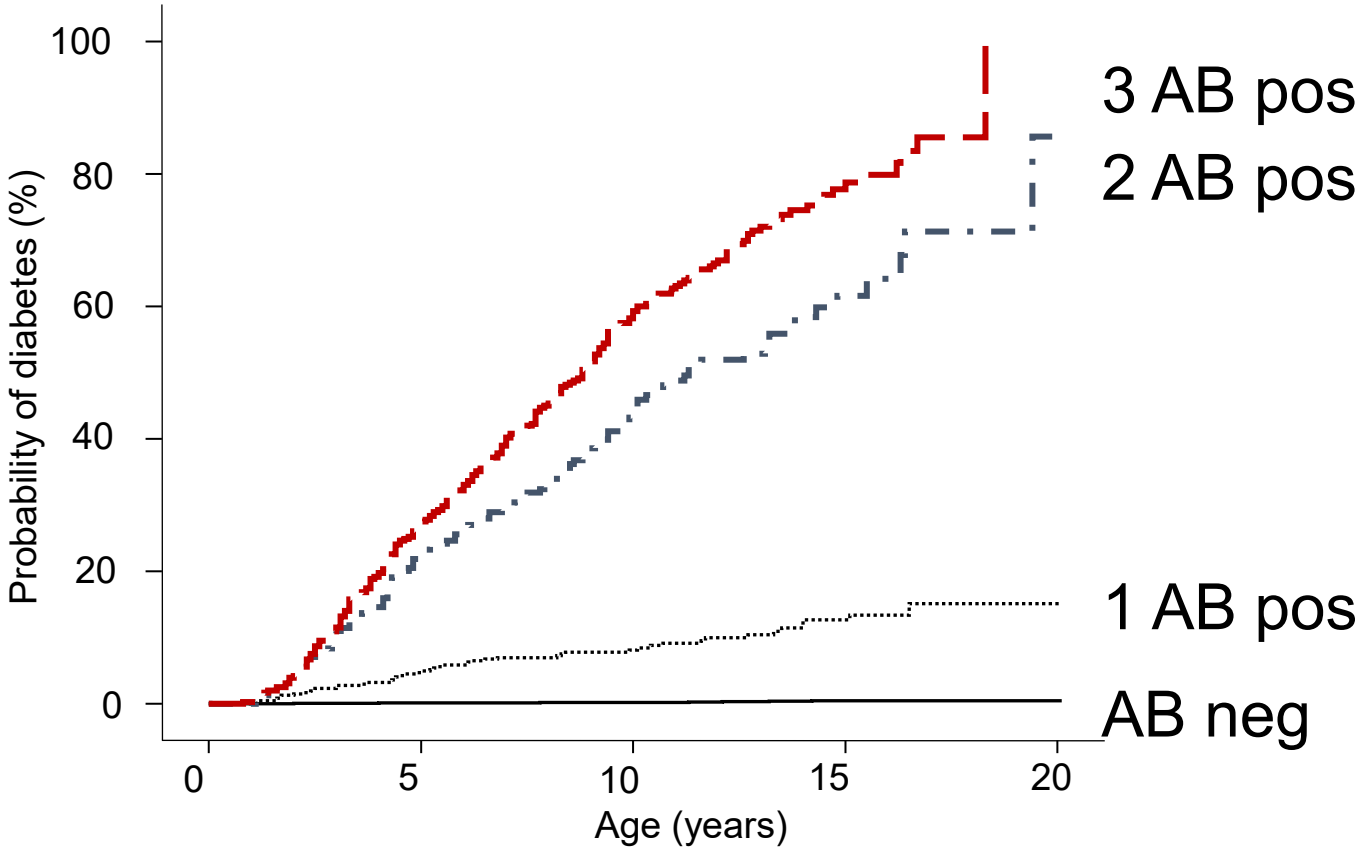
More signal, more certain of diagnosis

20-80 JDF units/ml

The more T1D associated elements that come together,
the more likely it will be T1D

Some of this is due to pathogenesis
Some is due to the mathematics of increased odds (Bayes theorem)

# Children with multiple islet autoantibodies progress to symptomatic type 1 diabetes




**Reversion** to negative with two or more islet autoantibodies **< 1 %**

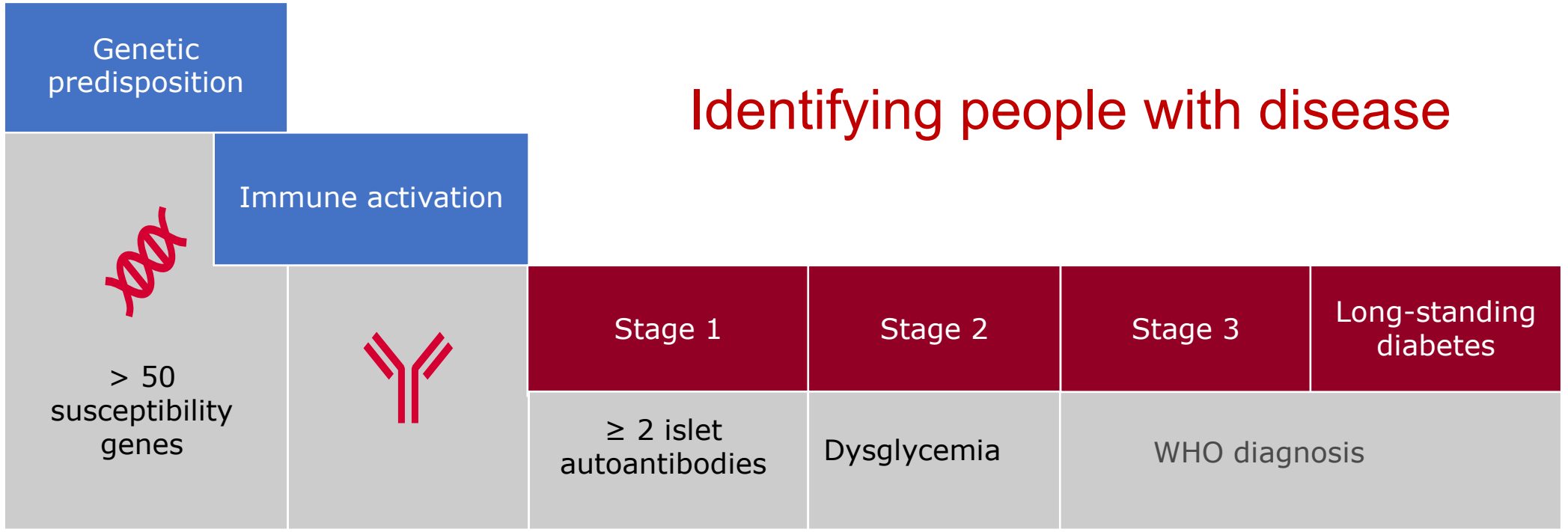
**Reversion** to negative with single islet autoantibodies ~ **24 %**
Most reversions (85%) occur within 2 years after seroconversion

*Ziegler, Rewers, Simell, et al JAMA 2013*

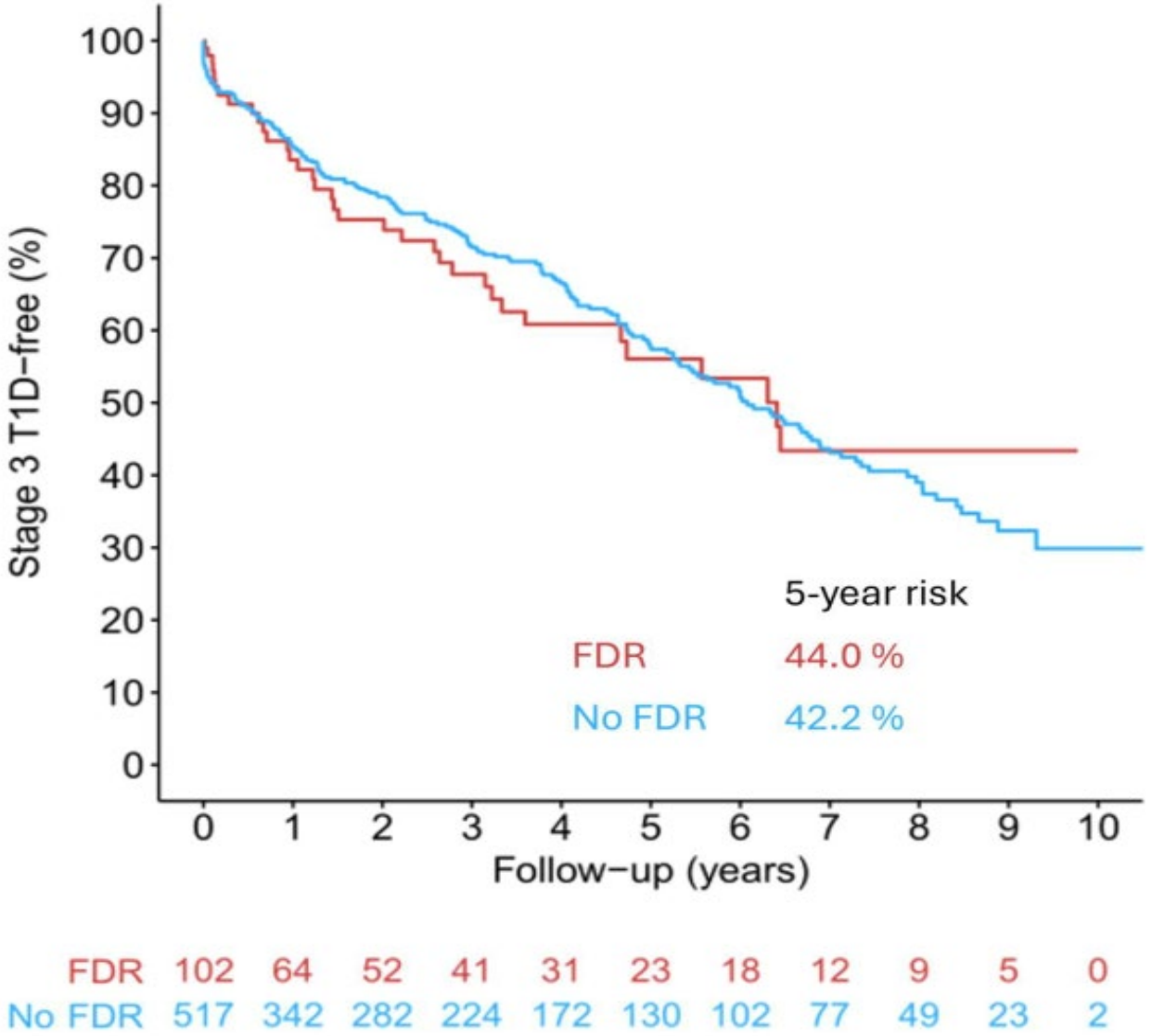
*Vehik et al, TEDDY 2016*

# Type 1 diabetes is now 2 or more autoantibodies

## Identifying people at risk



# Progression from early stage T1D to stage 3 T1D is the same in children with and without a frist-degree family history of T1D



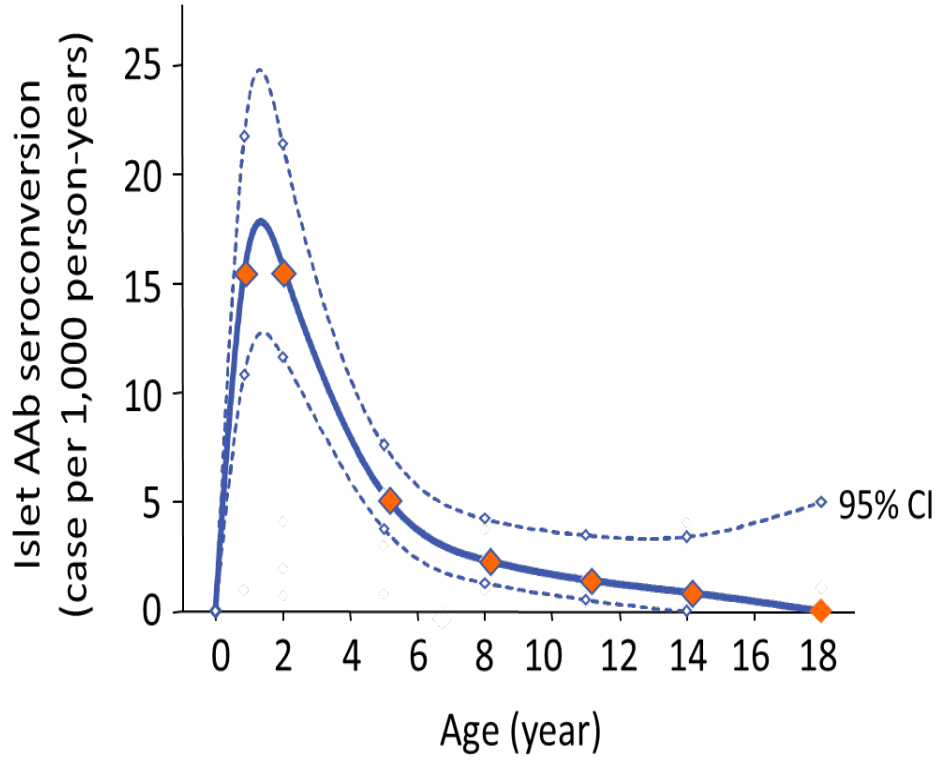

Fr1da

Typ 1 Diabetes: Früh erkennen – Früh gut behandeln

Screening does not need to be restricted to those with a prior genetic risk

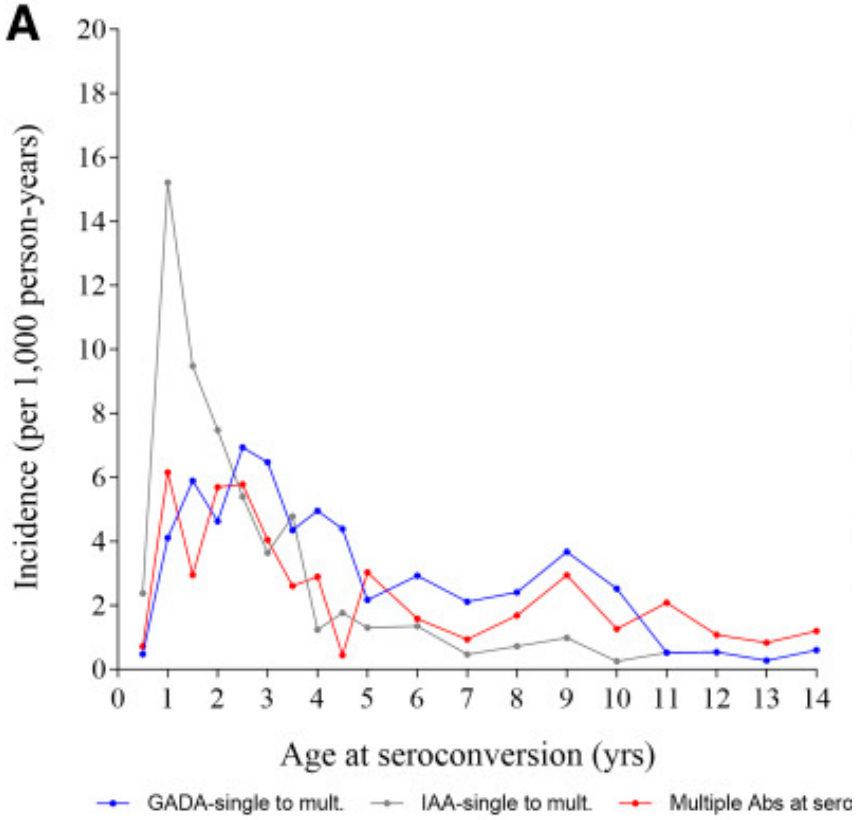
# The early peak incidence of islet autoimmunity

Germany




*Ziegler et al, 2012*

TEDDY (Finland, Sweden, Germany, USA)




*Rewers et al, 2025*

Screening can occur in early childhood

# New opportunites

Why does it start in some?

