GENETICS OF CHILDHOOD-ONSET DIABETES

Molecular Diagnostics Symposium 2021

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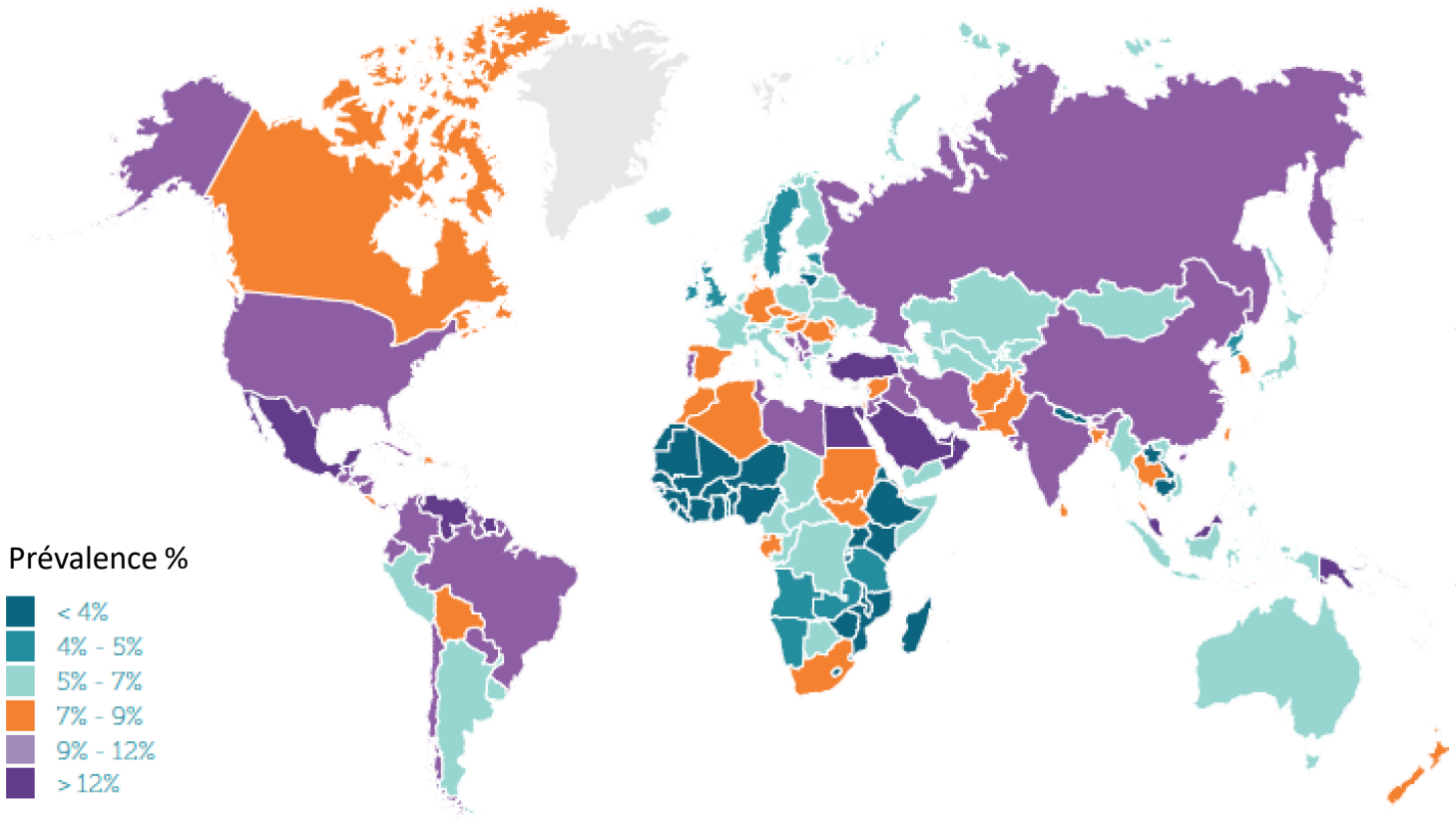
DIABETES

DEFINITIONS - PREVALENCE

Not a rare disease !

425 Millions of people worldwide with diabetes in 2017

625 Millions in 2045



International Federation of Diabetes 2017

Définitions

Diabetes

Glycemia ≥ 7mmol/l fasting

Glycémia 2h after meal ≥11.1mmol/l

HBA1c (glycated haemoglobin=[glucose]blood) ≥ 6.5%

Pre-Diabetes

Glycemia fasting ≥ 5.6-6.9 mmol/l,

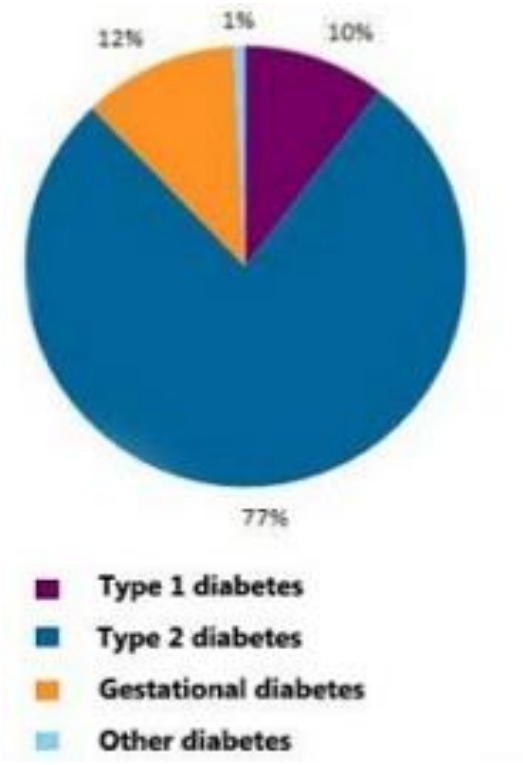
Glycémia 2h after meal at 7.8-11 mmol/l

HbA1c = 5.7-6.4%,

DIABETES
TYPES

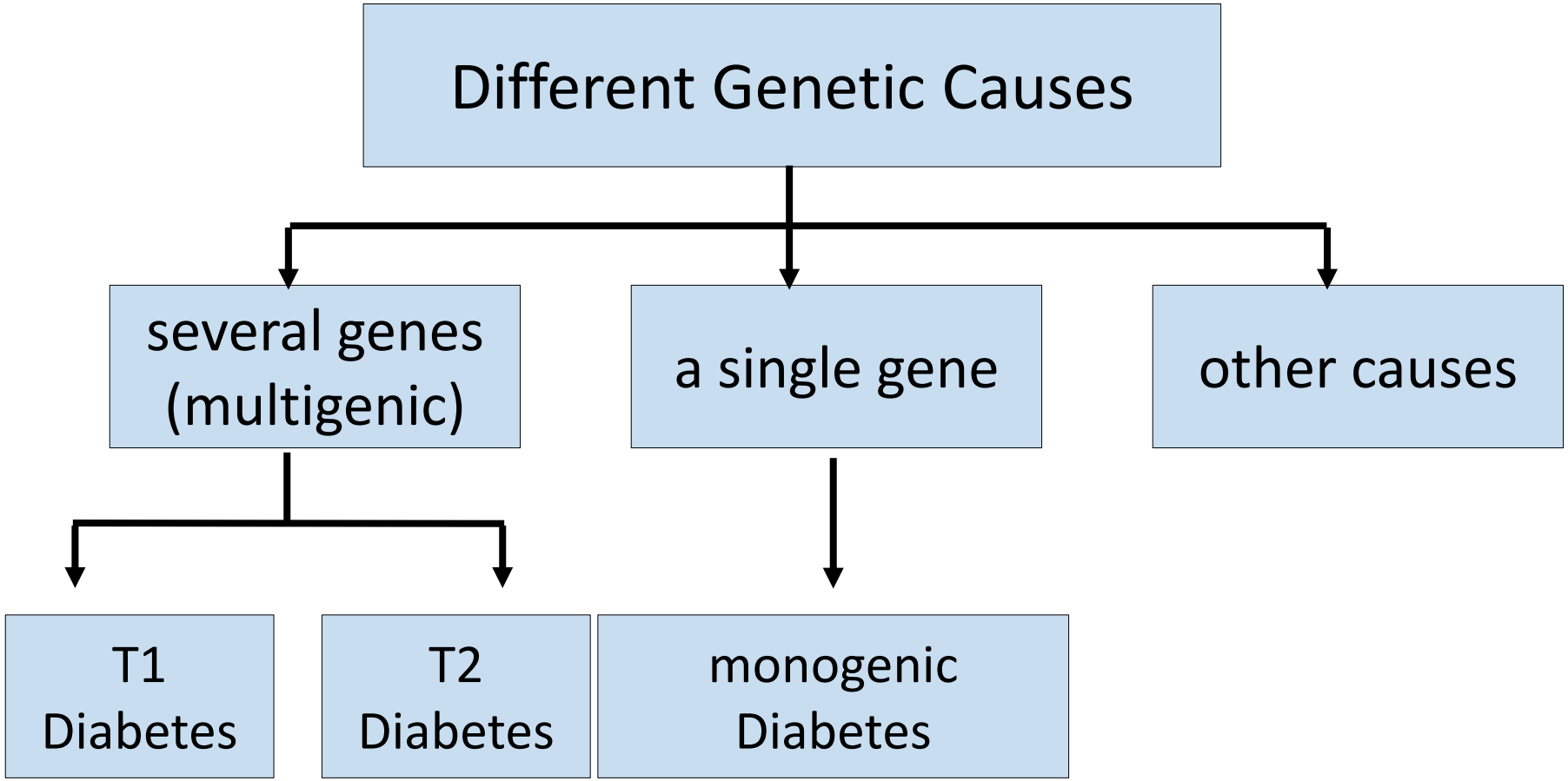
In general, a disease showing an abnormal elevated rate of glucose in blood.

1	**Type 1 (T1D)** *(insulin-dependent)*	• Destruction of beta cells • Absolute insulin deficiency
2	**Type 2 (T2D)** *(insulin-resistent)*	• Progressive deficit of adequate insulin secretion • Insulin resistance
3	**Gestational** *(2ème ou 3ème trimester)*	• Miscellaneous causes
4	**Specific diabetes of other origins**	• Monogenic diabetes syndromes (eg, neonatal diabetes, maturity onset diabetes of the young, MODY) • Diseases of the exocrine pancreas (e.g. Cystic Fibrosis) • Toxins or drugs (e.g. cyclosporine, glucocorticoids, HIV/AIDS, transplantation)



American Diabetes Association 2020

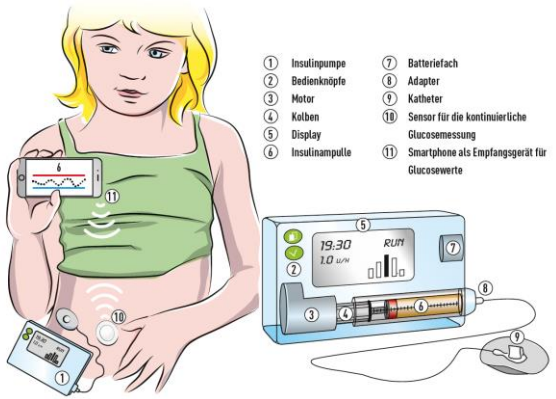
DIABETES AETIOLOGY

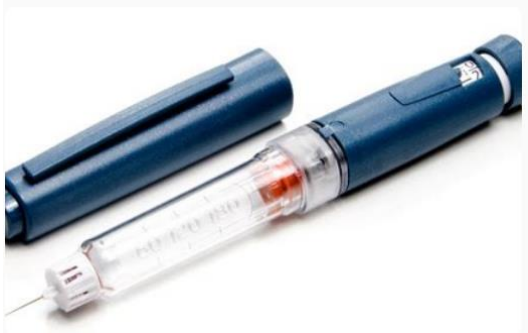


GENE DICTATES TREATMENT

«the right treatment in the right patient»

Type 1





Monogenic

- No therapy required

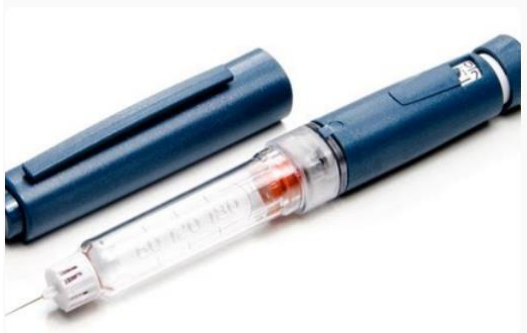
or

- Tablets

or

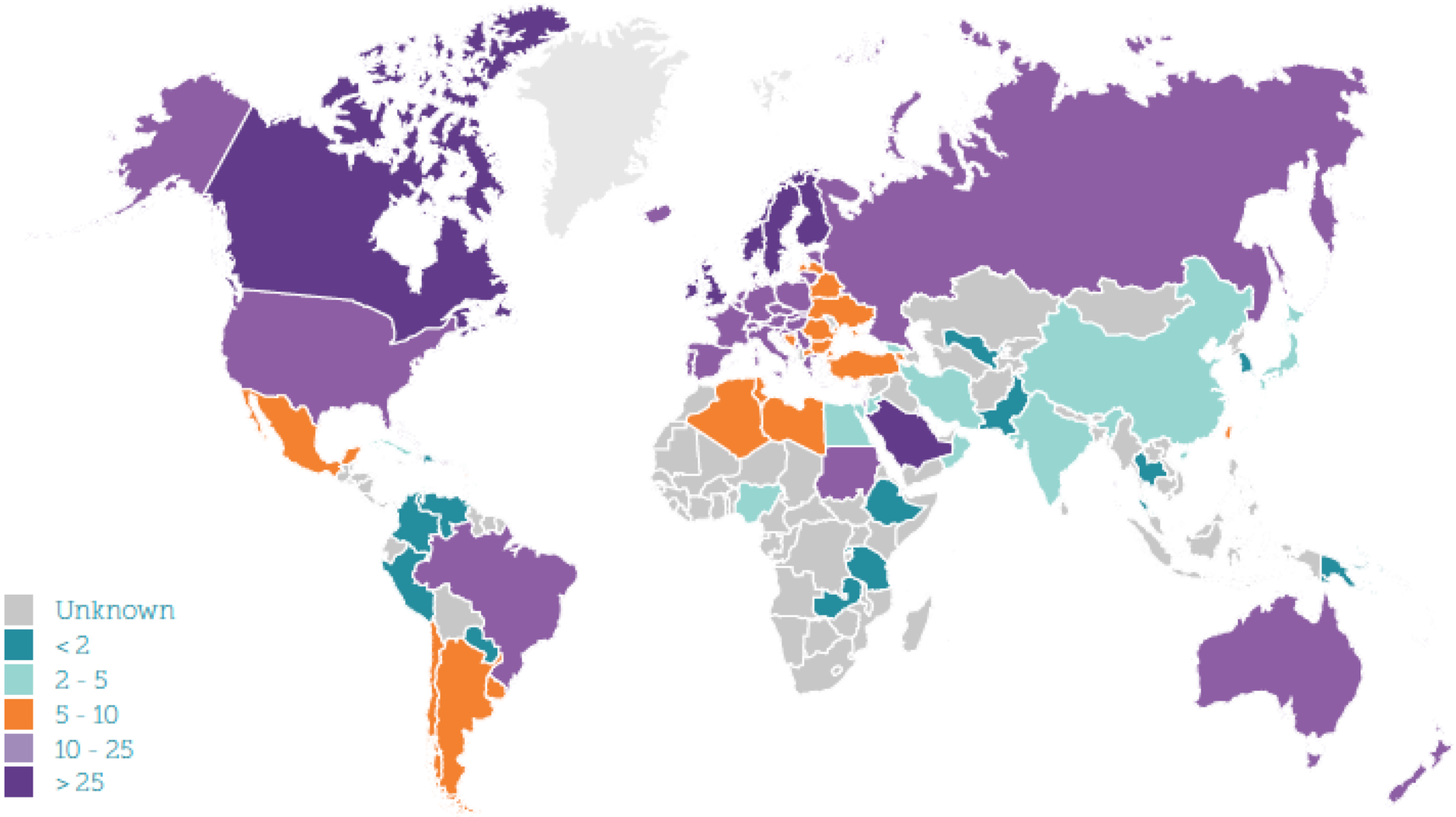
- Sometimes insulin

Type 2



Paediatrica, 27(1), 6-10. pdf

WORLDWIDE INCIDENCE OF TYPE 1 DIABETES

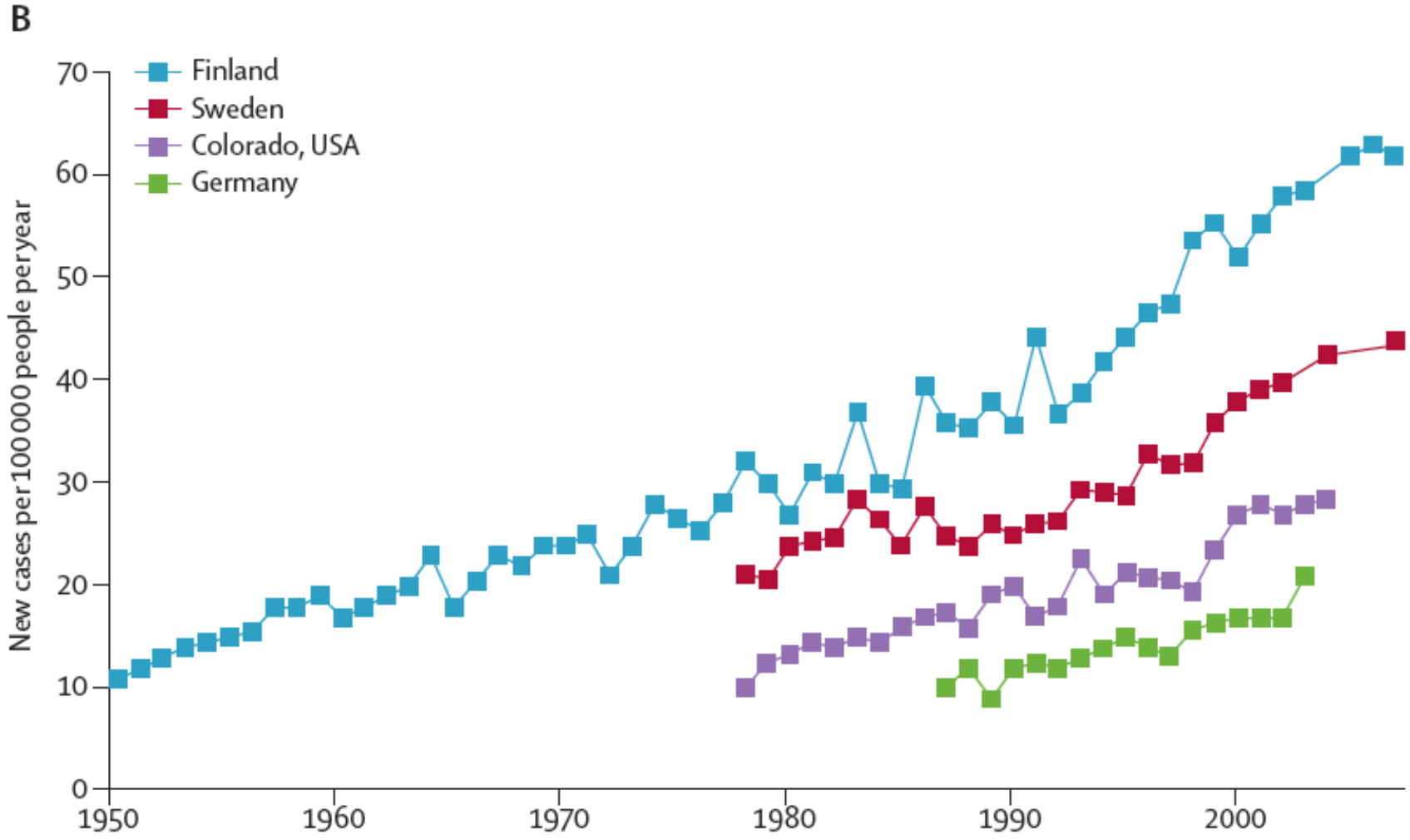


Incidence per 100’000 children/year

IDF 2015

EVOLUTION OF INCIDENCE OF TYPE 1 DIABETES

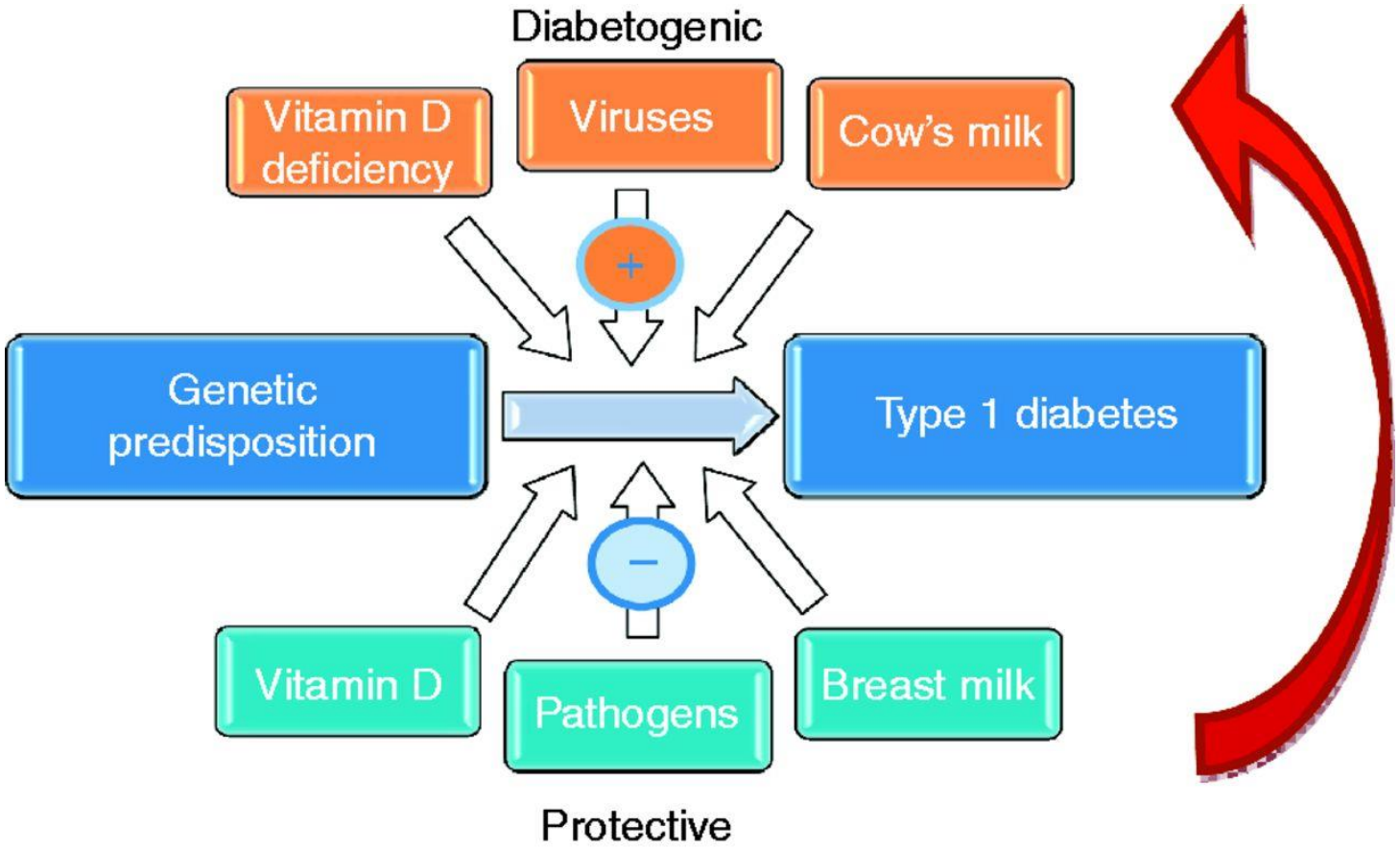
Constant increase of T1D
(since 1950)