The heterogeneity of progression.

# Lingering Questions

Is progression linear within an individual and can we predict at the level of the individual?

What drives progression?

What about Adults?

Remember that we are predicting the future from the past……..

# Start

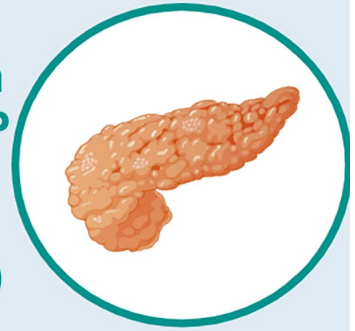
“Disaster risk depends on the vulnerability of the community it hits and how the community responds.”

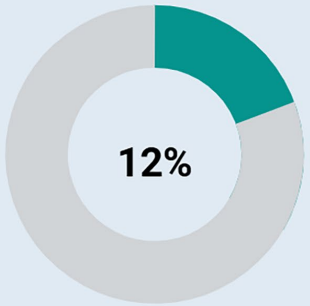
Beta cells are more vulnerable that alpha cells

# Pancreas damage and islet autoimmunity

Islet Autoantibodies and Their Association With β-Cell Function and Diabetes Measures in Children With Acute Recurrent and Chronic Pancreatitis. Diabetes Care 2025
Ginzburg…..Abu-El-Haija

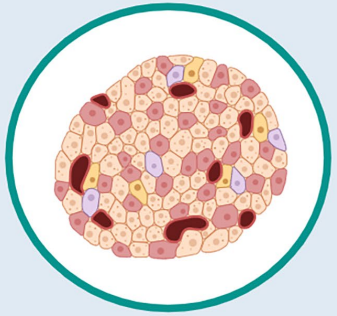
**Youth with ARP or CP and Ab testing (*n* = 234)**



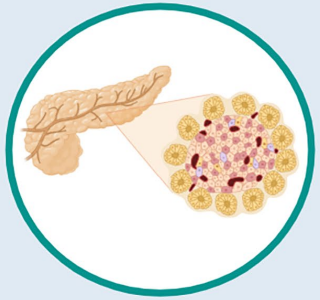



Ab+ patients **(*n* = 28)**
5.6%
multiple Ab

$HbA_{1c}$ higher in Ab+



C-peptide lower in Ab+



Ab+ had higher prevalence of prediabetes/ diabetes

Ab+ had shorter time to diabetes development

**Ab+ associated with higher risk of development, shorter time to diabetes development, β-cell dysfunction, higher $HbA_{1c}$ and lower C-peptide**

*Archives of Disease in Childhood*, 1988, **63**, 1234–1239

# HLA type, islet cell antibodies, and glucose intolerance in cystic fibrosis

P R STUTCHFIELD,* S M O'HALLORAN,* C S SMITH,* J C WOODROW,†
G F BOTTAZZO,‡ AND D HEAF*

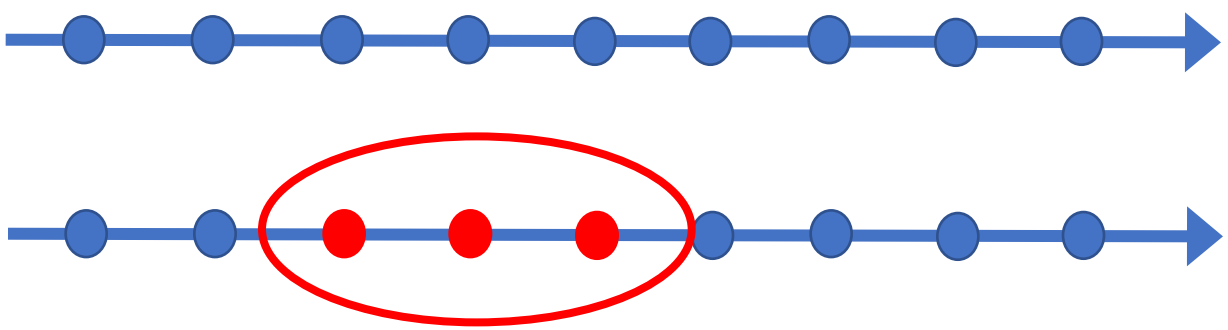
ICA: 7 (15%) of 45 patients with CF

increased incidence of islet cell antibodies in our patients with cystic fibrosis may reflect that in those patients who have a genetic predisposition an autoimmune response may be mounted to damaged pancreatic tissue with the production of antibodies to islet cells. This may result in further β cell destruction. Evidence of impaired β cell function was present in five out of seven (71%) patients positive for islet cell antibodies at the time of the study.

# Prolonged enteroviral infections

## Enterovirus B positivity in (monthly) stool samples increases susceptibility to islet autoimmunity

*Vehik K. et al. Nat Med 2019*



No infection

Prolonged infection

TEDDY

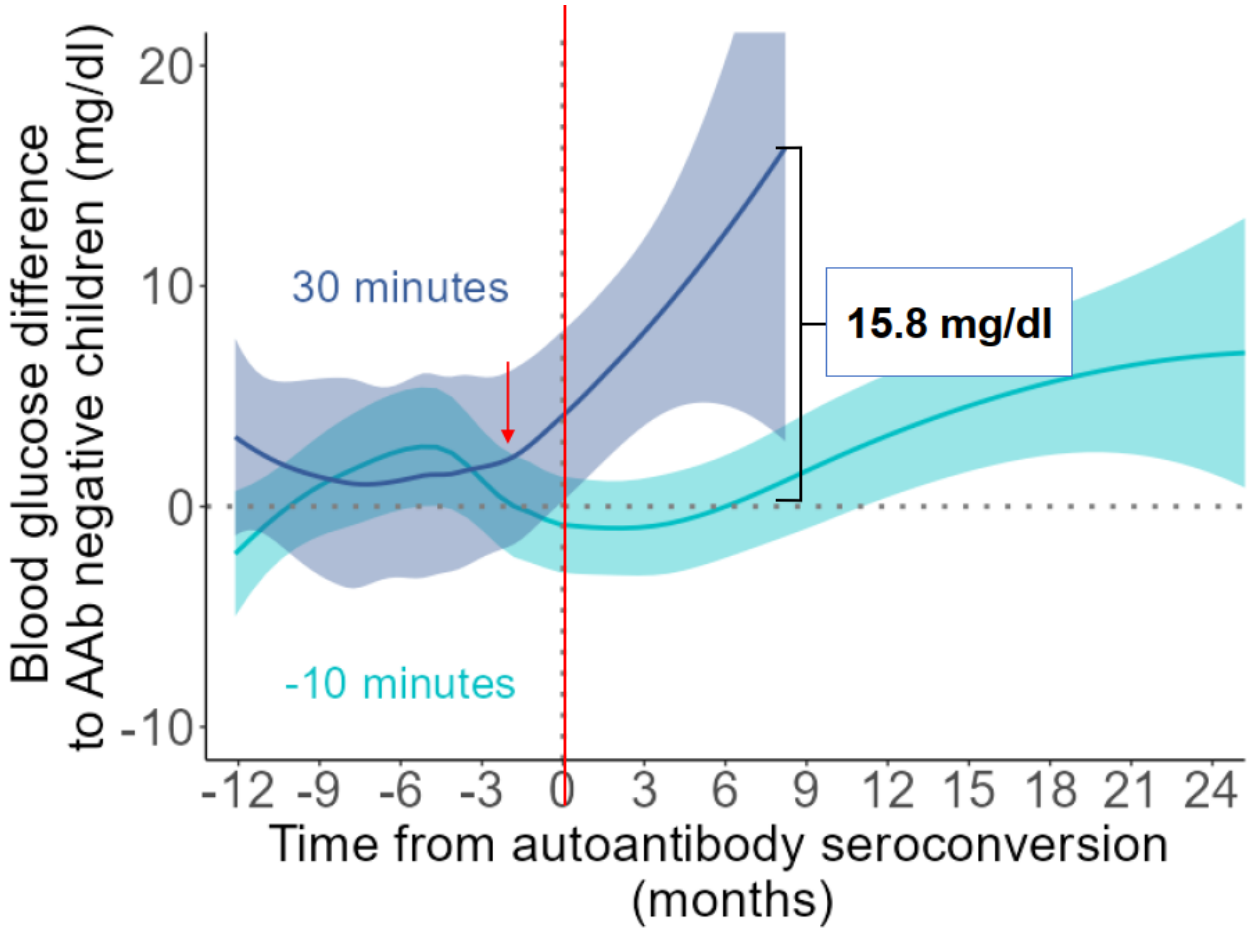
OR 3.1 (CI 1.6-5.7)
P=0.0005

GPPAD
GLOBAL PLATFORM FOR THE PREVENTION OF AUTOIMMUNE DIABETES

AVAnT1A study – weekly saliva samples from age 3 mo to 2 y

Marked variation in duration of virus detection
– not all virus infections are the same

# Sharp and sustained rise in glucose around islet autoantibody seroconversion




GPPAD
GLOBAL PLATFORM FOR THE PREVENTION OF AUTOIMMUNE DIABETES

*Warncke K, Weiss A, et al, J Clin Invest 2022*

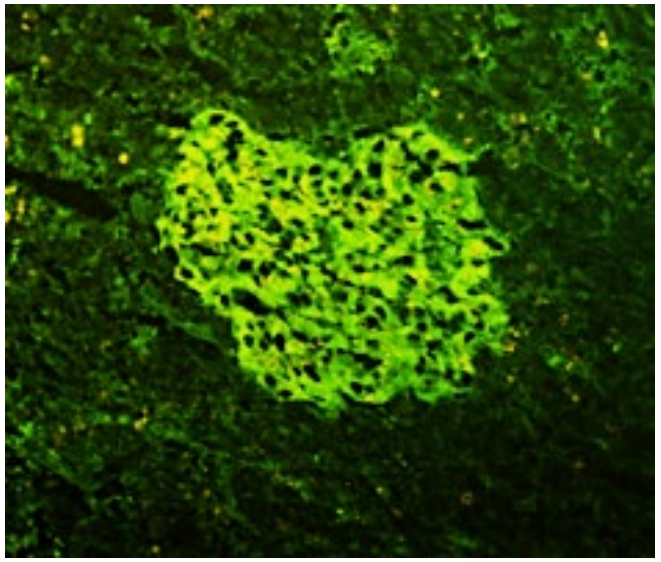
# Releasing the immune system and autoimmunity – CPI

100 patients (melanoma) treated with Checkpoint Inhibitors – Julian Steininger

Tested before and after 1 year trreatment of treatment for 37 autoantibodies (Mostly LIPS assays):

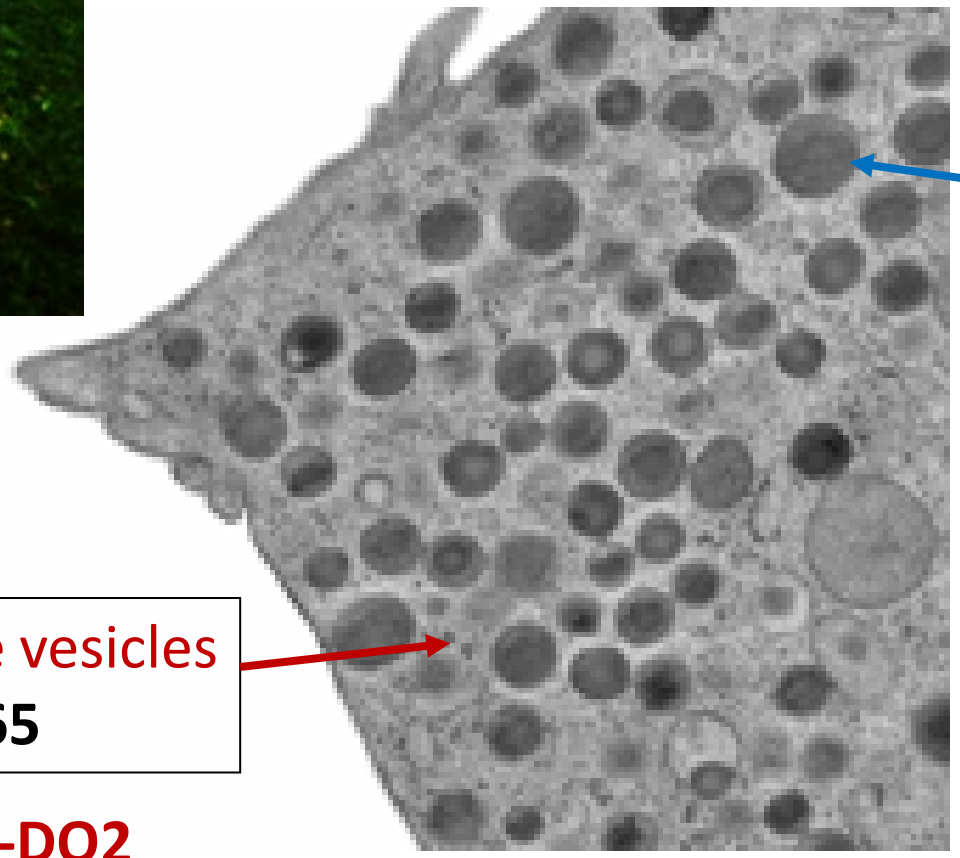
CDR2, La, Ro52, Ro60, ATP4A, CA, HARS1, GAD65, IL17A, IFNA1, IFNA2, ITM2B, GNAL, TPO, ISH1c, C1QA, CBLIF, TOP1, GRIN1, PTRN3, CENPA, ELAV4, TGM2, Col7A, DLAT, TG, TSHR, CCP2, RP11, RP155, PMS100, PMS75, SmD1, U1-snRNP, BP180, PB230