Atkinson, The Lancet 2013

CAUSES OF TYPE 1 DIABETES

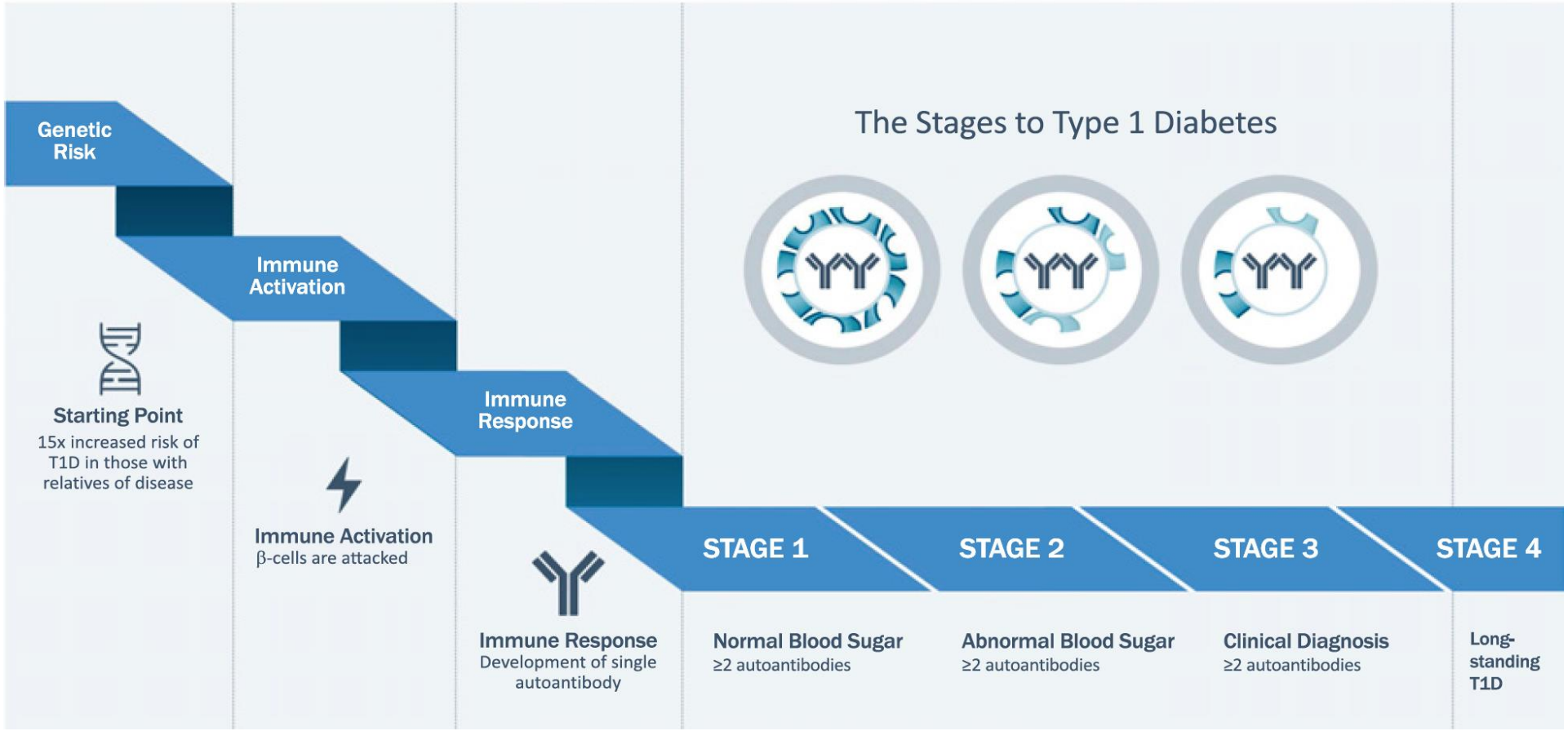
Increased incidence: Thought to be due to environmental factors ?
-hygiene, perhaps less exposure to infectious agents during childhood
-cow's milk: decrease breast feeding
-…



Egro 2013, Journal of Molecular Endocrinology 51, 1; 10.1530/JME-13-0067

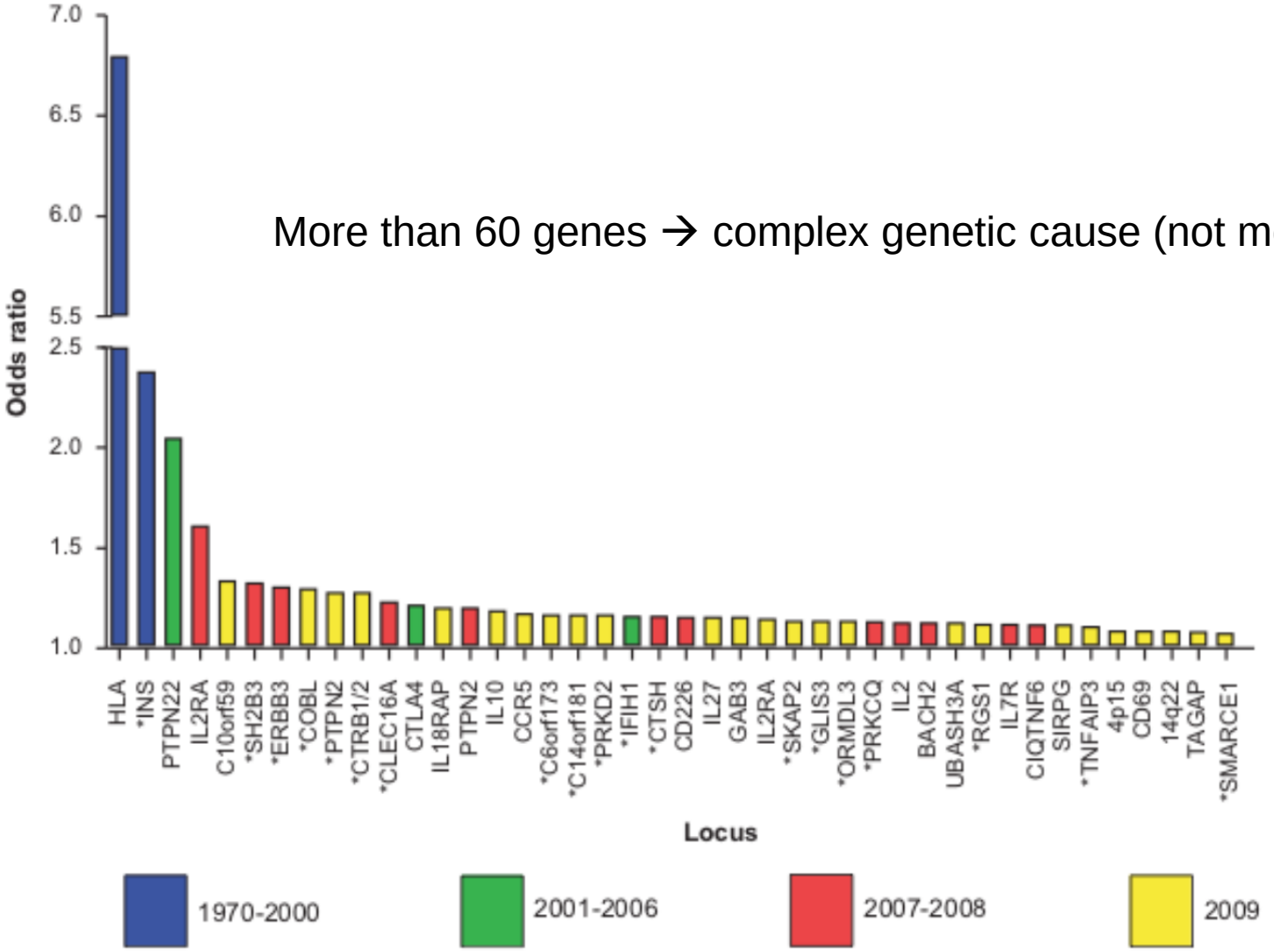
STAGES OF TYPE 1 DIABETES

- Study with people at high risk for diabetes (stage 2)



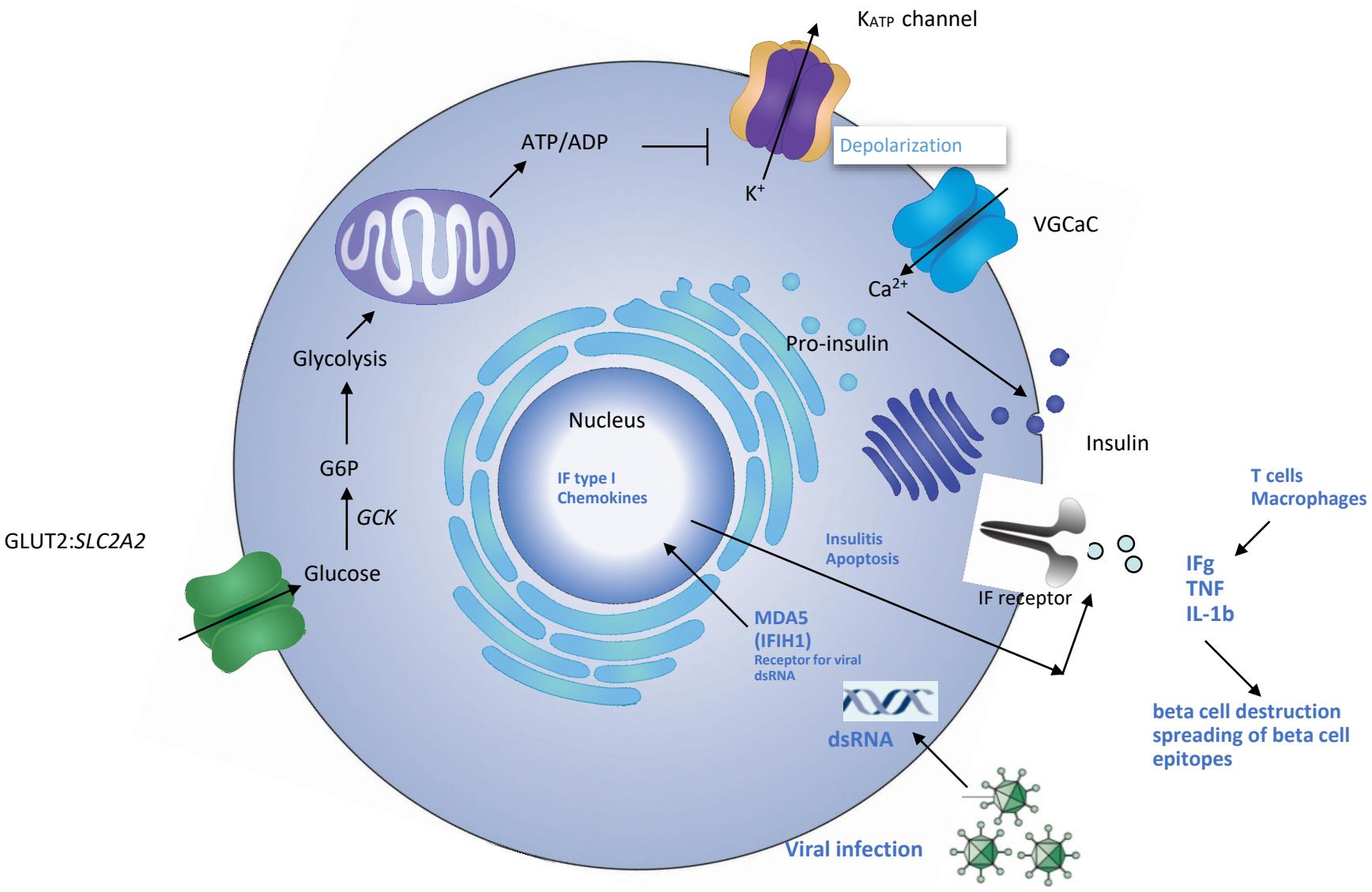
T1D PREDISPOSING GENES

More than 60 genes → complex genetic cause (not monogenic)