# Islet Autoantigens



GAD65 and IA-2 are the major antigens of ICA

*(Bonifacio et al, 1995, Genovese et al, 1994)*



Secretory granule
**Insulin/proinsulin**
**IA-2/IA-2β**
**ZnT8**
Tetraspanin 7

Synaptic-like vesicles
**GAD65**

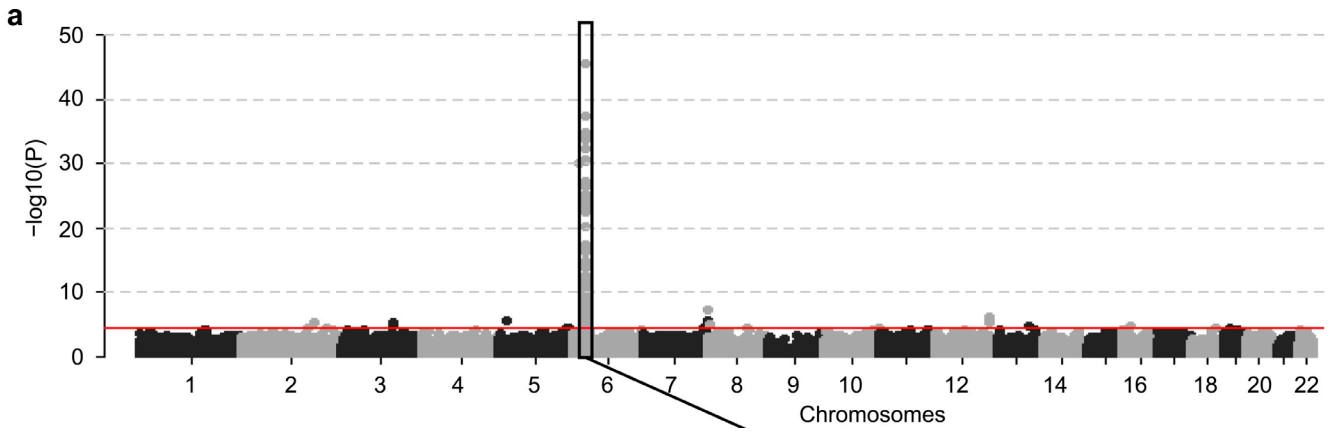
HLA DR4-DQ8
(IAA – *INS* genotype)
Insulin is classic target in children

**HLA DR3-DQ2**
Classic target in adults
(and neurological diseases)

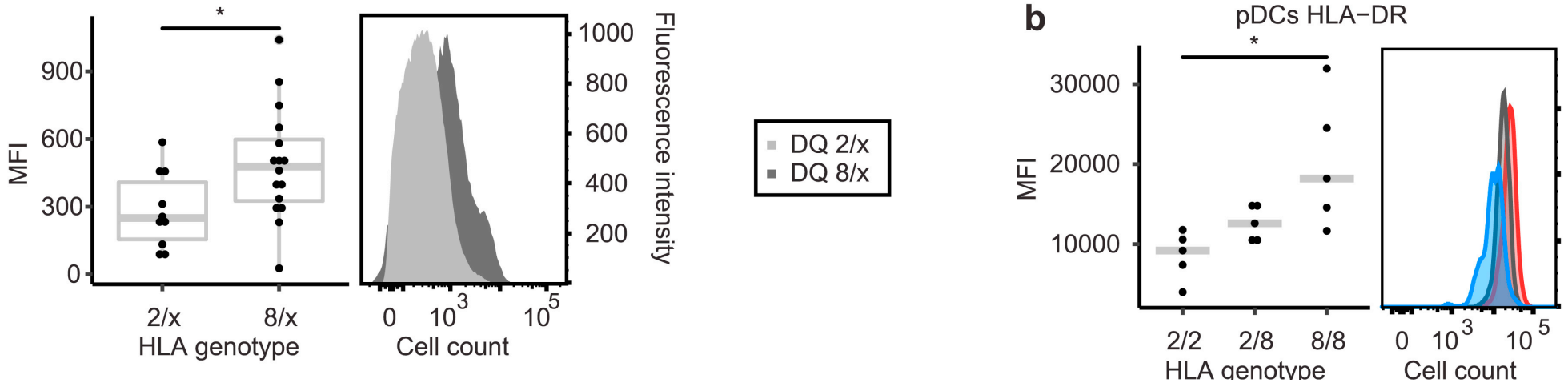
IGRP - ER

# HLA DR3 and DR4 have different expression levels on antigen-presenting cells

Large differences in CPG methylation in HLA region

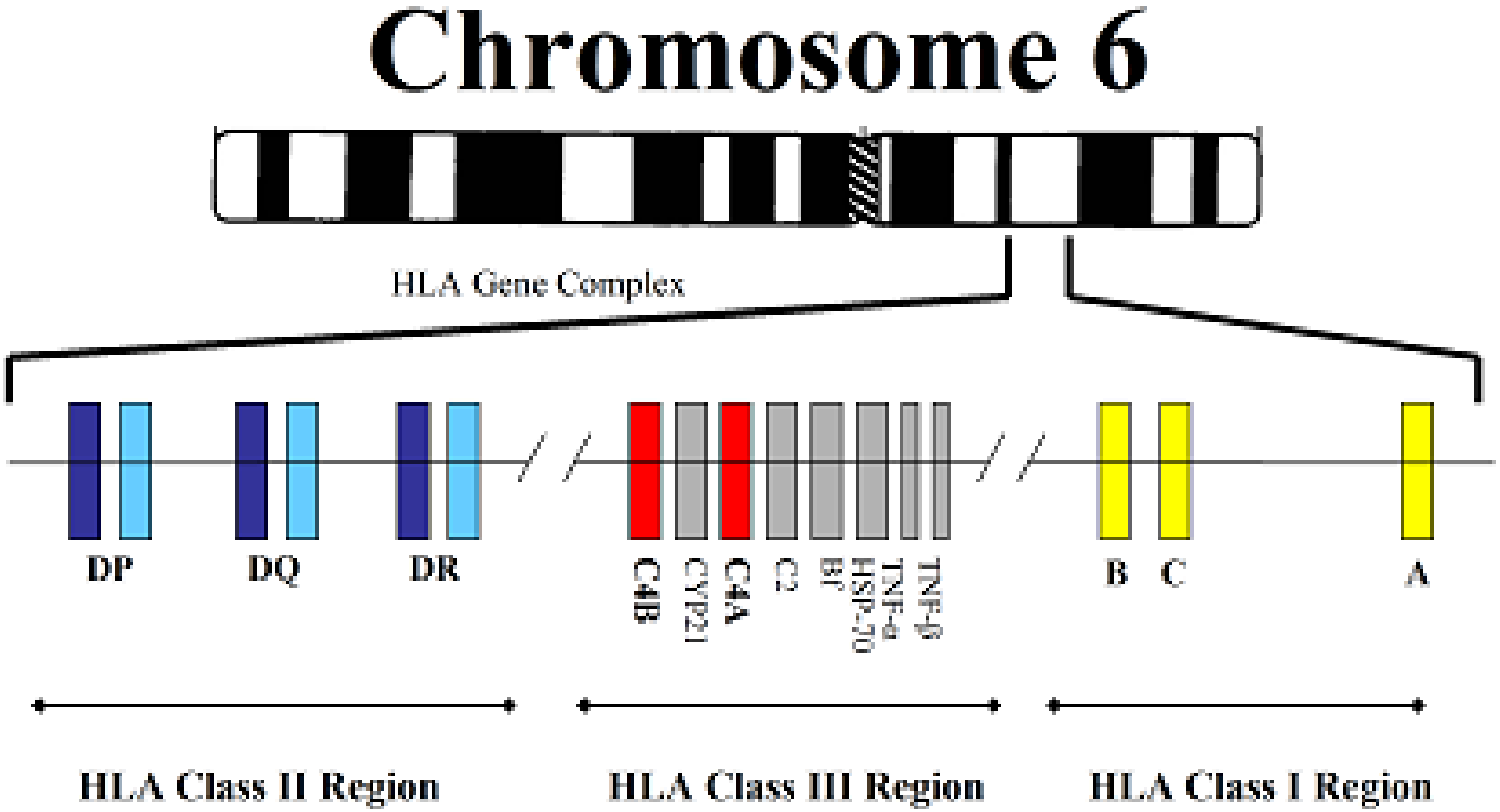



And surface expression of HLA DR protein




*Kindt et al, J Autoimmunity 2015*

# HLA gene complex includes multiple immune genes in linkage disequilibrium



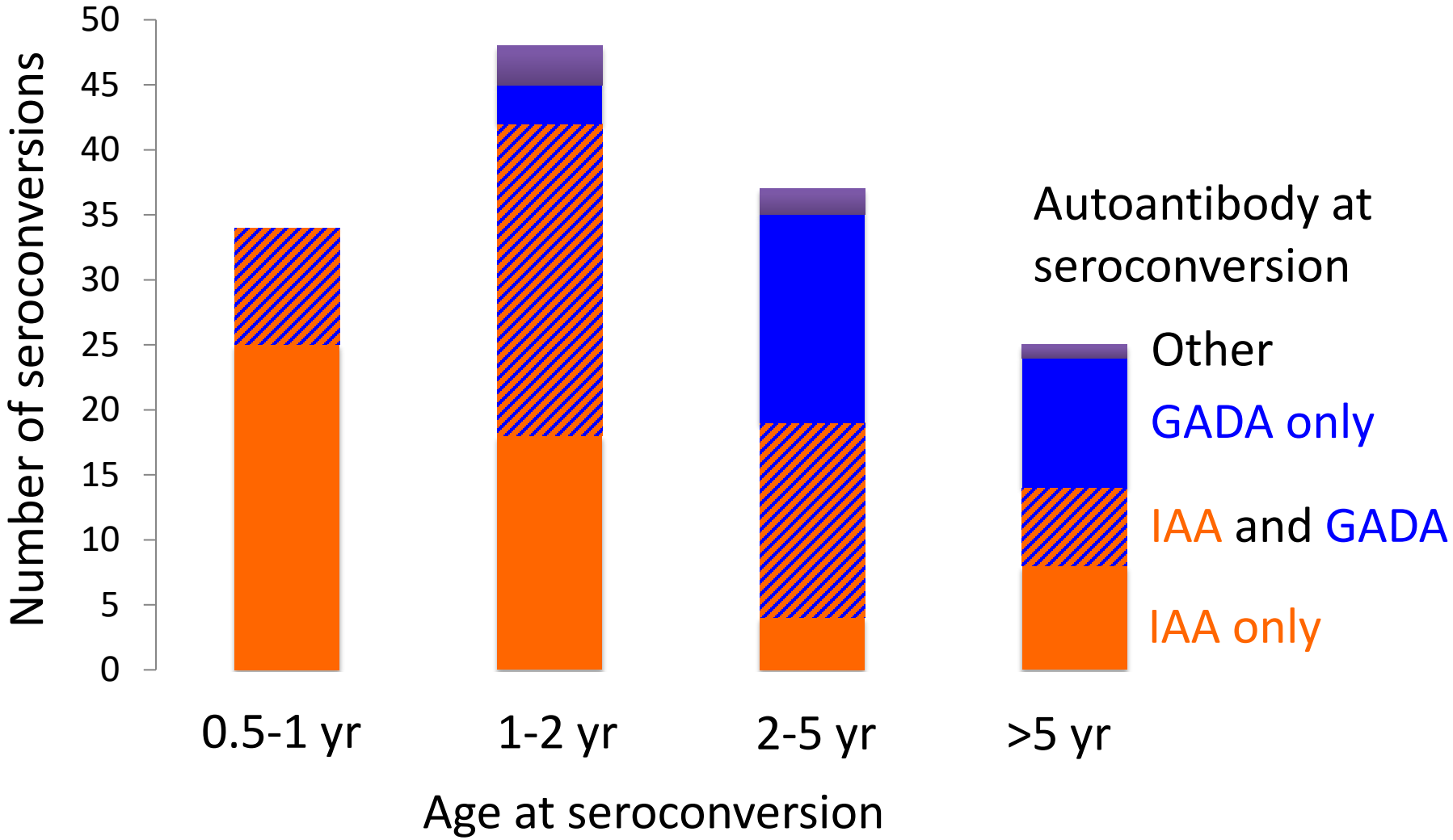

DR3-DQ2 often
has C4 null alleles

Insulin (DR4, children)