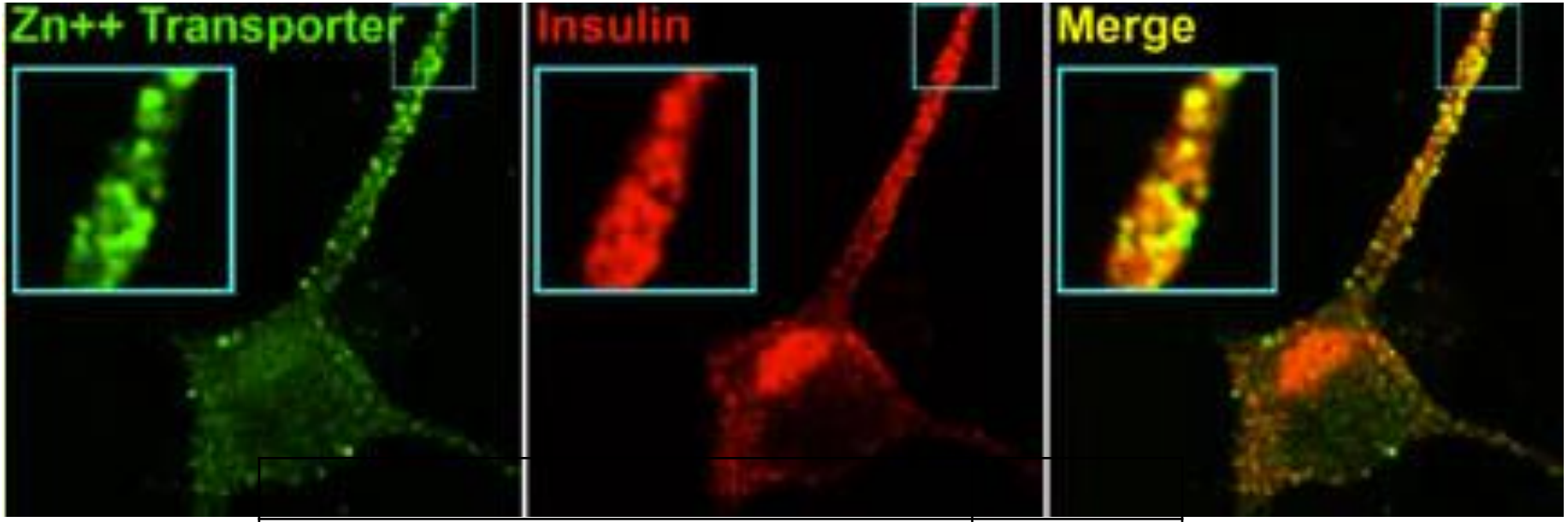
Pociot F et al., Diabetes 2010

VIRAL INFECTION HYPOTHESIS



De Beeck et al., Nat Rev Endocr 2016

AUTOIMMUNE ANTIBODIES: MARKERS LOSS

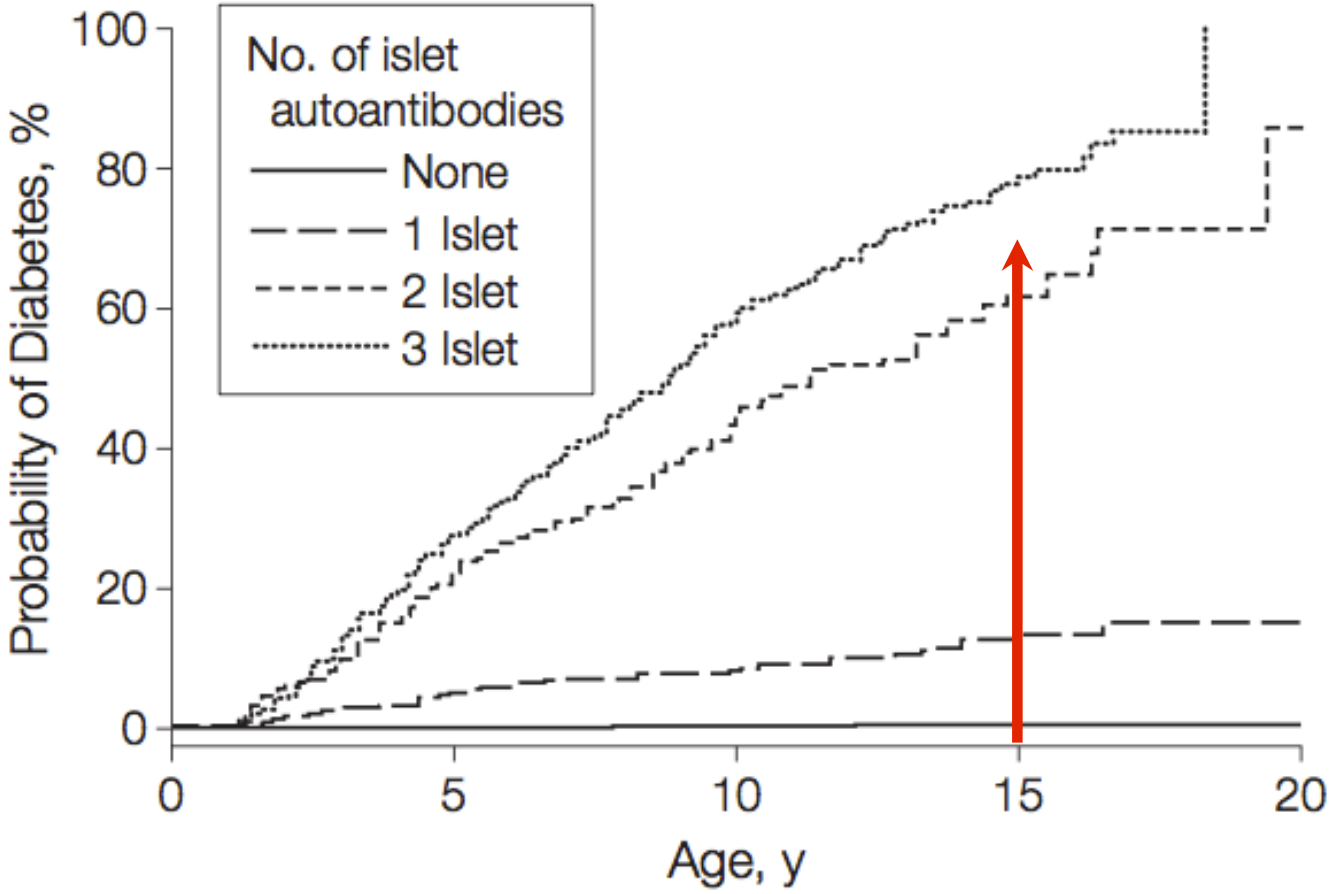


Anti-glutamate decarboxylase 65	GAD
Anti-IA2 (tyrosine phosphatase)	IA-2
Anti-insuline	IAA
Anti-îlots	ICA
Transporteur de Zinc	ZnT8

Achenbach et al.,Diabetologia 2009
Tsirogianni et al., Autoimmunity Reviews 2009
Solimena et al, EMBO 1996
Wenzlau et al., PNAS 2007

Courtesy of Antonio R. Lara-Lemus , M.D., Ph.D. and Peter Arvan, M.D., Ph.D., Department of Internal Medicine-Metabolism, Endocrinology & Diabetes, University of Michigan

RISK OF DIABETES - ANTIBODIES



Diabète

79.1% avec 3 AC

61.6% avec 2 AC

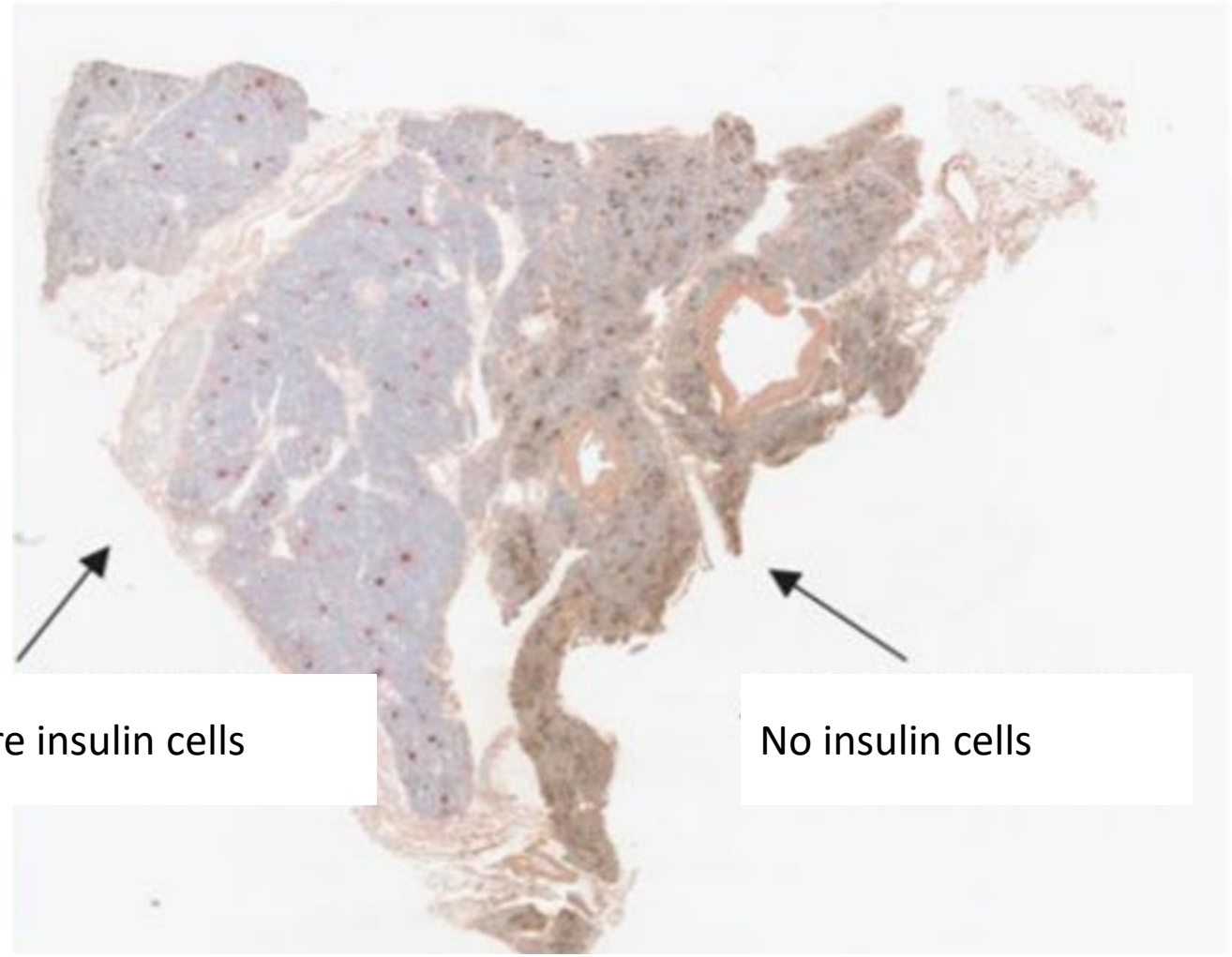
12.7% avec 1 AC

0.4% negative

A total of 13,377 children included, 92.1% neg, 7.9% pos
4% multiple antibodies
Among 1059 children pos, 428 children had diabetes.

Ziegler et al., JAMA 2013

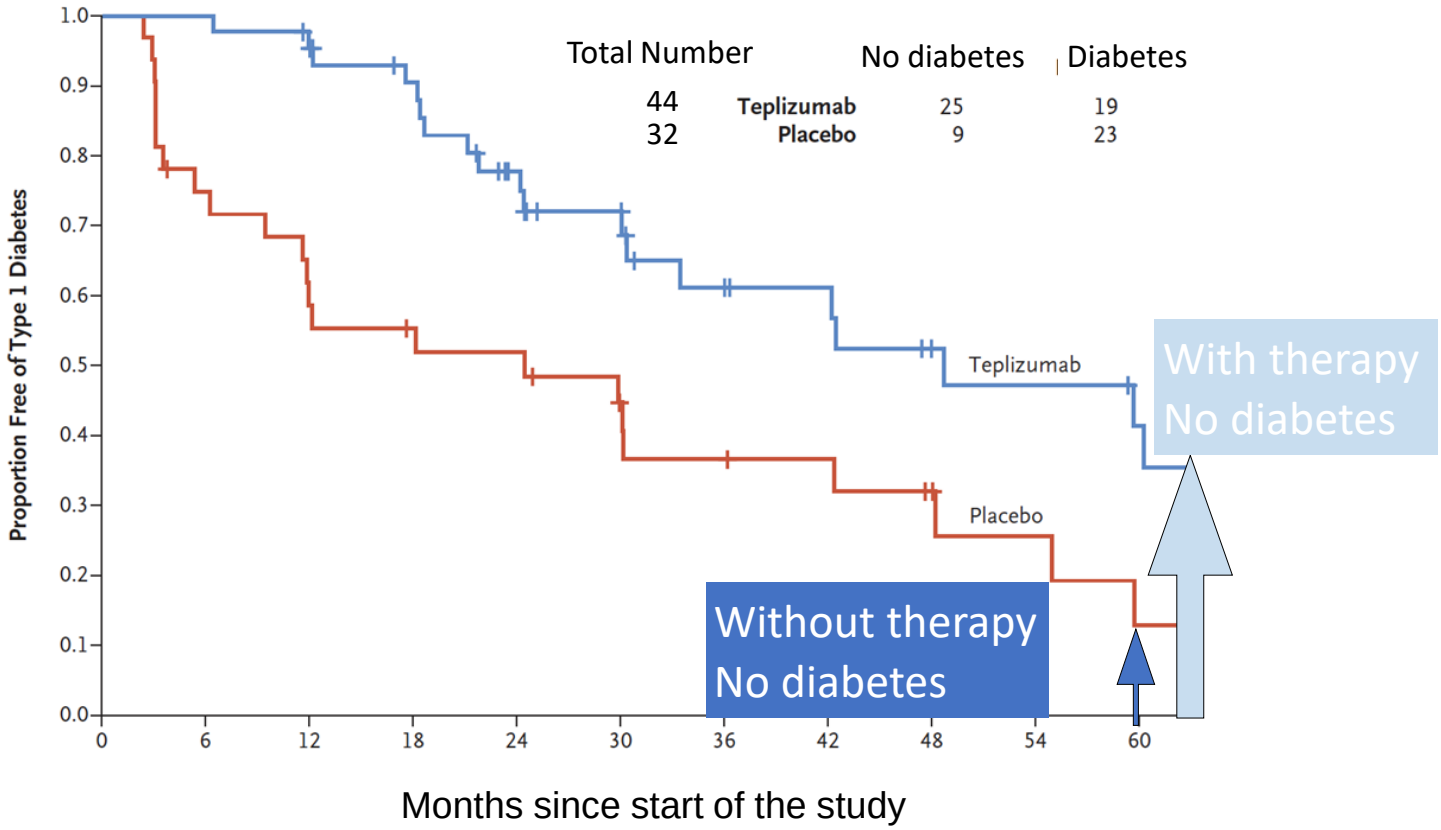
HISTOLOGICAL SECTION IN TYPE 1



JA Bluestone *et al.* *Nature* 2010

CAN TYPE 1 DIABETES BE PREVENTED?