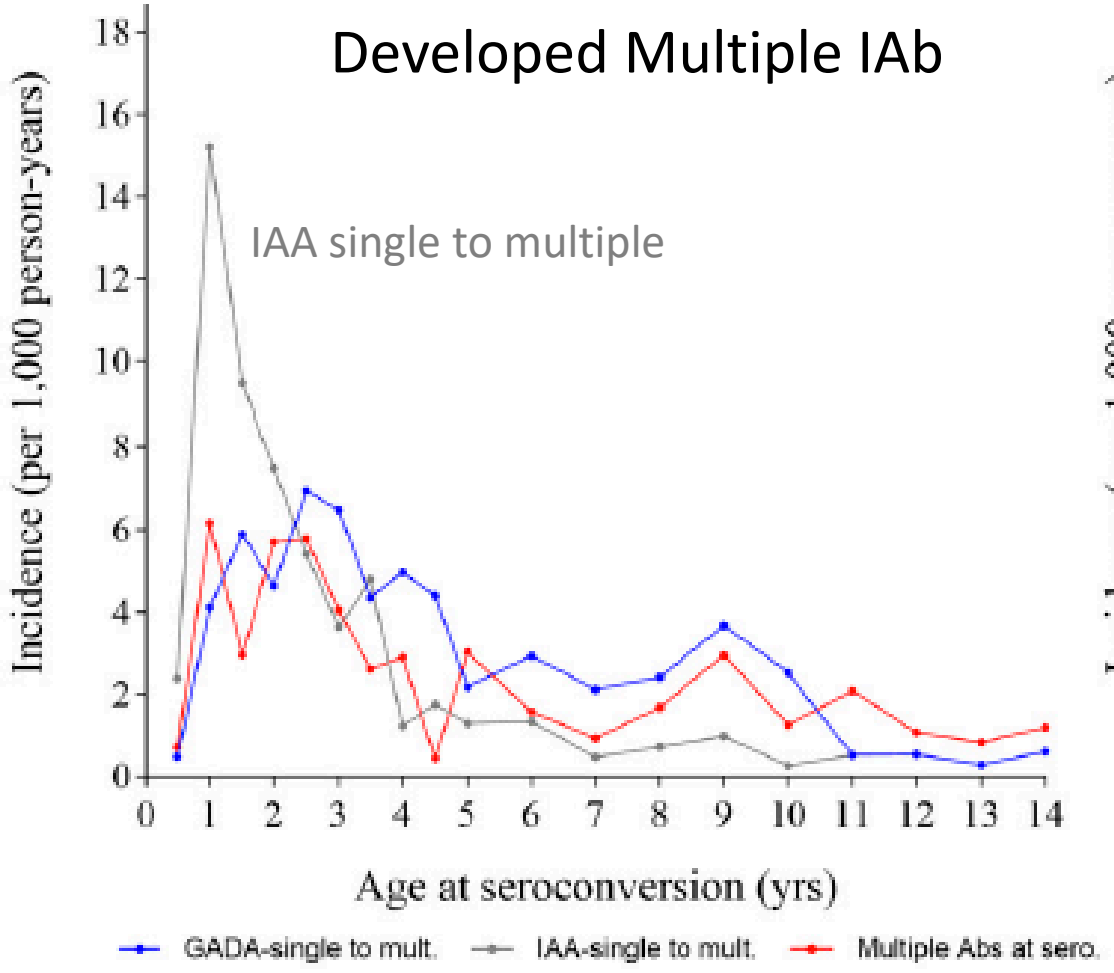
vs

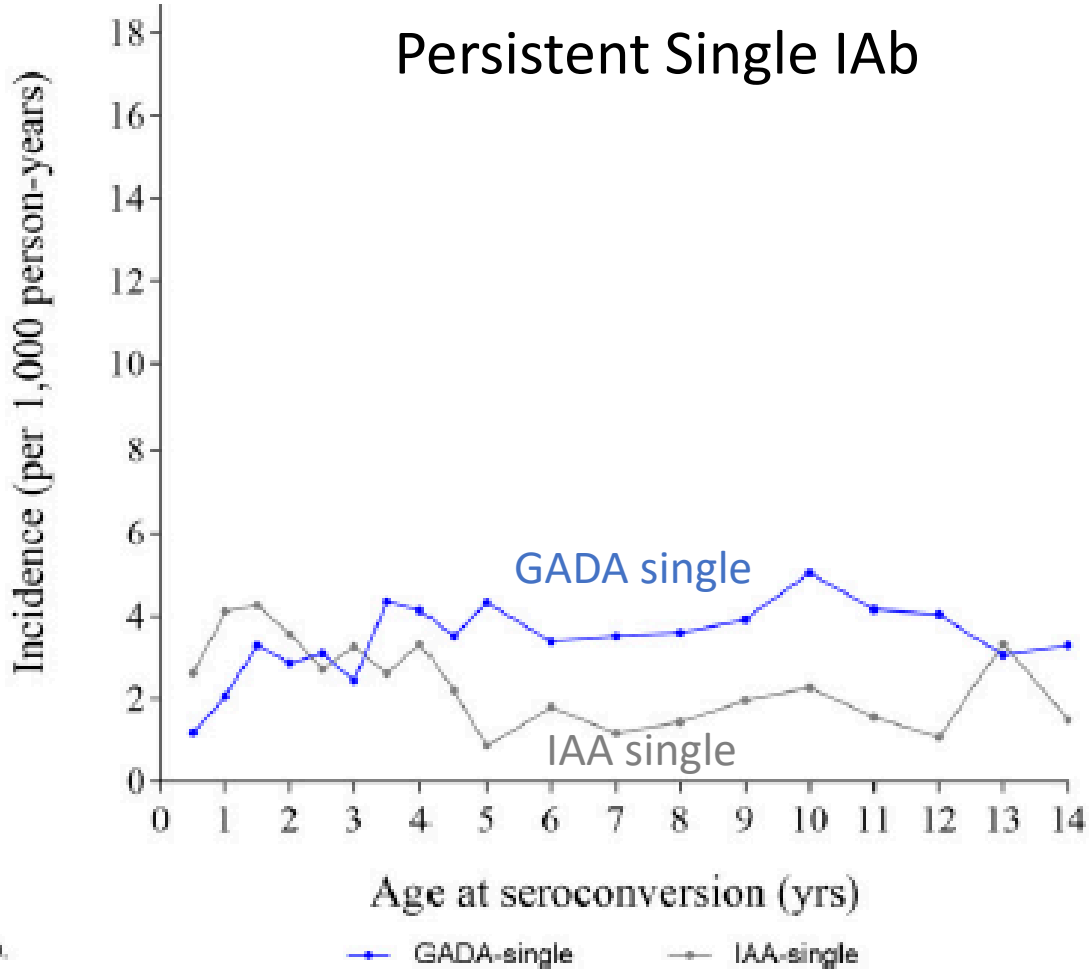
GAD65 (DR3, adults)

# First year is dominated by seroconversion to insulin autoantibodies BABYDIAB



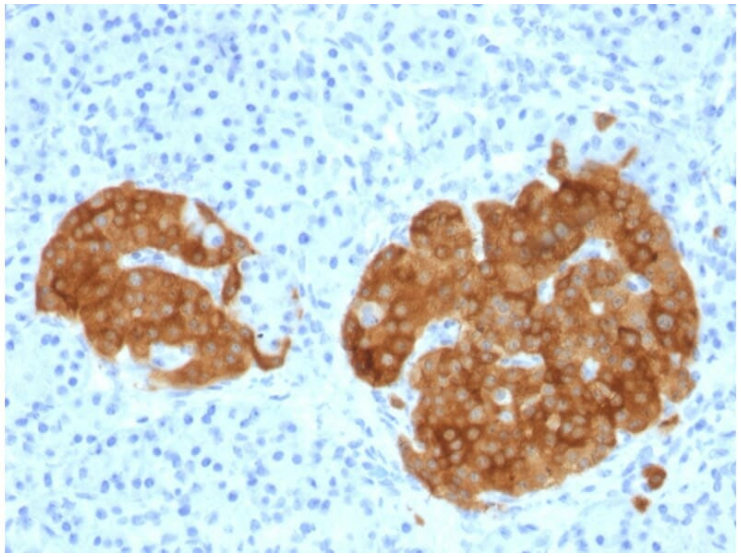
# TEDDY developed the notion of an IAA first vs GADA first autoimmunity



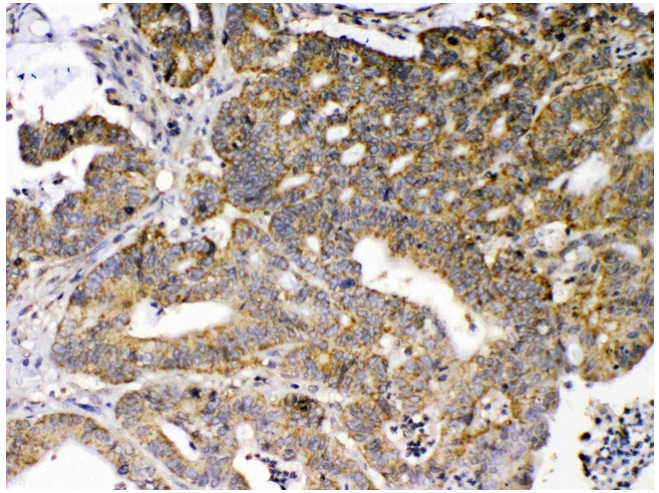




*Rewers et al, Diabetes Care, 2025*

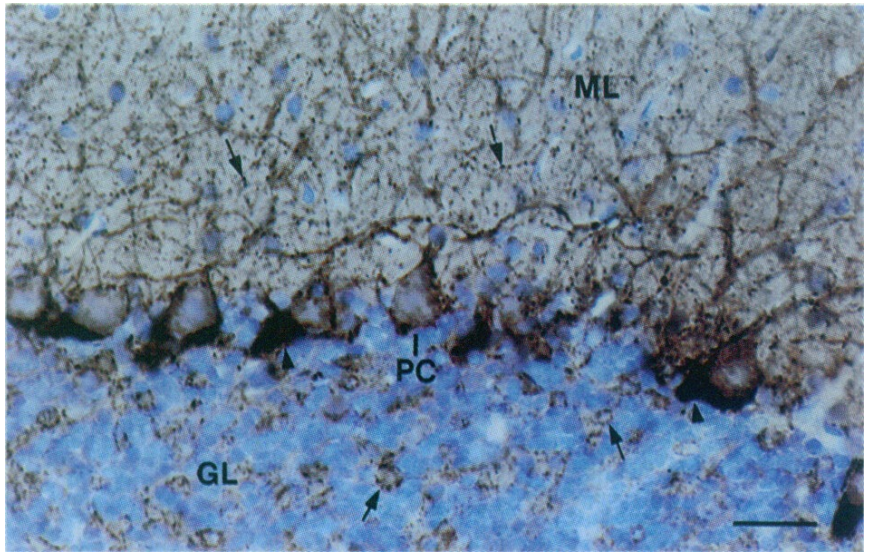
# GAD65 is not specific to the pancreatic islets



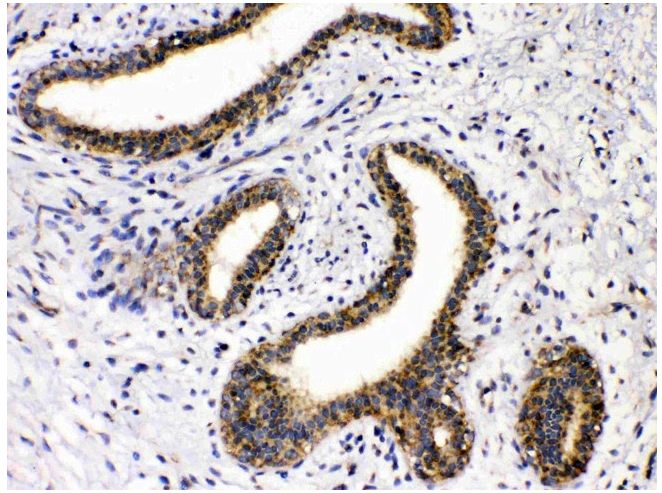
Pancreatic
Islets



Colorectal
cancer




Purkinje cells
Cerebellum



Breast
cancer

Triggers – make something happen
(often suddenly and causally)

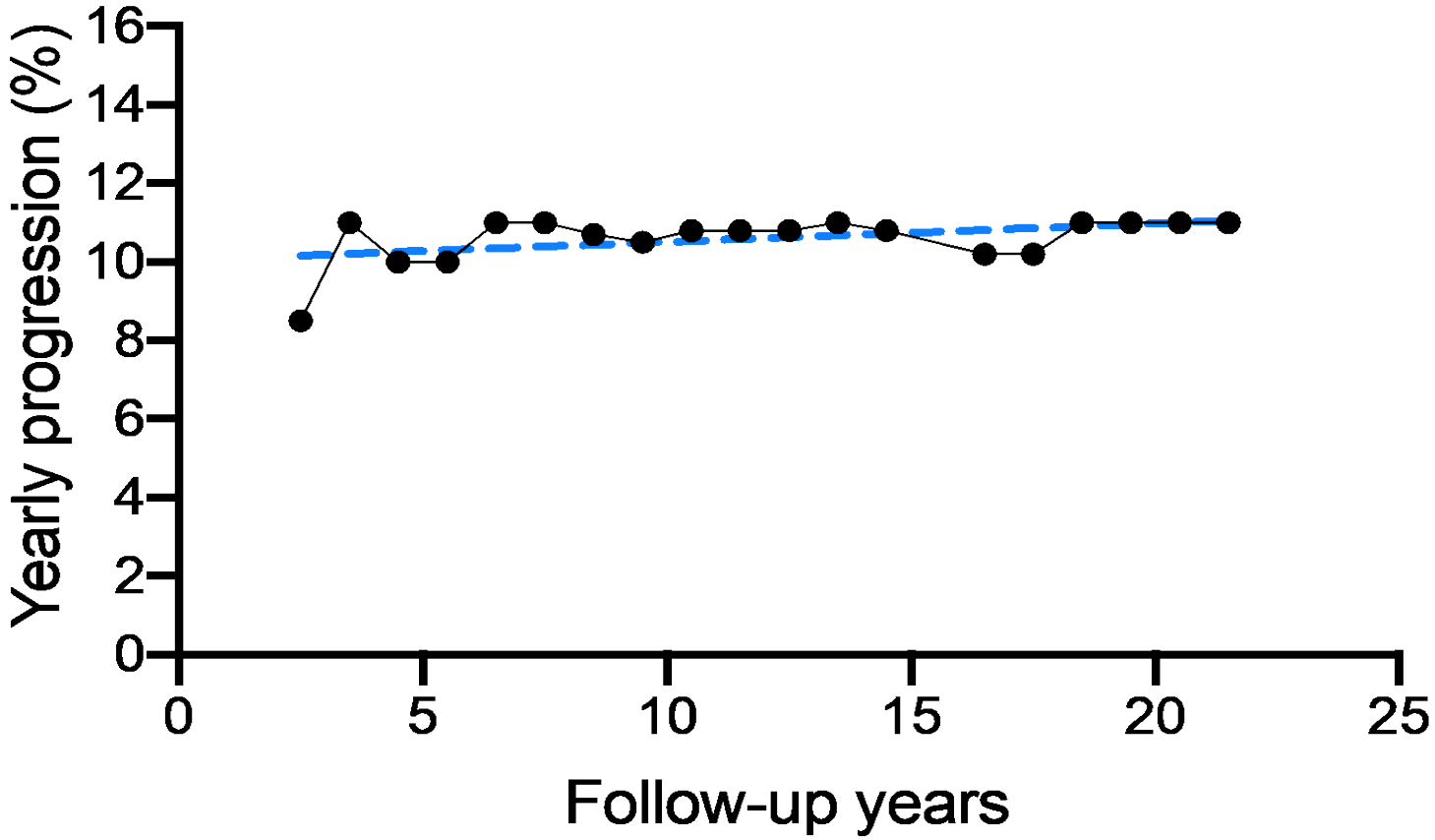
Modifiers of risk – increase or decrease the genetically defined risk for (early stage) T1D