- Study with people at high risk for diabetes (stage 2)
- 14-day therapy with teplizumab (AC anti-CD3 monoclonal)
- Immunomodulator: acts on inflammatory cells (CD8+ lymphocytes), which probably kill insulin-producing beta cells.



STUDY SUMMARY

- 14-day therapy with teplizumab (AC anti-CD3 monoclonal)

  →slows the onset of diabetes in high-risk individuals
- 2-year slowdown
- 43% of treated and 72% of untreated people developed diabetes.
- Treatment can be done in children and adults.
- One sub-group responds better than others
- → Precision medicine
- First study showing a slowing down of diabetes by immunotherapy

CONCLUSIONS

- Accurate diagnosis leads to accurate treatment
- Diagnosis with autoimmune antibodies
- Diagnosis by genetic analysis
- Measuring risk in family members of people with diabetes becomes possible
- Measuring the risk for all children will be possible in the future

MONOGENIC DIABETES
(MD)

- A heterogeneous group of disorders
- **Results from a defect in a single gene**
- Originally MD were classified in two groups depending on the age of diagnosis

Neonatal Diabetes Mellitus (NDM)	Maturity Onset Diabetes of the Young (MODY)

- Remains undiagnosed in probably more than 90% of patients
- Diagnosis often missed ← children diagnosed with T1D or T2D
- Switzerland: 350’000 diabetic individuals of whom 2% (≈ 7’000) are supposed to have MD
- **Definitive diagnosis by genetic analysis**
- **Genetic defect dictates the treatment = <u>precision medicine</u>**

Bopp M. Diabetes Care 2011
Pihoker C. JCEM 2013

NEONATAL DIABETES MELLITUS
(NDM)

Diagnosed within the first 6 months of life

- Prevalence is 1/90 000 to 1/160 000 live births
- Permanent (PNDM) and transient (TNDM) forms

22 Known NDM genes

- >50% of patients harbor activating mutations of
  - ✓ *KCNJ11*
  - ✓ *ABCC8* genes
    (coding for the K-ATP channel)

- 90% of patients respond to high doses of Sulfonylurea

✓ syndromic features
- pancreas agenesis
- delayed development
- epilepsy
- hearing loss
- cardiopathy

22 NDM Genes	
PLAGL1	***KCNJ11***
ZPF57	***ABCC8***
PDX1	***GCK***
PTF1A	***SLC2A2***
HNF1B	***SLC19A2***
RFX6	***INS***
GATA6	***FOXP3***
GLIS3	***EIF2AK3***
NEUROG3	***IER3IP1***
NEUROD1	***WFS1***
PAX6	***STAT3***

Rubio Cabezas O. Horm Res Paediatr 2013
Pearson E.R. NEJM 2006

MATURITY ONSET DIABETES OF THE YOUNG
(MODY)

MODY are diabetes **diagnosed before 25 years**

- -Autosomal dominant inheritance
- -Insulin independence
  (although insulin may be required for optimal control)

Usually absence of

- -features of insulin resistance
- -β cell autoimmunity
  (< 1 % positive GAD or IA2 Auto Ab)

Patients with *HNF1A* or *HNF4A* mutations

→ sensitive to low doses of Sulfonylurea

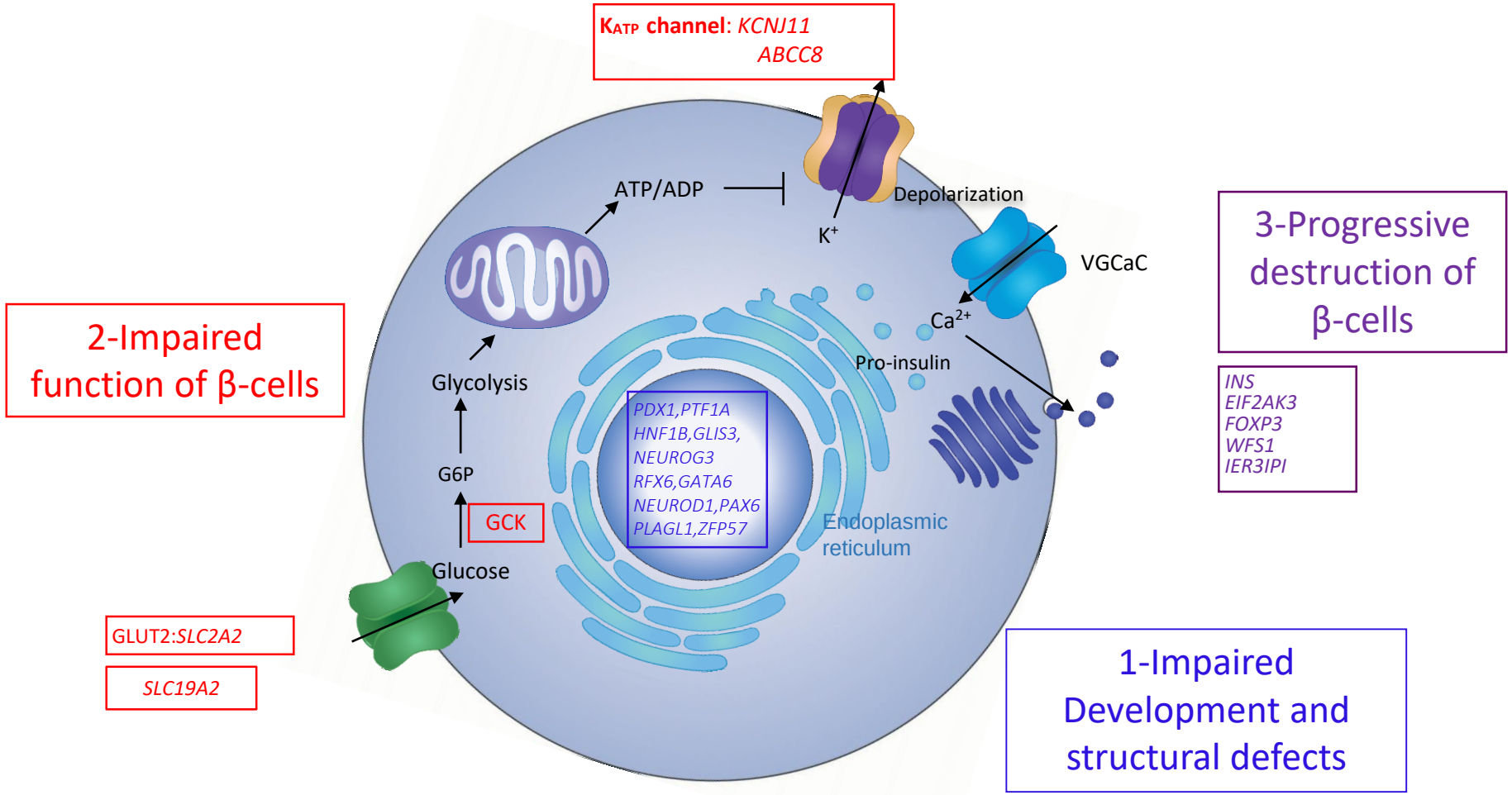
Patients with *GCK* mutations

→ do not require pharmacological treatment

13 MODY Genes	
HNF4A	**MODY 1**
GCK	**MODY 2**
HNF1A	**MODY 3**
PDX1	**MODY 4**
HNF1B	**MODY 5**
NEUROD1	**MODY 6**
KLF11	**MODY 7**
CEL	**MODY 8**
PAX4	**MODY 9**
INS	**MODY 10**
BLK	**MODY 11**
ABCC8	**MODY 12**
KCNJ11	**MODY 13**

Ellard, S. Diabetologia 2013
Mc Donald, T.J. Diab Med 2011

PANCREATIC BETA CELL



Stekelenburg & Schwitzgebel, Endocrine Development, 2016

GENETIC ANALYSIS IN PATIENTS WITH MONOGENIC DIABETES

- ✓ Probably more than 90% of patients with suspicion of MD remains **undiagnosed**
- ✓ Traditional testing for MD focuses only on one or a few genes depending on the clinical picture and the patient's phenotype

Aim

- ➢ Determining the respective prevalence of known MD genes
- ➢ Identify potential novel genes causing MD

Method

- ➢ Identify gene variants causing MD using next-generation sequencing (NGS)
- ➢ Selection of 323 potential diabetes genes
- ➢ Design of a capture assay for coding and splicing regions of these genes (HaloPlex technology)

PATIENT INCLUSION CRITERIA

Neonatal Diabetes

Suspicion of MODY

Presumed T1DM
No AB

Presumed T2DM
Diagnosis <45 yrs
Without metabolic features

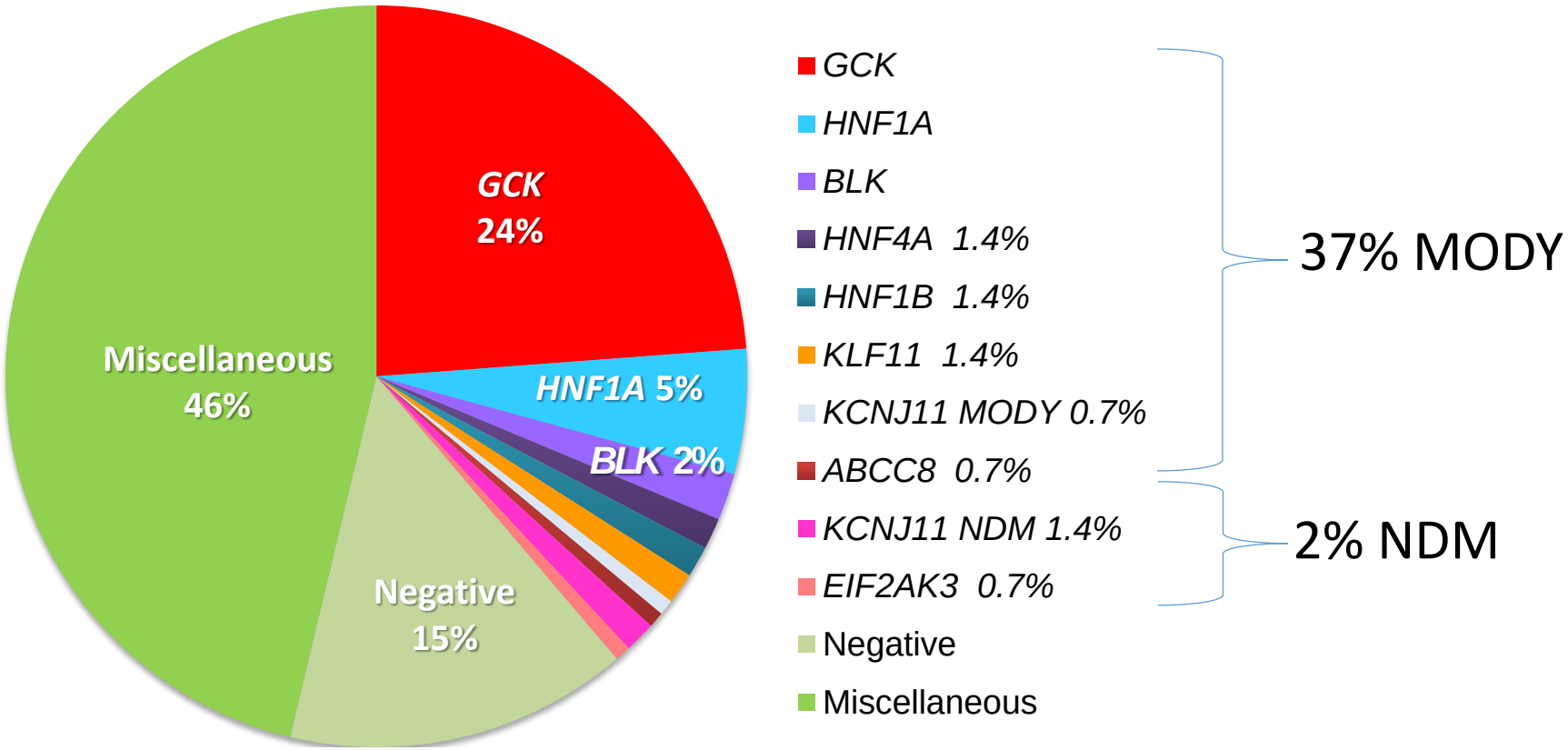
Syndromic diabetes with extra-pancreatic features such as:
Hearing loss
Developmental delay
Epilepsy
Cardiopathy