Drivers – keep the process going

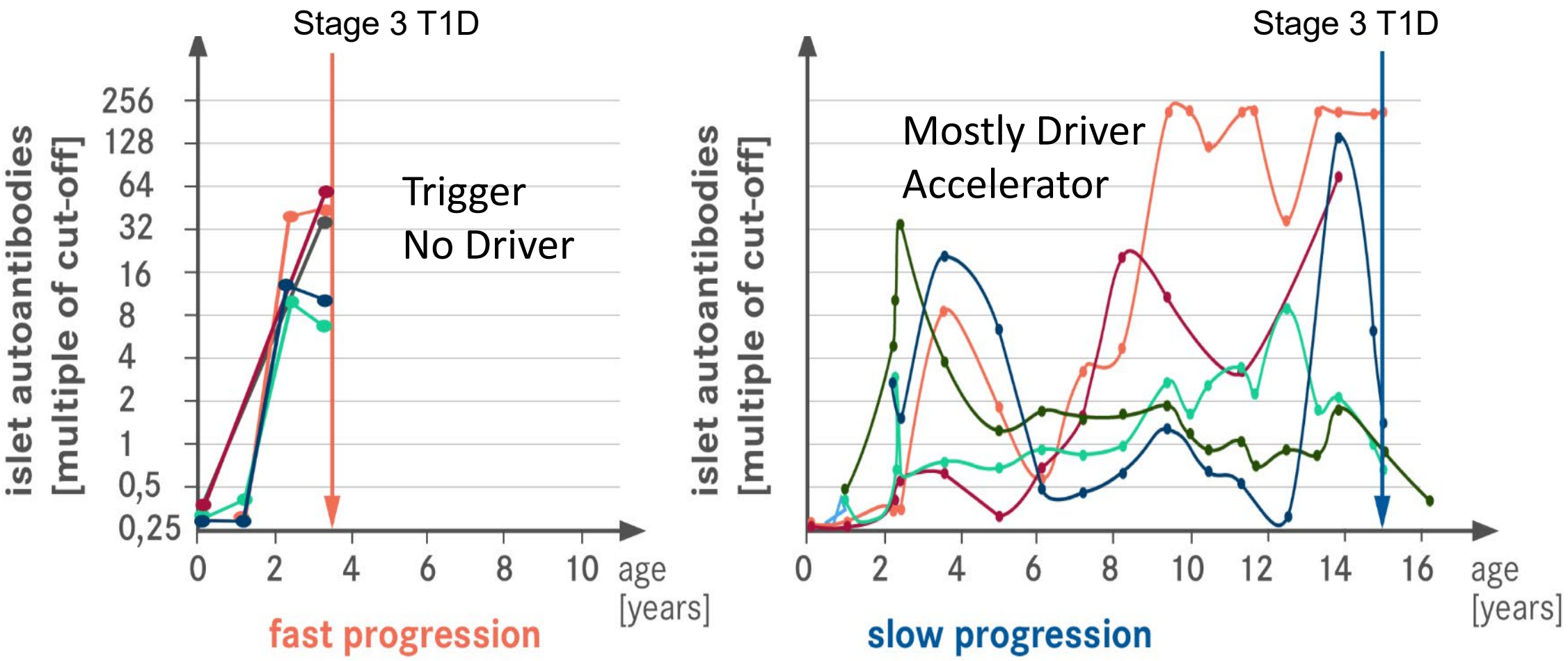
Accelerators – change (increase) rate of progression

# Progression to Stage 3 T1D

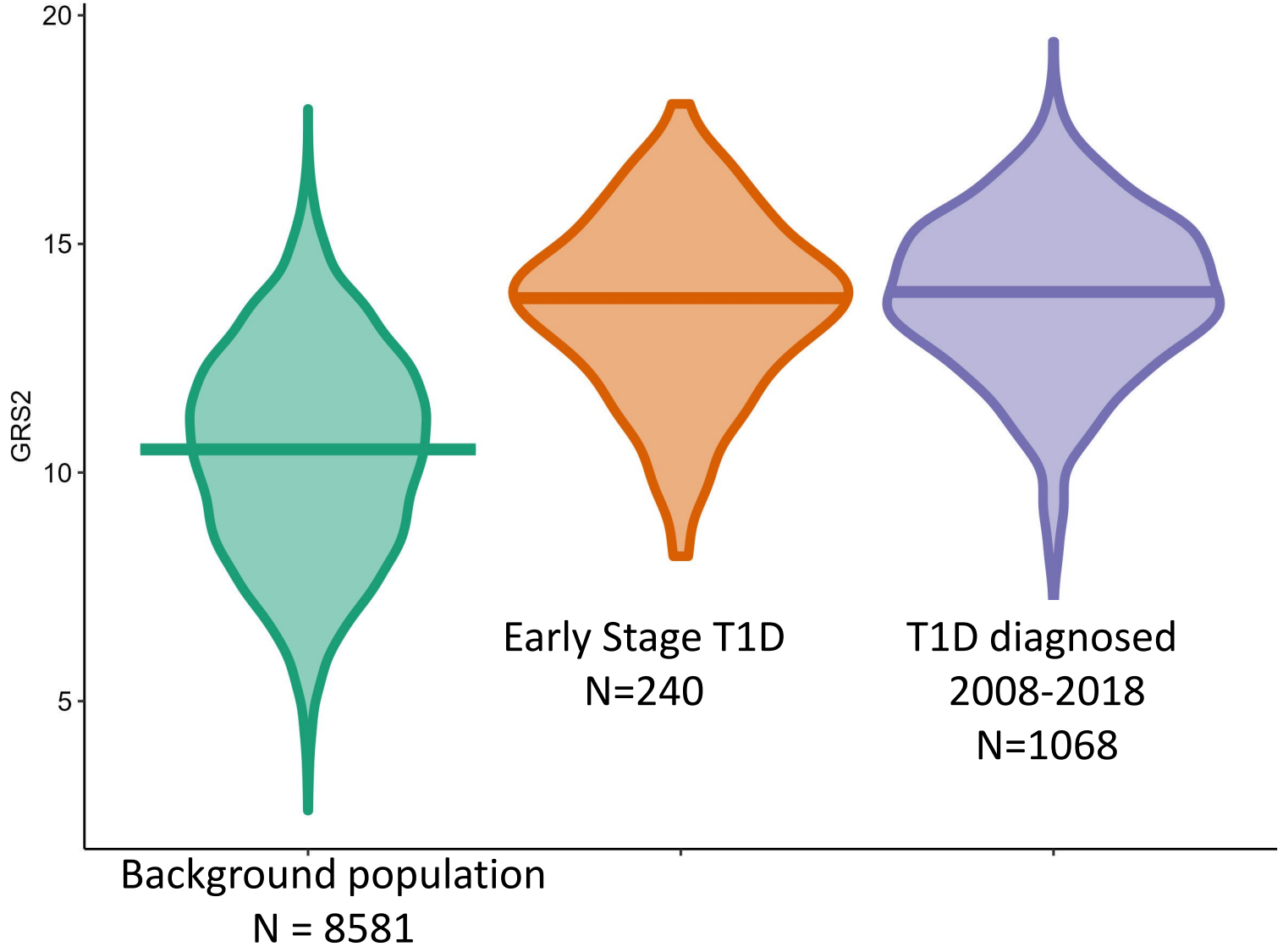
# Progression is stable over 20 years - BABYDIAB



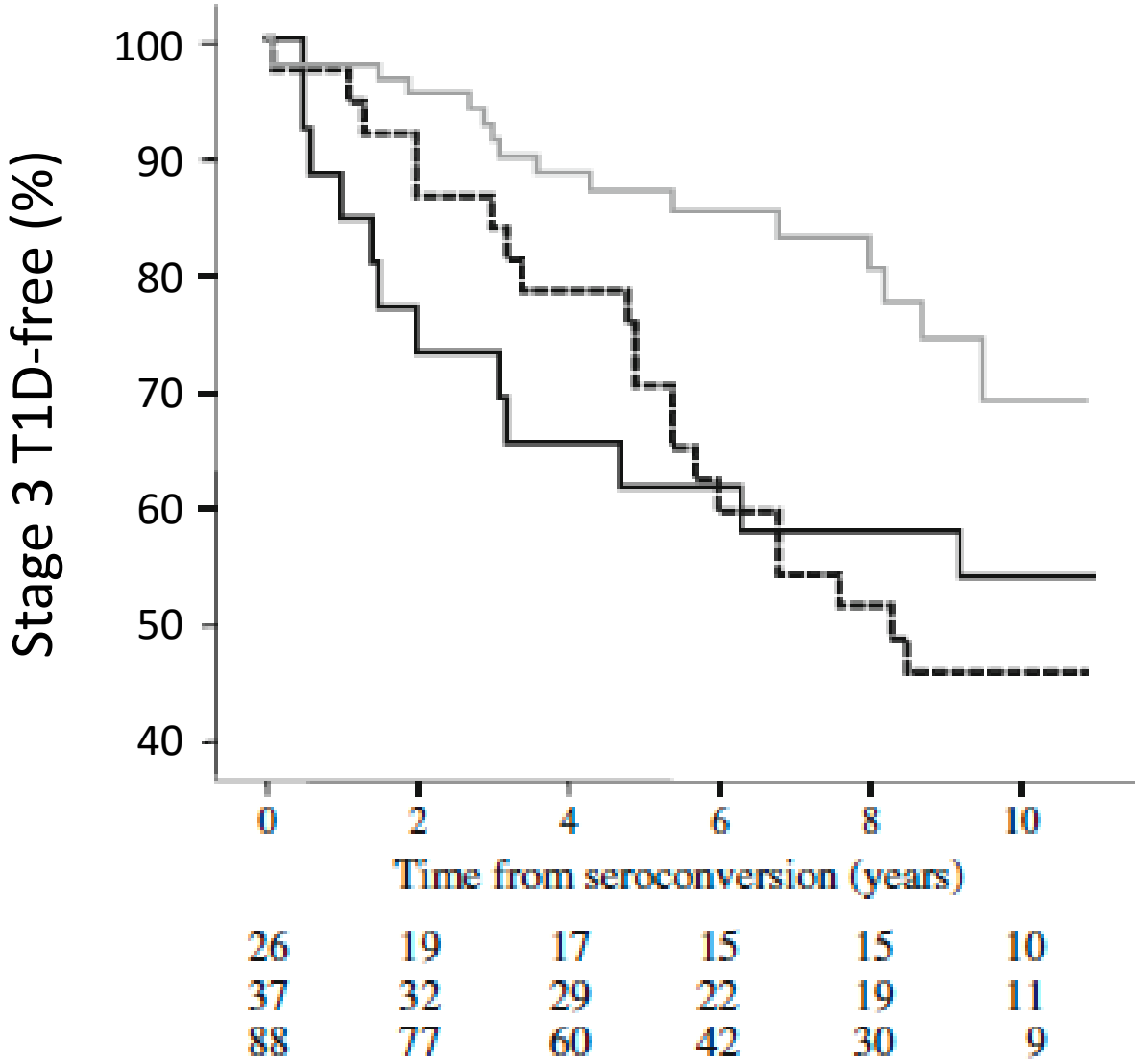
# There is marked variation in disease progression



# T1D genetics is the same in early stage and clinical stage 3 - Doesn’t affect progression of early stage



# Age (or phenotype) at start of the process matters

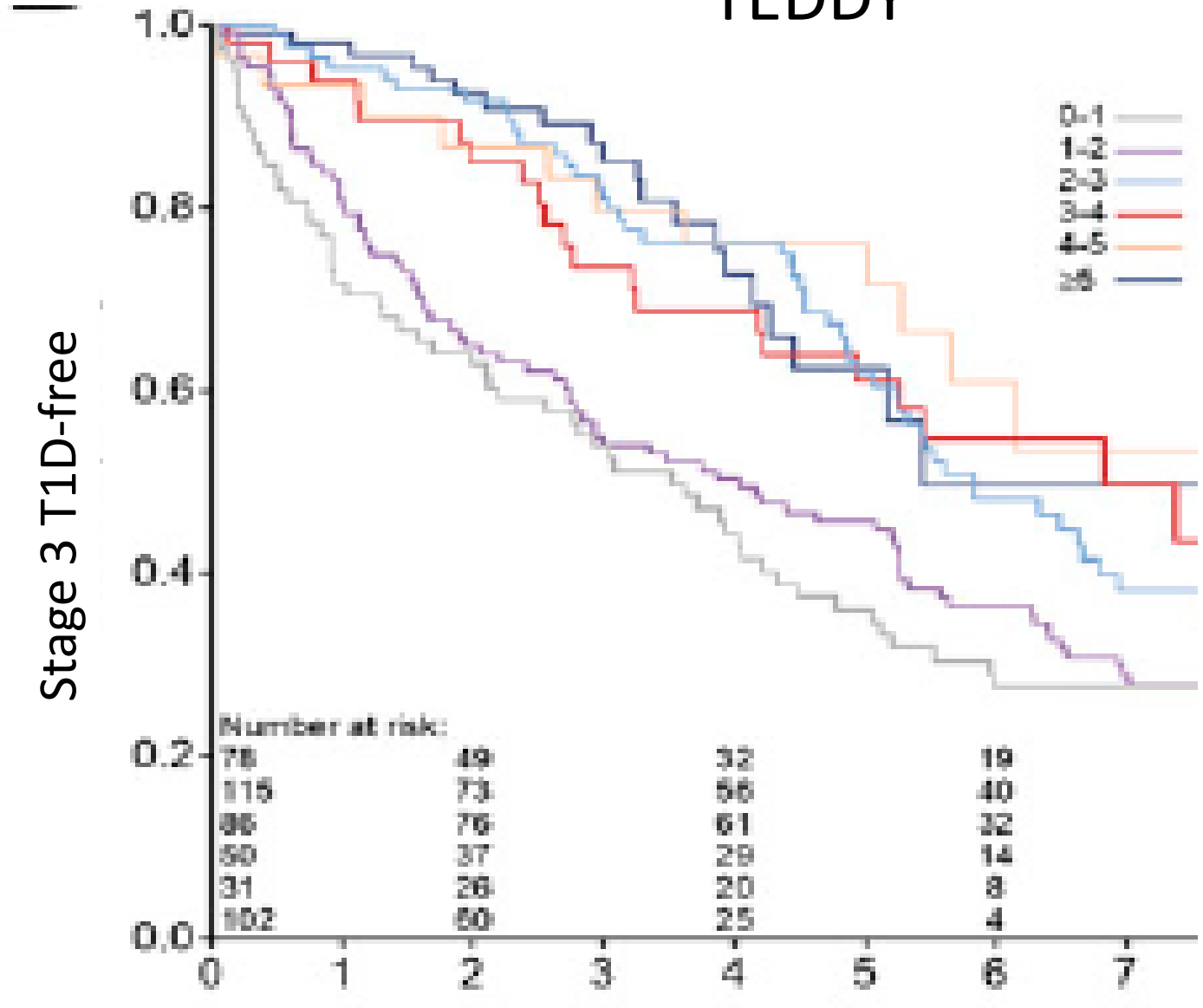



Start after age 2 yr

Start by age 2 yr

*Hummel et al Ann Int Med, 2004*
*Ziegler & Bonifacio, Diabetologia 2012*

# TEDDY




Start after age 2 yr

Start by age 2 yr

*Rewers et al, Diabetes Care, 2025*

# GAD antibodies are associated with relative protection

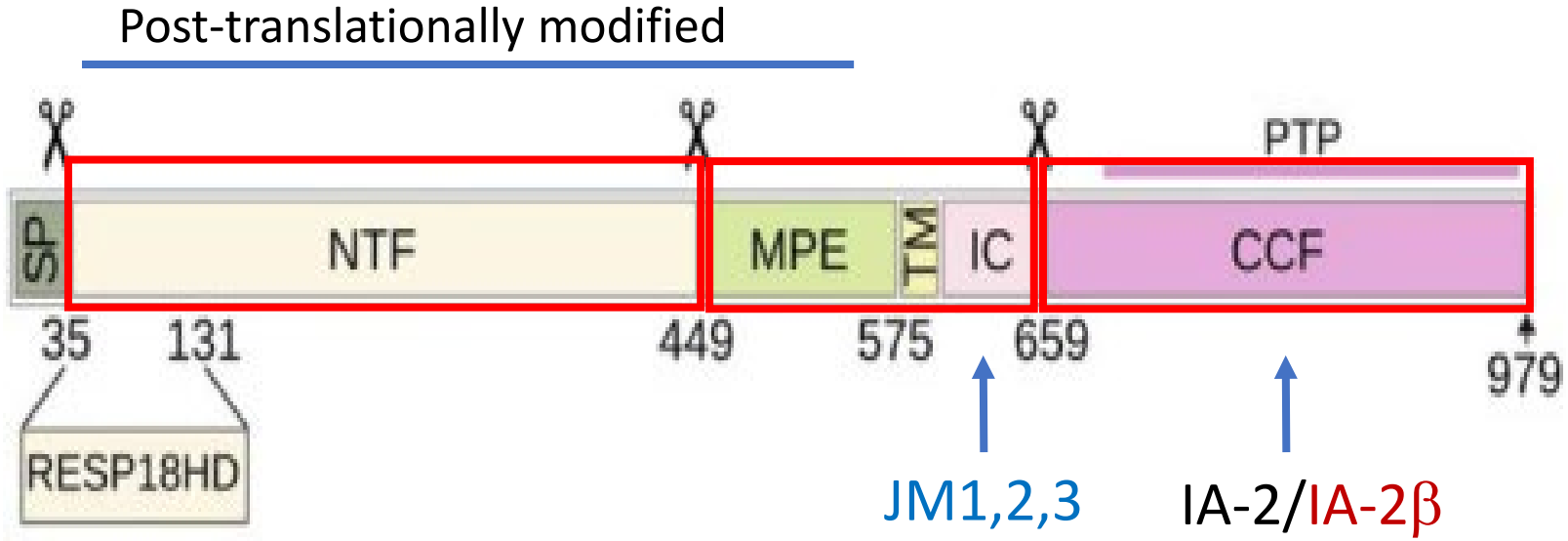
GADA positive multiple islet autoantibody positive children have >50% lower risk to progress to Stage 3 T1D than GADA negative

Fr1da: HR 0.43 (95% CI 0.25-0.75)
TrialNet: HR 0.35 (95% CI 0.22-0.57)

Are they an indicator of efforts to protect the beta cell?