147 diabetic patients

Age at diagnosis	Diabetes < 6 months	Diabetes ≥ 6m - 18 y	Diabetes ≥ 18 years
Number of patients	4	44	99
Mean age at diagnosis	1.5 month	130 months (10.8 years)	423 months (35.3 years)
Sexe	25% female	59% female	59% female
Mean HbA1c at diagnosis	3.4%	8.9%	8.1%
Mean Glycemia at diagnosis	37.8 mmo/l	14.3 mmol/l	10.2 mmol/l

MONOGENIC KNOWN DIABETES GENE PREVALENCE IN CH

147 CH patients



8 novel *GCK* mutations, 2 novel *HNF1A* mutations, 1 novel *KLF11* mutation

NEONATAL RESULTS

4 patients with Neonatal diabetes

Age at diagnosis	Diabetes < 6 months
Number of patients	4
Mean age at diagnosis	1.5 months
Genes	*KCNJ11/HNF4A, EIF2AK3, IL18RAP*

1 patient with *KCNJ11* PNDM

1 patient with a digenic *KCNJ11/HNF4A* TNDM

1 patient with a Wolcott Rallison syndrome

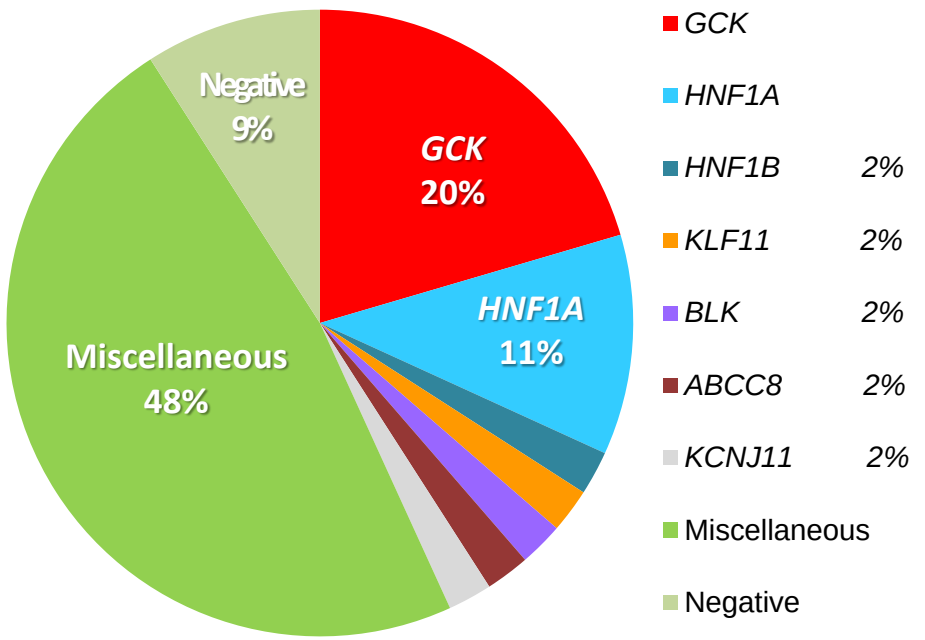
1 patient with an neonatal T1DM

Anderson de la Llana S.
Horm Res Paediatr 2015

PEDIATRIC RESULTS

44 paediatric patients with clinical suspicion of monogenic diabetes

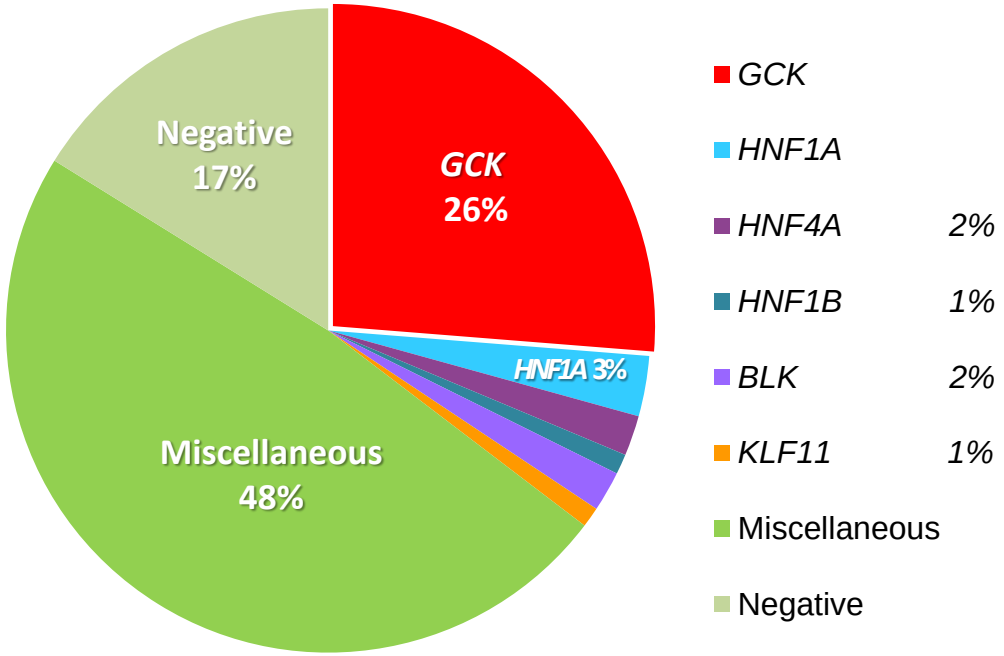
Age at diagnosis	**Diabetes ≥ 6-months to 18 years**
Number of patients	44
Mean age at diagnosis	130 months (10.8 years)
Genes	*GCK* *HNF1A* *HNF1B* *KLF11* *BLK* *ABCC8* *KCNJ11*



ADULT RESULTS

99 adult patients with clinical suspicion of monogenic diabetes

Age at diagnosis	Diabetes ≥ 18 years
Number of patients	99
Mean age at diagnosis	423 months (35.3 years)
Genes	*GCK* *HNF1A* *HNF4A* *HNF1B* *KLF11* *BLK*



DEVELOPMENT OF A GENETIC DIAGNOSTIC TOOL

Following this study we launched a **diagnostic tool for monogenic diabetes** genes

- **Gene targeted NGS Based**

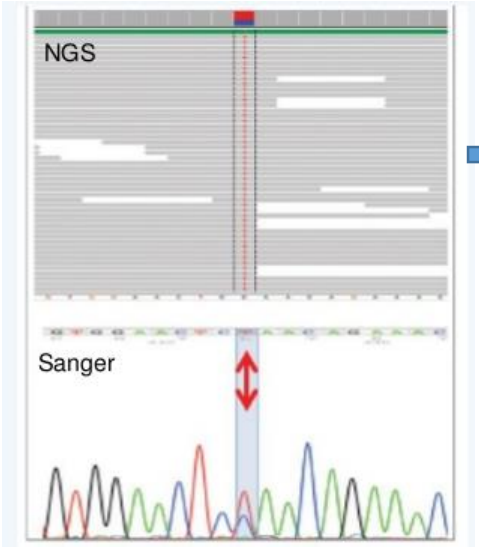
- Advantages:
  - ✓ More comprehensive: allows the analyzis of several/many genes in one step
    (instead of the traditional « one-by-one » successive serial test
  - ✓ Cheaper
    (compared to more than one gene analysis by traditional methods)
  - ✓ Very suitable for pathologies with high genetic heterogeneity (several to many genes involved)
  - ✓ Flexibility in choice of genes at the bioinformatic analysis stage
    (all sequenced genes, or a subpanel of few genes analyzed)

- Disadvantages:
  - TAT are longer than for a single gene analysis by classical Sanger method
  - Some types of variants (still) not yet detected (complex deletions/duplications, repeats, …)

DNA SEQUENCING SCALE

Years 1990-2000

Sequencing few parts of genes
(1 individual at a time)

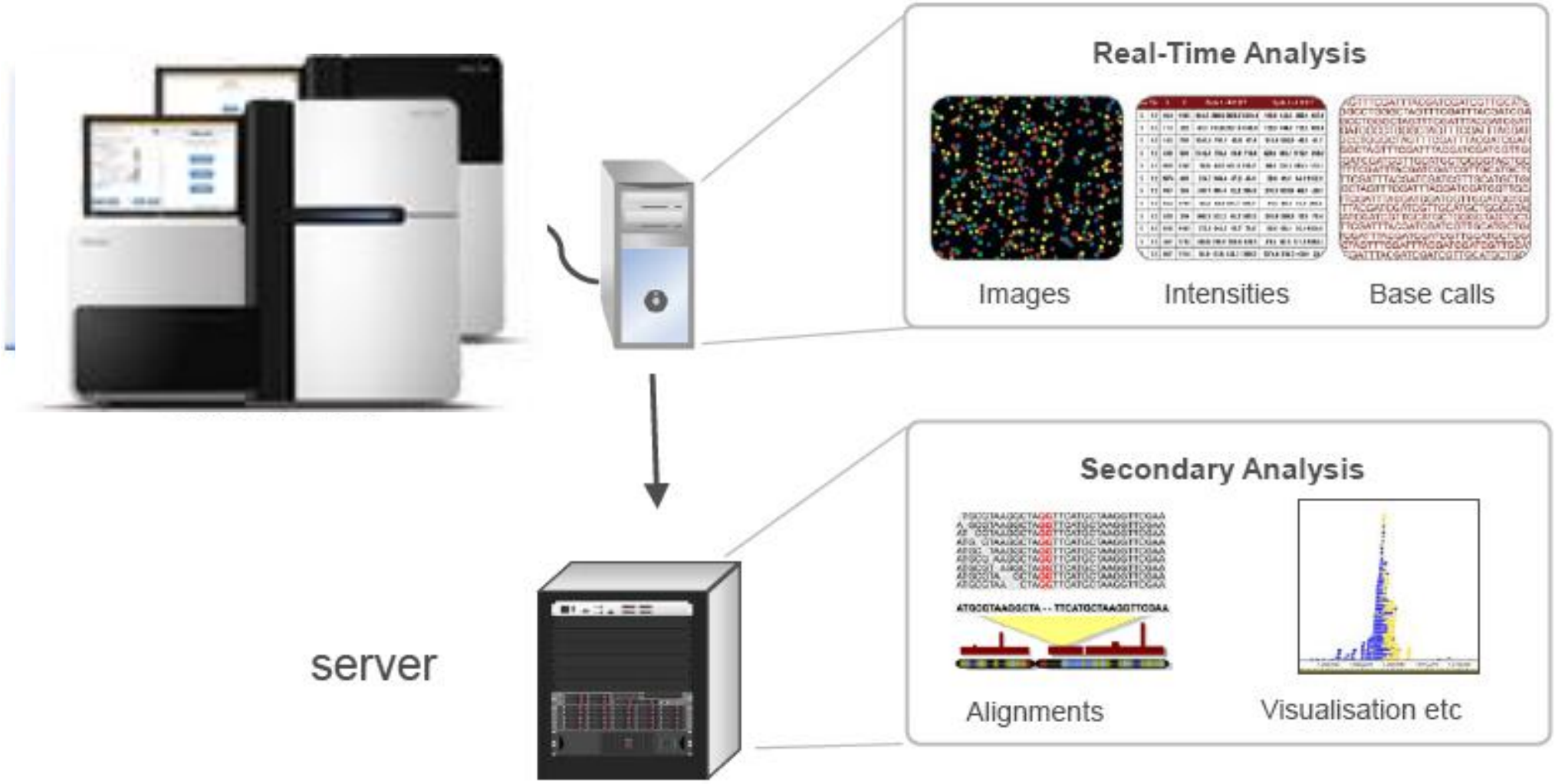


from year 2005

Sequencing of dozen to thousands genes
(in several individuals at a time)

	1st Sanger Sequencing	2nd "Next Generation" Sequencing	3rd « Single molecule sequencing »
Information Capacity:	100's of reads per experiment	10,000,000's of reads per experiment	1,000,000,000's of reads per experiment
Cost per Human Genome:	$1,000,000's	1'000-10'000	1'000s

NGS DATA WORKFLOW



From M. Guipponi

NGS ON GENE PANEL

It is required **to trap the coding portions of patient DNA to be analyzed**,……
…….if one do not want to sequence entire genome (~more than 98% of the data to discard).
→ Need a way **to capture the portions of DNA of interest**

Make library of patient DNA
(DNA fragmented in small pieces)

↓

Capturing of the pieces of coding part of DNA
(discarding the unwanted pieces: non coding)

↓

High Throughput Sequencing

↓

Bioinformatic Analysis
(alignement reads to reference genome, identification of variants)

↓

Gene panel variants analysis and clinical classification
(frequency, biological filtering and clinical role assessment)

↓

Complementary analyses
Selected variant confirmation + eventual complementary tests

NGS DIAGNOSTIC TOOL GENE PANEL
v.1.0 DESIGN

- **43 genes + non coding regions chosen from**
  - Monogenic diabetes genes: MODYs (1-13)
  - other neonatal, early or late onset monogenic Diabetes (14-43)

Gene name	RefSeq accession number (Genbank)	Chromos. Location	Theoretical coverage (%)	Missing nucleotides (n)	non covered (%)
HNF4A	NM_000457	Chr.20	100	-	-
GCK	NM_000162	Chr.7	100	-	-
HNF1A	NM_000545	Chr.12	100	-	-
PDX1	NM_000209	Chr.13	100	-	-
HNF1B	NM_000458	Chr.17	100	-	-
NEUROD1	NM_002500	Chr.2	100	-	-
KLF11	NM_003597	Chr.2	100	-	-
CEL	NM_001807	Chr.9	100	-	-
PAX4	NM_006193	Chr.7	100	-	-
INS	NM_000207	Chr.11	100	-	-
BLK	NM_001715	Chr.8	100	-	-
ABCC8	NM_000352	Chr.11	100	-	-
KCNJ11	NM_000525	Chr.11	100	-	-
SLC19A2	NM_006996	Chr.1	100	-	-
DNAJC3	NM_006260	Chr.13	100	-	-
PLAGL1	NM_001080954	Chr.6	100	-	-
GATA6	NM_005257	Chr.18	100	-	-
GATA4	NM_002052	Chr.8	100	-	-
SLC2A2	NM_000340	Chr.3	100	-	-
NKX2-2	NM_002509	Chr.20	100	-	-
NEUROG3	NM_020999	Chr.10	100	-	-
GLIS3	NM_152629	Chr.9	99.7	9	0.30%

Gene name	RefSeq accession number (Genbank)	Chromos. Location	Theoretical coverage (%)	Missing nucleotides (n)	non covered (%)
RFX6	NM_173560	Chr.6	100	-	-
MNX1	NM_005515	Chr.7	100	-	-
EIF2AK3	NM_004836	Chr.2	100	-	-
WFS1	NM_006005	Chr.4	100	-	-
IER3IP1	NM_016097	Chr.18	100	-	-
PAX6	NM_000280	Chr.11	100	-	-
FOXP3	NM_014009	Chr.X	100	-	-
STAT3	NM_139276	Chr.17	100	-	-
PCBD1	NM_000281	Chr.10	100	-	-
SIRT1	NM_012238	Chr.10	100	-	-
LRBA	NM_001199282	Chr.4	99.98	2	0.02%
ZPF57	NM_001109809	Chr.6	100	-	-
PTF1A enhancer	hg19	Chr.10	96.6	25	3.40%
INS intron	hg19	Chr.11	100	-	-
PPP1R15B	NM_032833	Chr.1	100	-	-
TMRT10A	NM_152292	Chr.4	100	-	-
KMT2D	NM_003482	Chr.12	98.87	~200	1.13%
KDM6A	NM_021140	Chr.X	100	-	-
RAP1A	NM_001010935	Chr.1	100	-	-
RAP1B	NM_015646	Chr.12	100	-	-
CISD2	NM_001008388	Chr.4	100	-	-
PTF1A	NM_178161	Chr.10	100	-	-

GENE SUBPANELS ANALYZIS

- **The bioinformatic analyzis of NGS raw data produced can be restricted to a subpanel**
  - MODYs (1-13), or Some few MODY genes, or other neonatal monogenic Diabetes genes, or….
  - **Raw data a conserved** by laboratory for years: a **further second bioinformatic analysis** (other subpanel) can be performed
- Reminder: as required by Federal Office of Public Health (OFSP/BAG/UFSP), any request of an analysis of more than 10 genes can be made only by a medical genetics FMH title holder
- We still carry analysis on single gene base, by Sanger (traditional sequencing)

HNF4A
GCK
HNF1A
PDX1
HNF1B
NEUROD1
KLF11
CEL
PAX4
INS
BLK
ABCC8
KCNJ11

MODY 1-13

HNF4A
GCK
HNF1A
PDX1
HNF1B
NEUROD1
KLF11
CEL
PAX4
INS
BLK
ABCC8
KCNJ11
SLC19A2
DNAJC3
PLAGL1
GATA6
GATA4
SLC2A2
NKX2-2
NEUROG3
GLIS3

All MODYs

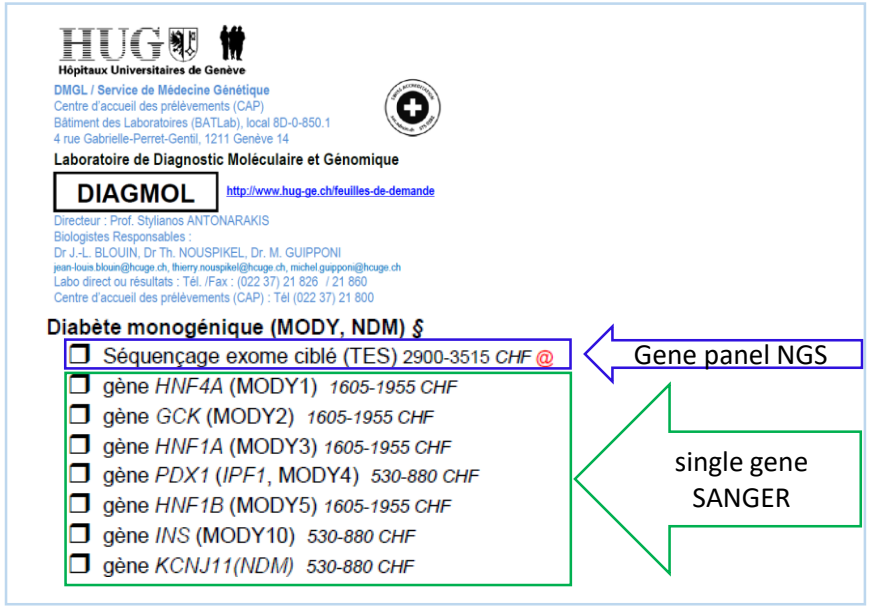
RFX6
MNX1
EIF2AK3
WFS1
IER3IP1
PAX6
FOXP3
STAT3
PCBD1
SIRT1
LRBA
ZPF57
PTF1A enhancer
INS intron
PPP1R15B
TMRT10A
KMT2D
KDM6A
RAP1A
RAP1B
CISD2
PTF1A

NDM

?
?
?
?

YOUR CHOICE

Request of analysis to laboratory


HUG
Hôpitaux Universitaires de Genève
DMGL / Service de Médecine Génétique
Centre d'accueil des prélèvements (CAP)
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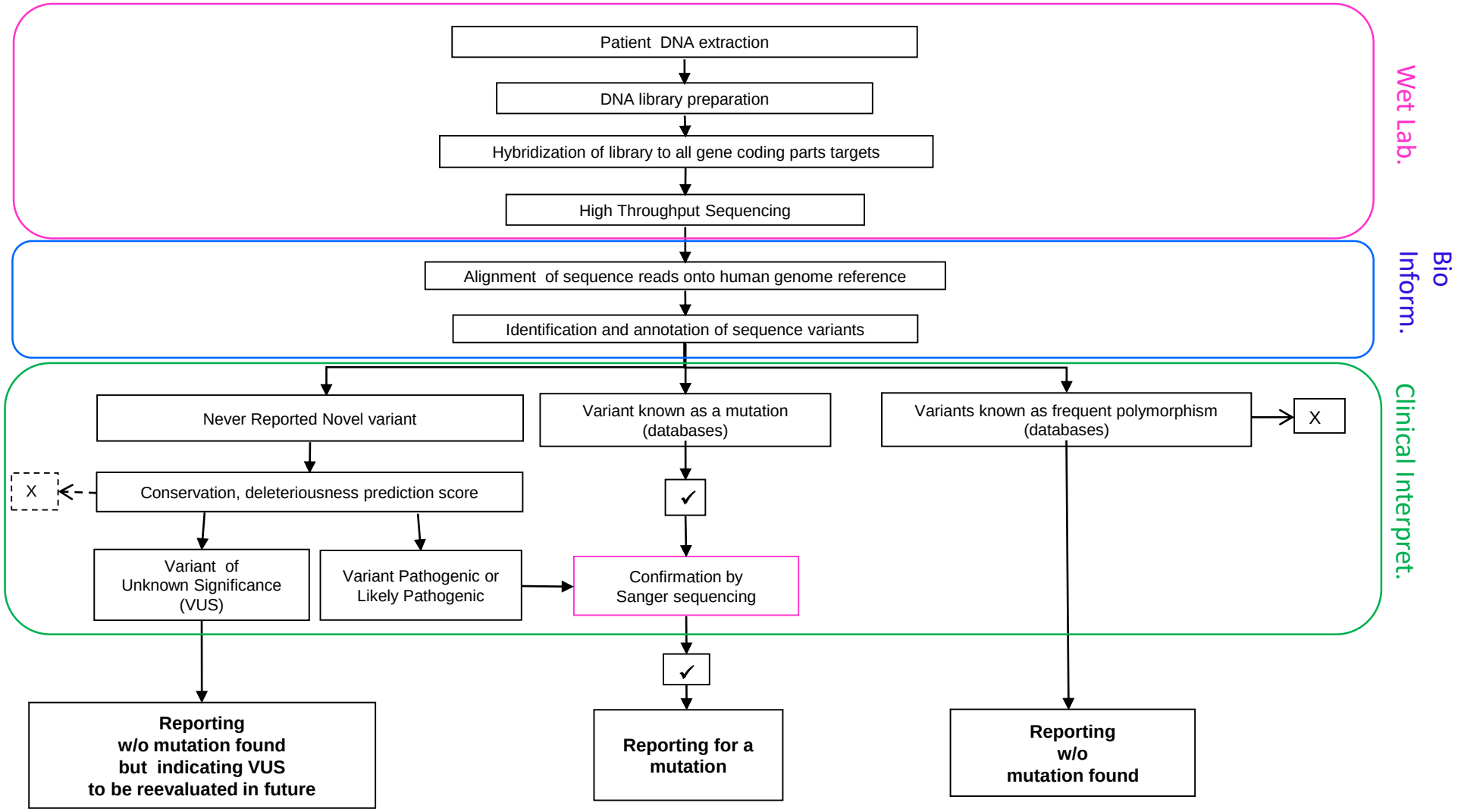
Diabète monogénique (MODY, NDM) §
❒ Séquençage exome ciblé (TES) 2900-3515 CHF @
❒ gène HNF4A (MODY1) 1605-1955 CHF
❒ gène GCK (MODY2) 1605-1955 CHF
❒ gène HNF1A (MODY3) 1605-1955 CHF
❒ gène PDX1 (IPF1, MODY4) 530-880 CHF
❒ gène HNF1B (MODY5) 1605-1955 CHF
❒ gène INS (MODY10) 530-880 CHF
❒ gène KCNJ11(NDM) 530-880 CHF

NGS FLOWCHART



NGS SEQUENCE READ TILING

ALIGNMENT OF SEQUENCE TO GENOME REFERENCE : DETECTION OF NUCLEOTIDE VARIANTS

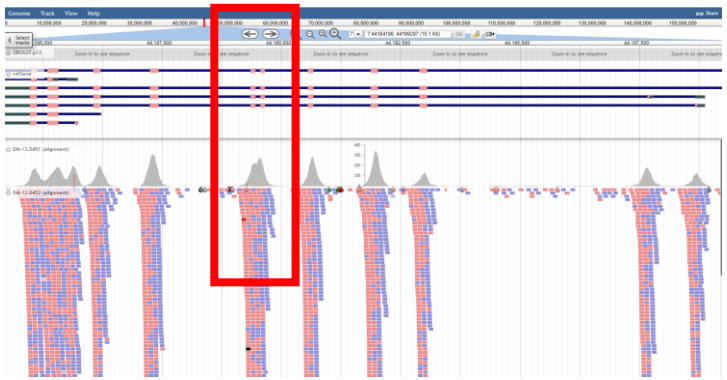


Fig. A

Example of gene *GCK*

Fig.A: *sequencing coverage in short-reads for entire gene.*
-Pink color boxes (on the blue bars) show respective location of the exons (each of the blue bars show distinct transcripts of the gene
-Each «grey peaks» depicts distribution of coverage depth in exon and close vicinity

Fig.B: *zoom of the red squared section of fig.A*
Below the grey area (enlarge grey peaks) are the distribution of forward (red) and reward (blue) reads of sequences

Fig. B

HIGH THROUGHPUT SEQUENCING

Identify thousands of variant (exome) / millions of variants (genome) per individual

That is necessary to class, filter, then to interpret in order to sort out the causative variant

***Fig.A**: example of files of sequence reads*
Each line is a read followed by sequence quality)

***Fig.B**: example of aligned sequence reads*
Each line is a read
Each letter A,C,G,T or N depicts a variant or a non assigned call
Here, only the « G » and « A » are true calls (not errors)
All the other dispersed letters are sequencing errors