*Bonifacio, Ziegler, Diabetologia 2020*

# IA-2 antibodies are a collection of antibodies

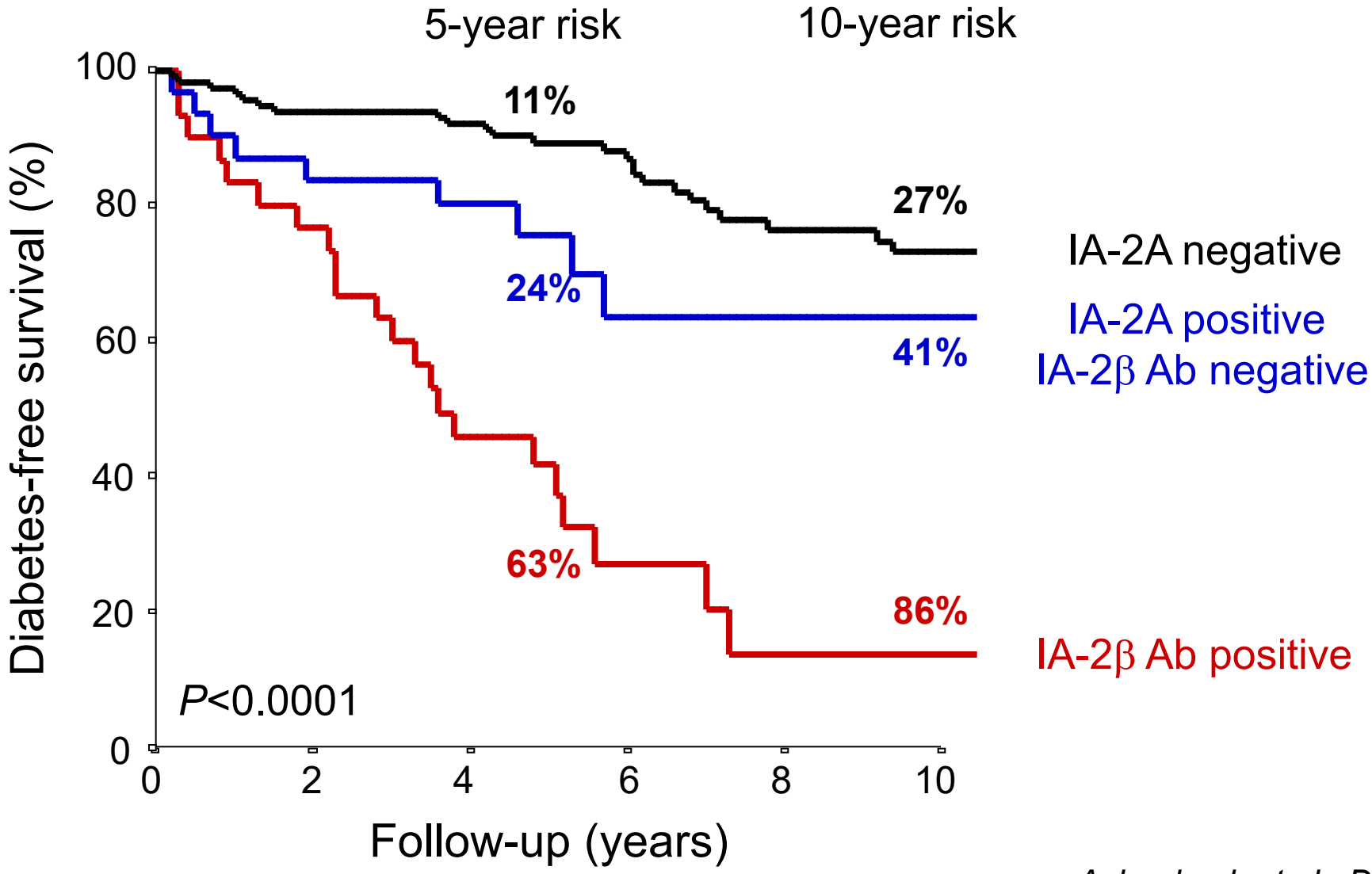



*Toledo et al, J Biol Chem, 2019*
*Bonifacio et al, J Immunol, 1998*
*Bearzatto et al, J Immunol, 2001*

All associated with HLA DR4
(JM1 epitope antibodies found in non-DR4)

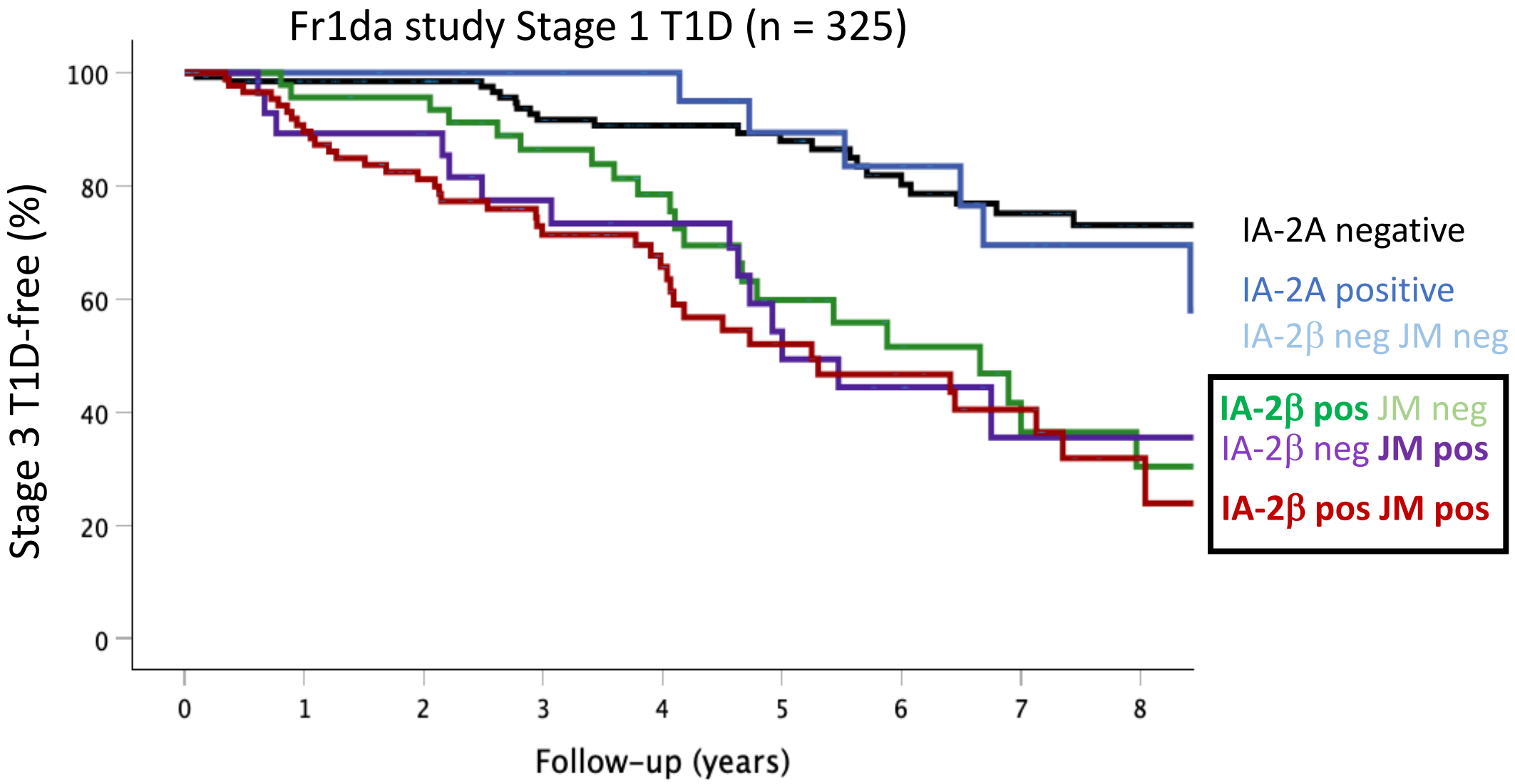
Christie et al, 1992, Christie et al 1994, Bingley et al, 1994, Genovese et al, 1994, Achenbach et al, 2001, Decochez et al, 2002

# High risk in IA-2A positive individuals is stratified by IA-2β autoantibodies

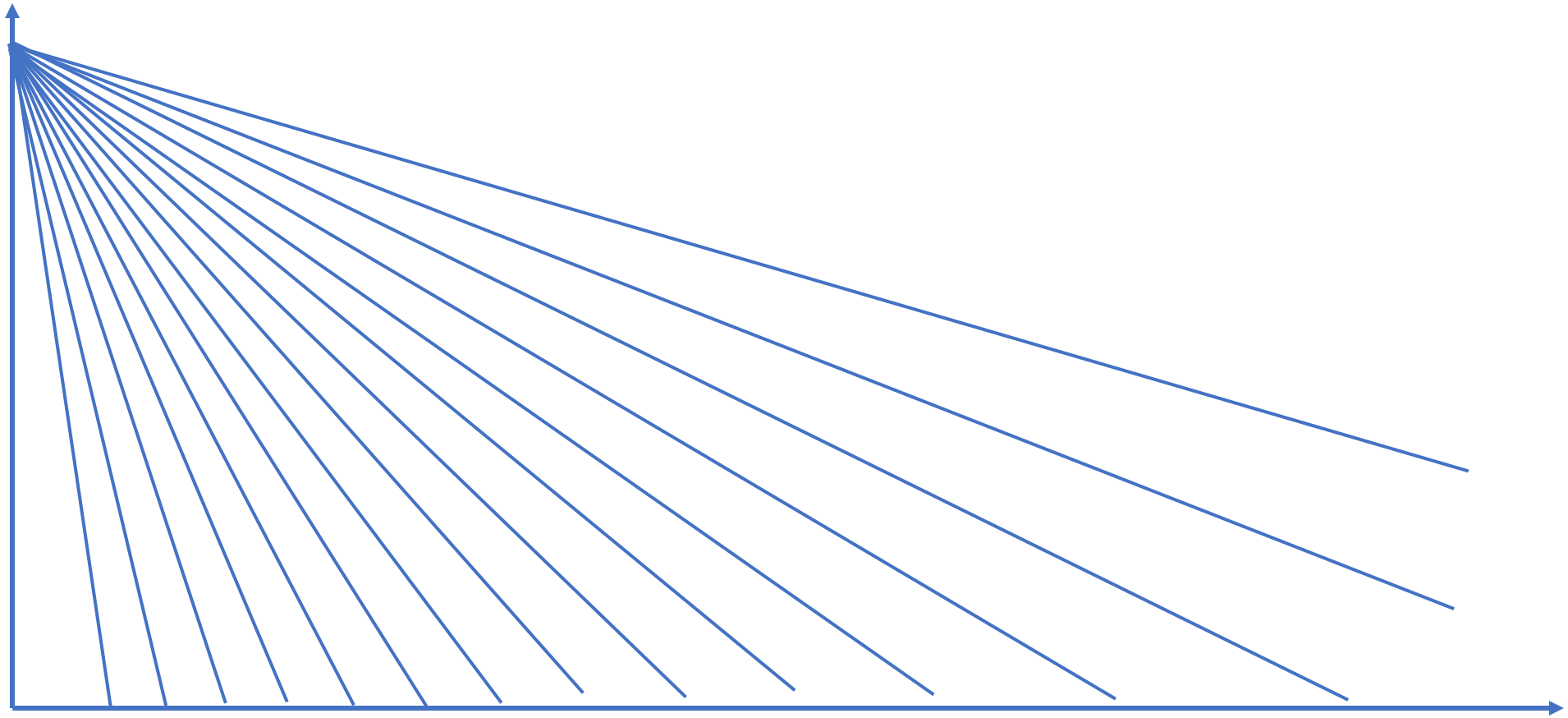



*Achenbach et al., Diabetes 2004*

# IA-2A against IA-2β PTP and IA-2 JM epitopes define faster progression

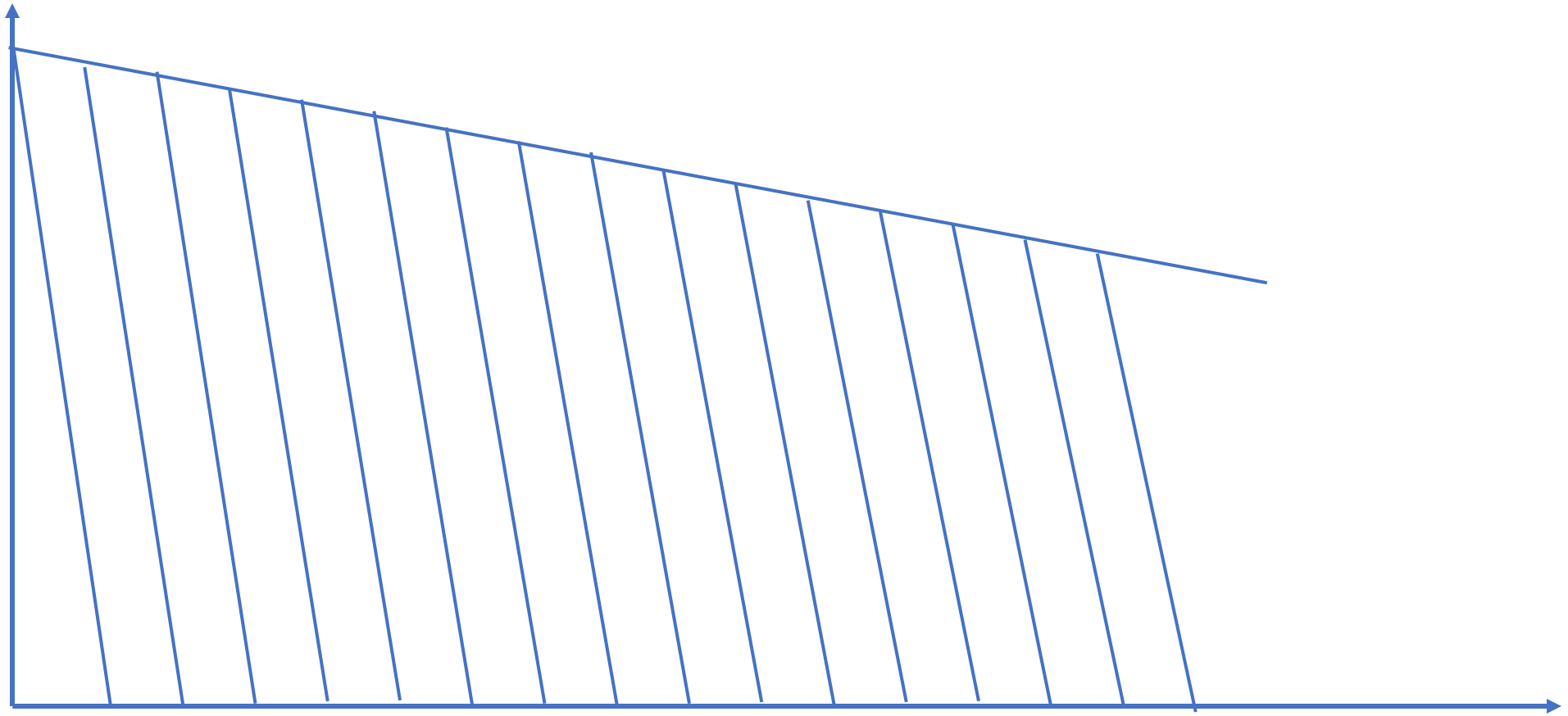


# Is it a linear progression for each individual?



Progression time to Stage 3 T1D

# Or is there an accelerator



Progression time to Stage 3 T1D

# Accelerated Progression following Viral Infection

## Enterovirus

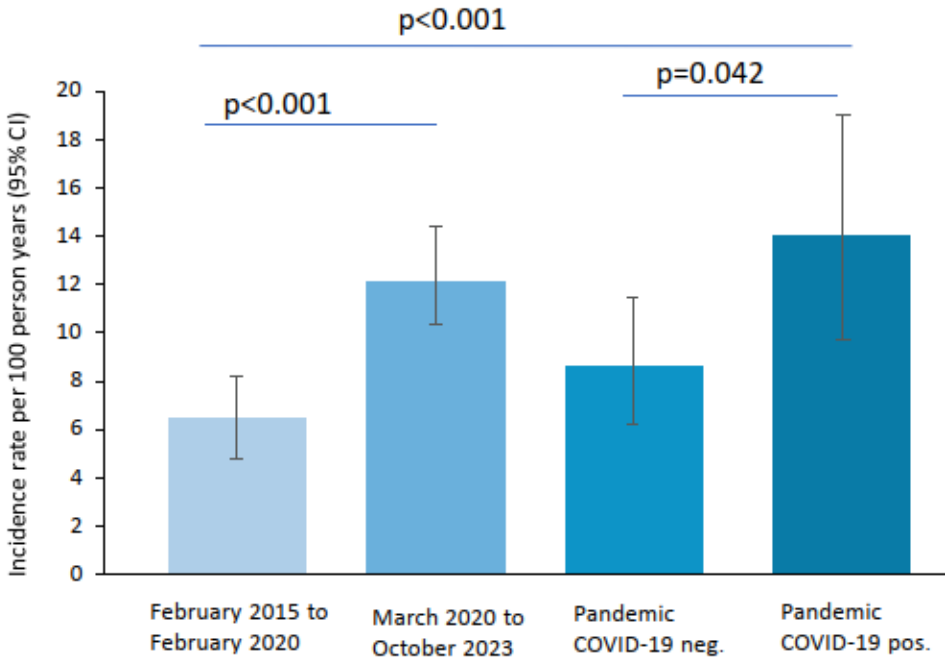
| Type of sample | Unadjusted HR (95% CI) | HR (95% CI) adjusted for islet autoantibodies |
|---|---|---|
| Serum | | |
| No enterovirus RNA in previous sample | 1.00 (ref.) | 1.00 (ref.) |
| Enterovirus RNA in previous sample | 6.36 (1.89–21.4)† | 7.02 (1.95–25.3)† |
| Rectal swab | | |
| No enterovirus RNA in previous sample | 1.00 (ref.) | 1.00 (ref.) |
| Enterovirus RNA in previous sample | 0.93 (0.12–6.90) | 0.79 (0.10–5.92) |

*Stene L, Diabetes 2010*

Frida
Typ 1 Diabetes: Früh erkennen – Früh gut behandeln

## SARS-CoV-2

*Friedl N et al, JAMA 2024*

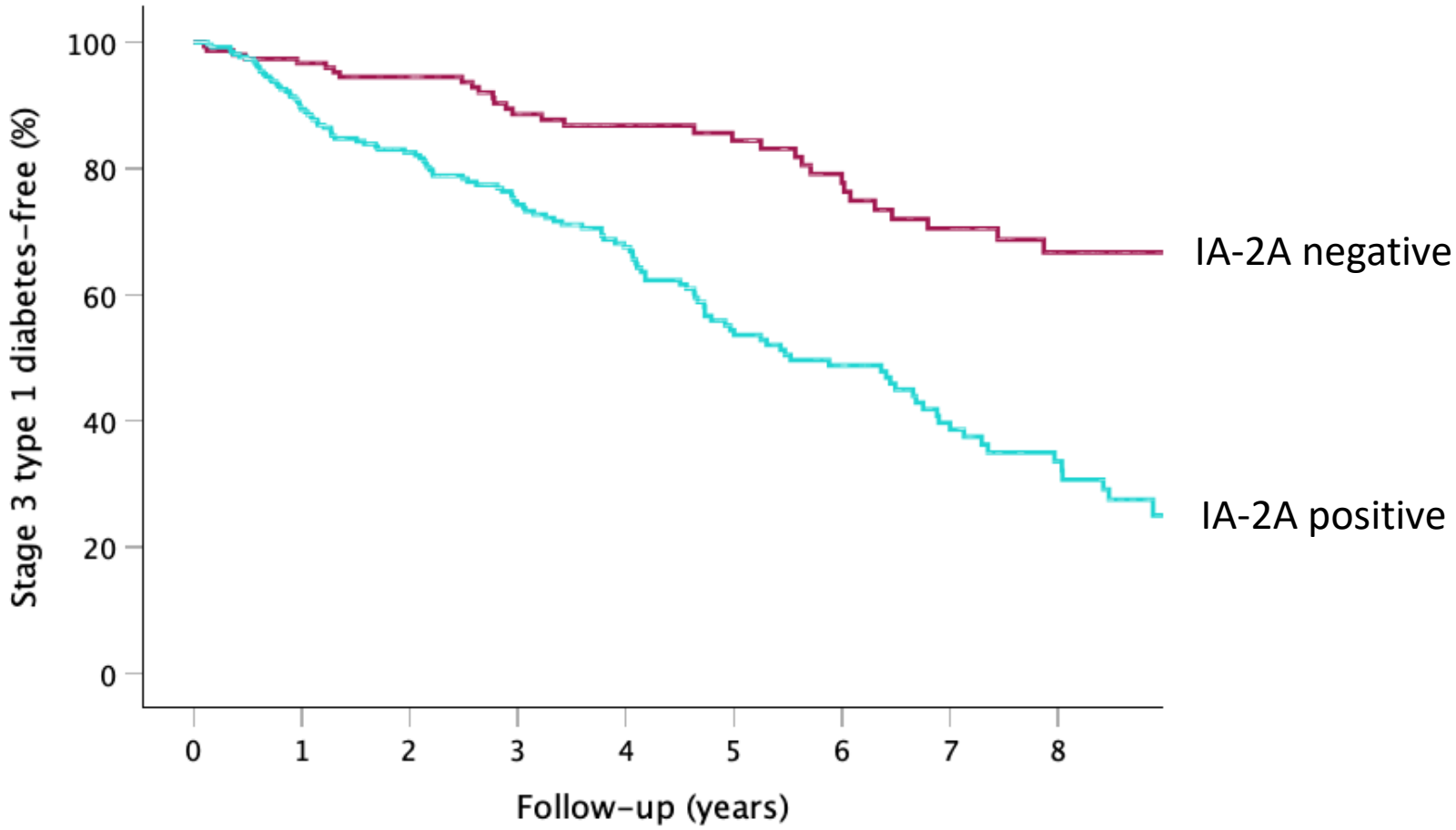


Pancreatic Injury Patterns in Patients With Coronavirus Disease 19 Pneumonia. Wang et al, Gastroenterology 2020

**Results:** None of the patients studied developed clinical signs or morphological alterations compatible with acute pancreatitis. However, it was found that **24.5% of patients had amylase values >53 IU/L** and **16.4% had lipase values >300 IU/L**.

# Adults and a forgotten setting

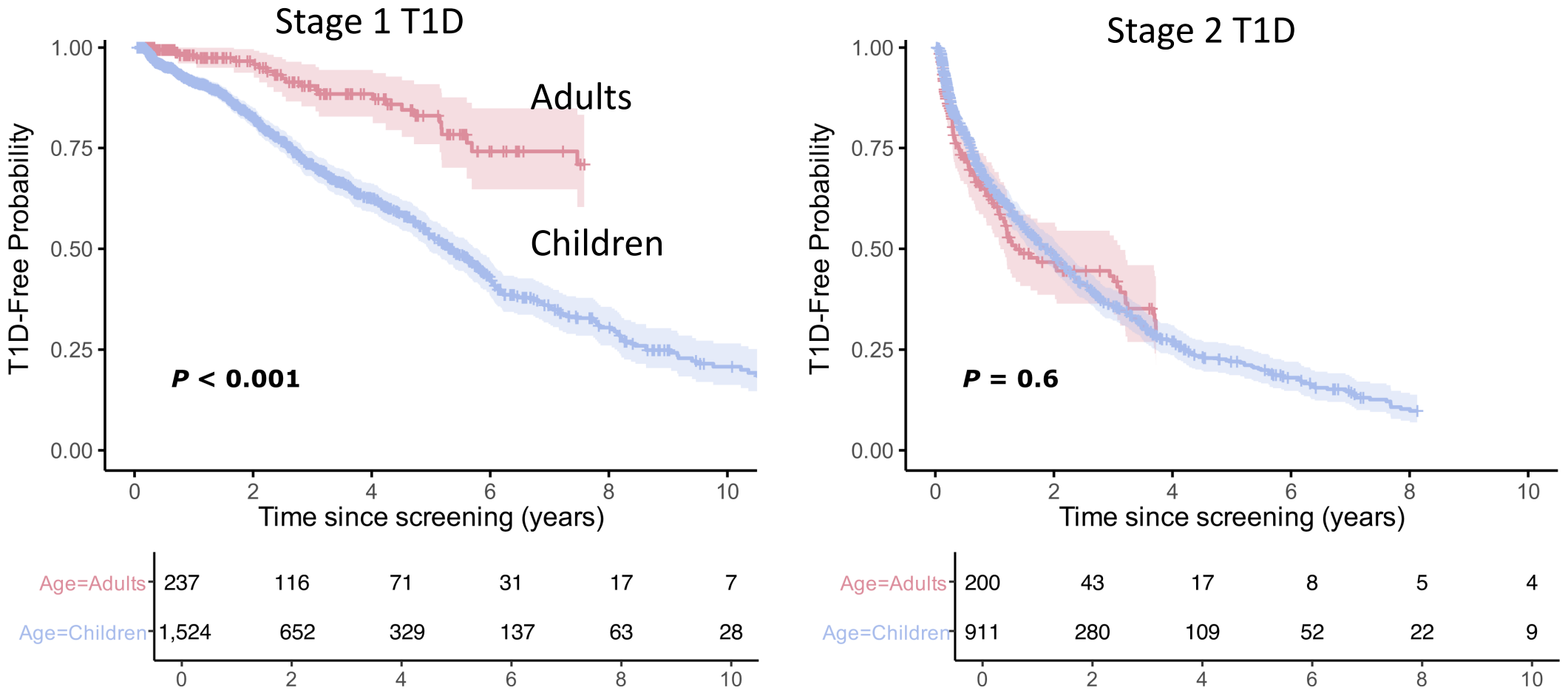
# Is early stage T1D in adults what is left from those who have not progressed?




70% of those with islet autoantibodies at age 25 years were already positive at age 15 years

# Progression in adults is the same as children in Stage 2 T1D - TrialNet

*Templeman et al, Diabetes Care, 2025*




End stage has a similar rate and/or

multiple antibodies + FDR + dysglycemia is required for true diagnosis in adults

# Screening in adults

Could be performed by:

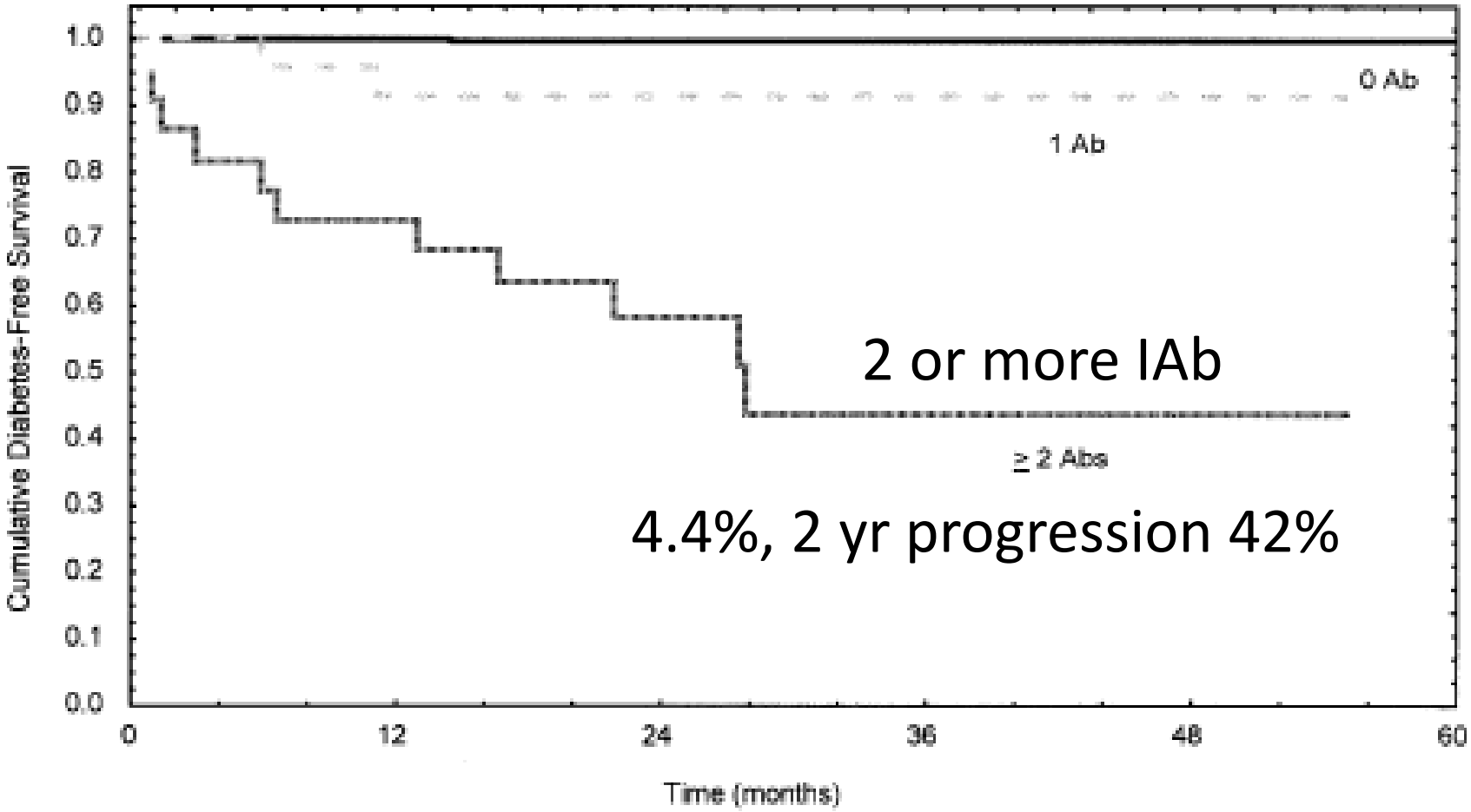
Antibodies (GADA)

And/or

Dysglycemia (eg HbA1c)

Plus genetics eg GRS

# Children with Incidental Hyperglycemia




| | | | | | | |
|---|---|---|---|---|---|---|
| 0 Ab | 452 | 416 | 340 | 218 | 145 | 87 |
| 1Ab | 24 | 22 | 17 | 11 | 6 | 4 |
| ≥2Abs | 22 | 17 | 10 | 7 | 5 | 4 |

*Lorini et al, Diabetes Care, 2001*

The more elements that come together, the more certain is disease
- Relevant for early stage diagnosis

The process appears heterogeneous between individuals – some elements are more relevant that others
- Relevant for prognosis

Different elements are relevant to different phases of the process
- Relevant for modifying strategies/therapies

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BABY DIAB

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Typ 1 Diabetes: Früh erkennen – Früh gut behandeln

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Breakthrough T1D™
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HELMSLEY CHARITABLE TRUST

NIDDK

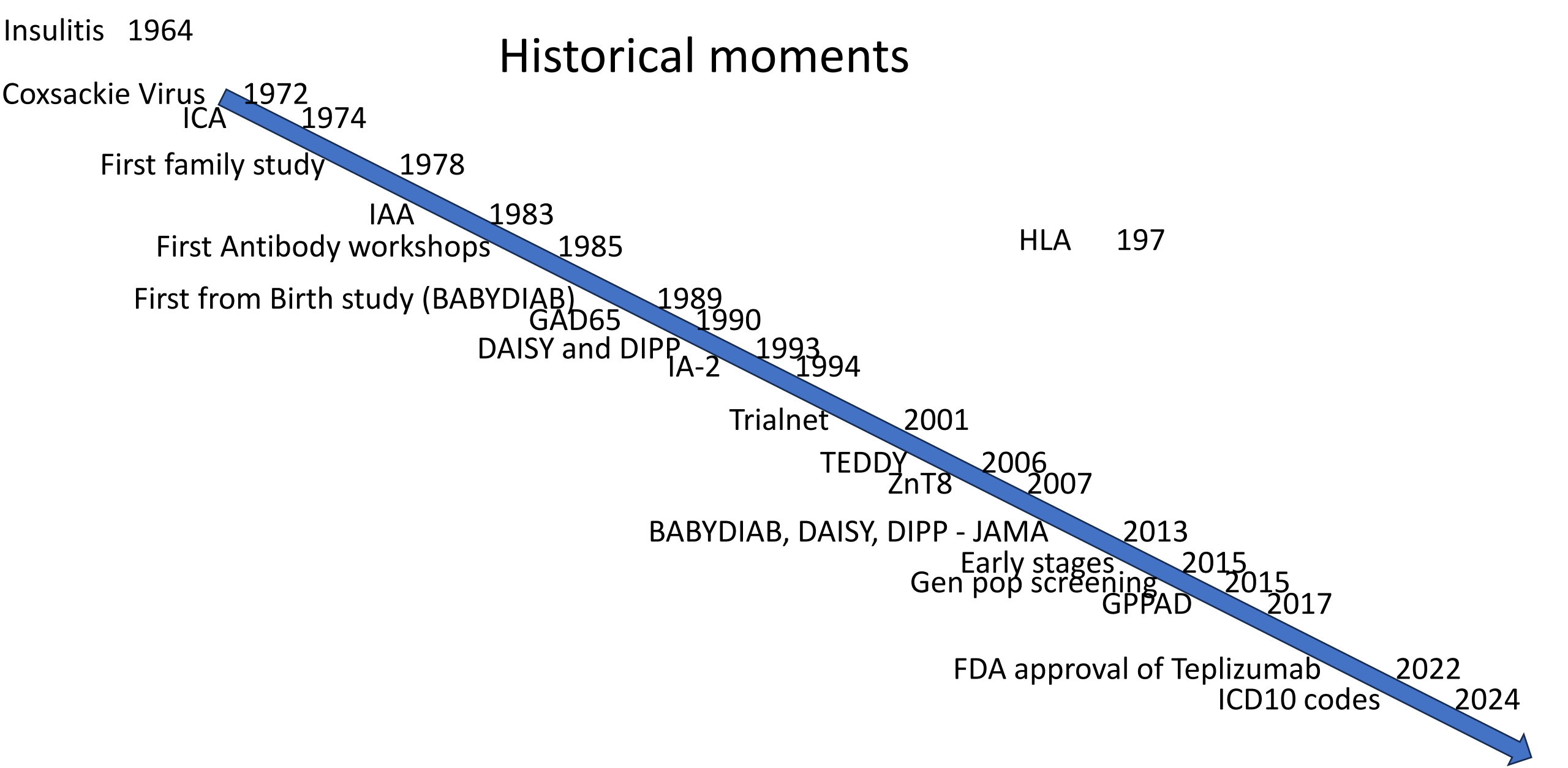
NATIONAL INSTITUTES OF HEALTH

Gesund. Leben. Bayern.

Bundesministerium für Bildung und Forschung

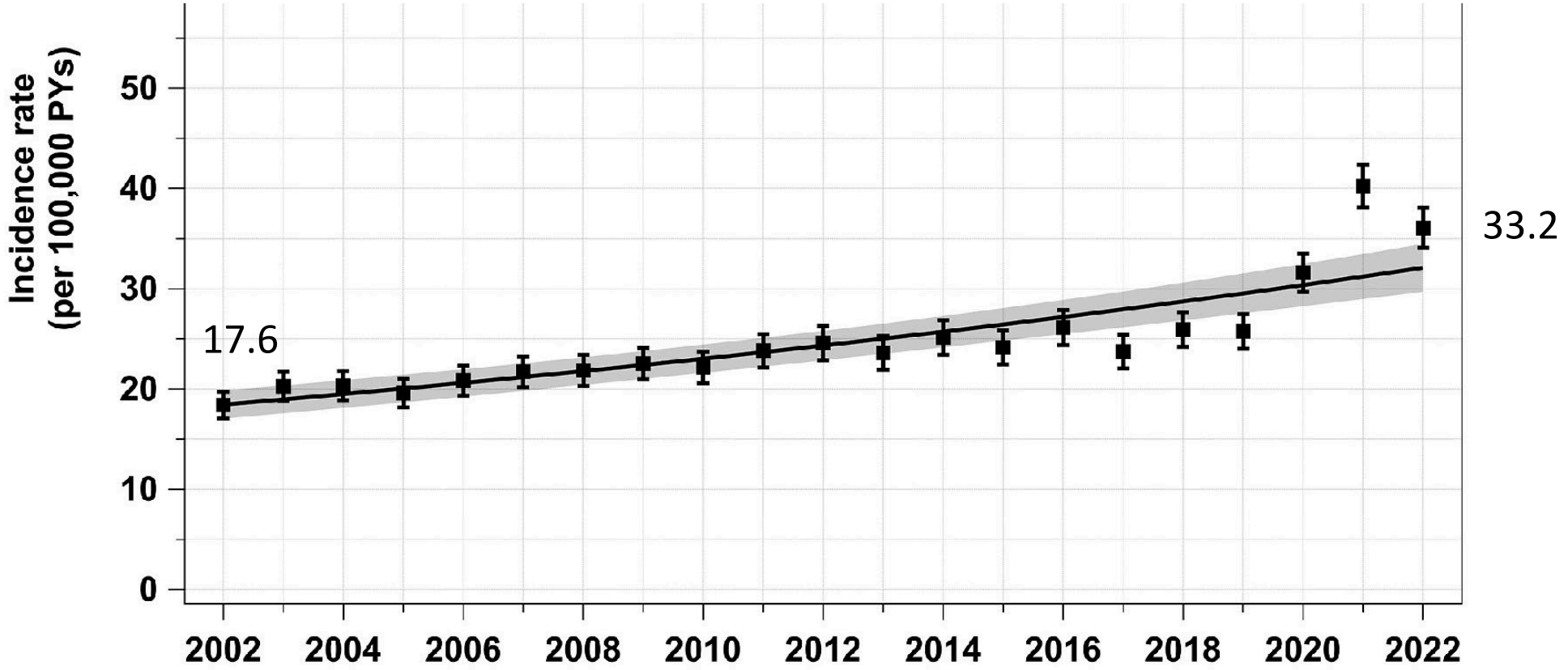
DZD
Deutsches Zentrum für Diabetesforschung

# Historical moments

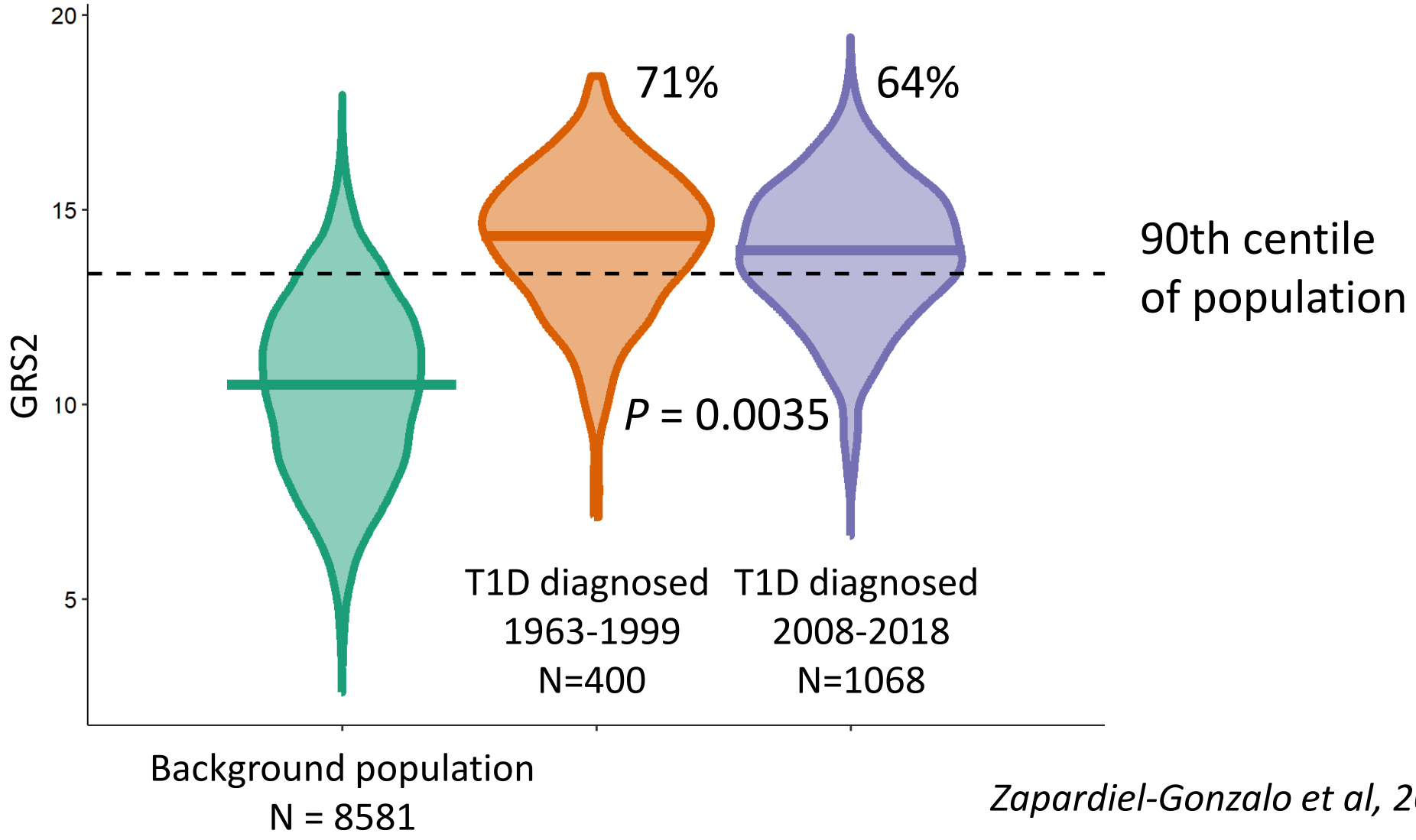


# Predicting the future from historical data

T1D incidence (0-19 yo) in Germany over 20 year period



# Genetic Architecture of T1D changes over time




*Zapardiel-Gonzalo et al, 2025*