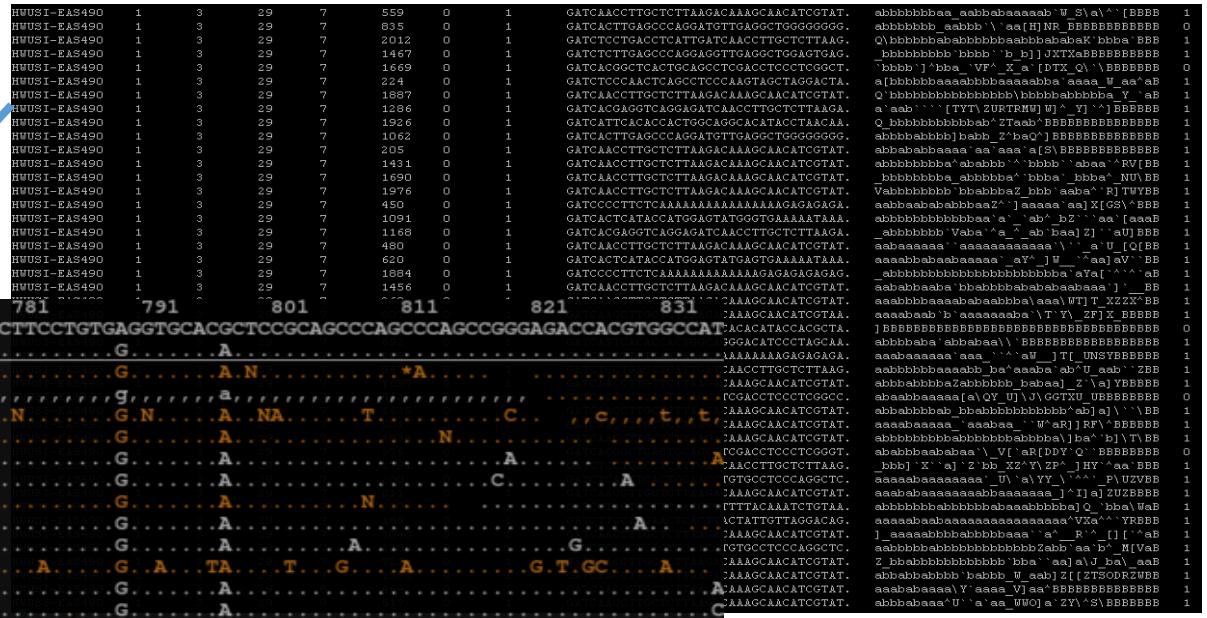


Fig.A

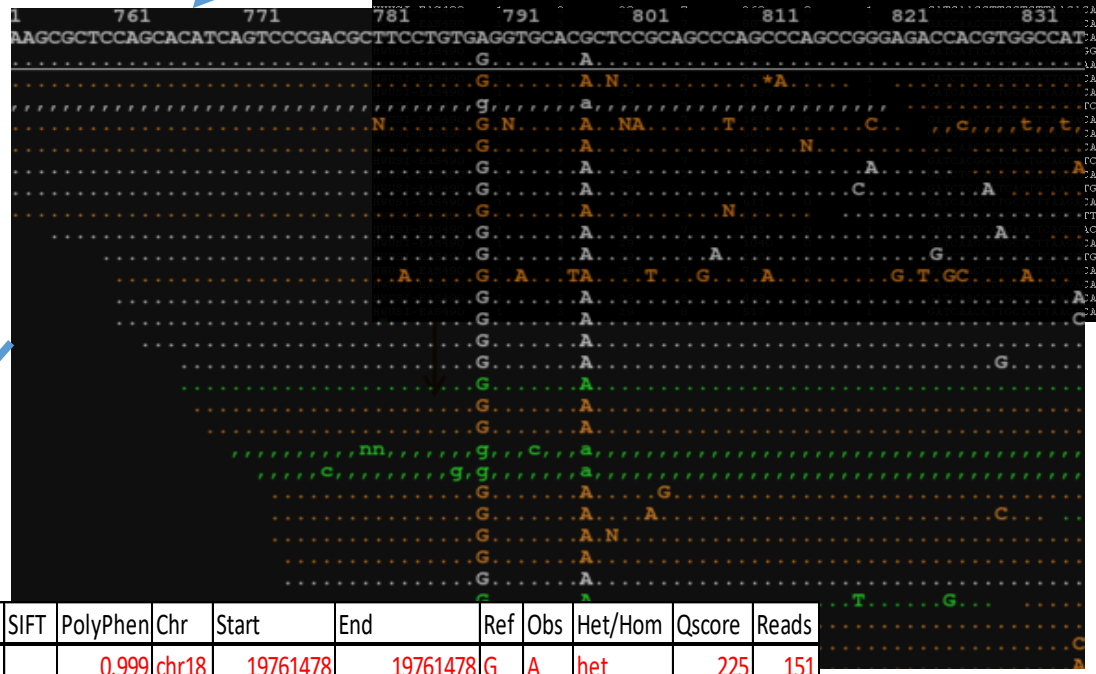


Fig.B

***Fig.C**: Table of selected variants*
Each line show a variant with informations regarding its position in gene, gene name, type, annotation, prediction, and geneome localisation

Func	Gene	ExonicFunc	AAChange	SIFT	PolyPhen	Chr	Start	End	Ref	Obs	Het/Hom	Qscore	Reads
exonic	GATA6	nonsynonymous SNV	p.R456H		0.999	chr18	19761478	19761478	G	A	het	225	151
exonic	C5orf22	nonsynonymous SNV	p.D155Y	0.12	0.503	chr5	31538452	31538452	G	T	het	225	42

Fig.C

EXOME SEQUENCING VARIANT LIST

ANNOVAR: ANNOTATED VARIANTS

All coding variants compared to human reference sequence (including false calls)

Automated variant filtering based on

- technical characteristics (quality) /
- population frequency

	Chr	Start	End	Func	Gene	ExonicFunc	AAChange	Obs	Ref	Zg	Mol	QS	C	INFO	nce46way	segdup	avsnp142	xavier_20150923	ExAC_Freq	ExAC_Adj	ExAC_Afr	ExAC_Amr	ExAC_Eas	ExAC_Nfe	ExAC_Oth	ExAC_Sas	ljb2_sift	ljb2_pp2hvar	ljb2_mt	ljb_gerp++	dbscsnv11	clinvar_20160302
224	chr5	89988464	89988464	exonic	ADGRV1	nonsynonymous SNV	ADGRV1:NM_032119:exon32:c.6994A>T:p.I2332F	T	A	het	AR/Digenic	1699.77	165	AC=1;AF=0.;	Score=701;cod=944	.	rs193030567	0.000207	0.0002	0.0003	0.0002	0	0	0.0003	0	0	0	0.701	0.289388	.	.	Uncertain significance|Benign
225	chr20	32080242	32080242	exonic	AHCY	nonsynonymous SNV	AHCY:NM_000687:exon8:c.367G>A:p.G123R,AHC	T	C	het	AR	592.77	92	AC=1;AF=0.;	Score=576;cod=293	.	rs41301825	0.0002884	0.0041	0.001	0.0034	0	0.0008	0.0062	0.0022	0.0012	0.01	0.828	0.581163	4.79	.	.
226	chr20	32081965	32081965	splicing	AHCY	.	NM_000687:exon3:c.136-3T>C,NM_000687:exon	T	A	het	AR	301.77	22	AC=1;AF=0.;	.	.	.	6.50E-06	8.63E-06	0	0	0	0	1.56E-05	0	0	.	.	.	.	0.0001	.
900	chr10	97386497	97386497	exonic	ALDH18A1	nonsynonymous SNV	ALDH18A1:NM_000135423:exon10:c.1169C>A:p.S	T	G	het	AD/AR	816.77	66	AC=1;AF=0.;	Score=583;cod=312	.	rs3765571	0.0175095	0.0172	0.0056	0.0081	0.0342	0.00656	0.02	0.0176	0.0156	0.03	0.029	0.687192	5.58	.	.
916	chr13	52598272	52598272	exonic	ALG11	nonsynonymous SNV	ALG11:NM_001004127:exon3:c.406C>T:p.R136C	T	G	het	AR	2761.77	247	AC=1;AF=0.;	Score=528;cod=186	.	rs150101415	0.0002523	0.0002	0	0.0002	0	0	0.0003	0.0001	0.0002	0	0.13	0.000093	5.81	.	.
227	chrX	110965944	110965944	splicing	ALG13	.	NM_001099922:exon12:c.1435+5A>T,NM_001259	T	A	hom	XL	1909.77	64	AC=2;AF=1.;	.	.	rs5985638	0.0694105	0.131	0.4888	0.0768	0.0003	0.0004	0.0094	0.0466	0.0054	.	.	.	.	0.0001	.
228	chr9	101984147	101984147	exonic	ALG2	nonsynonymous SNV	ALG2:NM_033087:exon1:c.30C>G:p.D10E	G	G	het	AR	1248.77	97	AC=1;AF=0.;	Score=583;cod=1	.	rs7023652	0.0059637	0.0083	0.0037	0.0022	0	0	0.0002	0.0052	0.0227	0.77	0	.	-4.75	.	.
229	chr16	89347441	89347441	exonic	ANKRD11	nonsynonymous SNV	ANKRD11:NM_001256183:exon9:c.5509C>T:p.P18	A	G	het	AD	2883.77	282	AC=1;AF=0.;	.	.	rs145906515	0.0038551	0.004	0.0008	0.0038	0	0.0038	0.0055	0.0059	0.0022	0.9	0.003	0.194961	-1.71	.	.
233	chrX	138757177	138757177	exonic	ARHGEF6	nonsynonymous SNV	ARHGEF6:NM_004840:exon19:c.2024G>T:p.G675V	A	C	hom	XL	2377.77	86	AC=2;AF=1.;	Score=409;cod=61	.	.	.	.	.	.	.	.	.	.	.	0.22	0.101	0.476956	3	.	.
237	chr1	27102188	27102188	exonic	ARID1A	nonsynonymous SNV	ARID1A:NM_006015:exon19:c.5114A>G:p.N1705S	G	A	het	AD	1531.77	145	AC=1;AF=0.;	Score=637;cod=517	.	rs61756316	0.0002276	0.0085	0.0892	0.0043	0	0	0.0007	0.0066	6.07E-05	0.14	0.056	0.960382	3.7	.	Likely benign
238	chr1	197055361	197055361	splicing	ASPM	.	NM_018136:exon28:c.10332-5A>TA,NM_001256836	TA	T	het	AR	335.73	34	AC=1;AF=0.;	.	.	rs200839523	0.0001164	0.2816	.	.	.	.	.	.	.	.	.	.	.	.	.
250	chr1	17332241	17332241	exonic	ATP13A2	nonsynonymous SNV	ATP13A2:NM_001141973:exon2:c.43G>A:p.G15S,A	T	C	het	AR	1642.77	196	AC=1;AF=0.;	.	.	.	6.50E-06	1.04E-05	0	0	0	0	1.86E-05	0	0	0.52	0.003	0.19219	-5.33	.	.
257	chr1	235623040	235623040	exonic	B3GALNT2	nonsynonymous SNV	B3GALNT2:NM_152490:exon8:c.896A>T:p.E299L	A	T	het	AR	1242.77	109	AC=1;AF=0.;	.	.	.	.	.	.	.	.	.	.	.	.	0.67	0.055	0.965538	3.6	.	.
281	chr13	31868092	31868092	exonic	B3GLCT	nonsynonymous SNV	B3GLCT:NM_194318:exon12:c.1000T>G:p.F334V	G	T	het	AR	531.77	54	AC=1;AF=0.;	Score=548;cod=225	.	.	.	.	.	.	.	.	.	.	.	1	0.741	0.999887	5.08	.	.
282	chr2	62449763	62449763	exonic	B3GNT2	nonsynonymous SNV	B3GNT2:NM_006577:exon2:c.408T>A:p.D136E	A	T	.	.	3787.77	356	AC=1;AF=0.;	.	.	rs78968142	0.0082793	0.0086	0.0441	0.0038	0	0.0189	0.0055	0.0166	0.0026	0.97	0.001	0.003127	-4.85	.	.
283	chr11	73753250	73753250	splicing	C2CD3	.	.	C	CAA	het	AR	155.5	17	AC=1;AF=0.;	.	.	.	0.0434859	0.1645	.	.	.	.	.	.	.	.	.	.	.	.	.
304	chrX	41469284	41469284	splicing	CASK	.	NM_003688:exon12:c.1034-6C>T,NM_001126054:ex	A	G	hom	XL	2399.77	85	AC=2;AF=1.;	.	.	rs73476569	0.0250514	0.0357	0.3161	0.016	0	0	0.0004	0.0151	0.0007	.	.	.	.	0.0001	Benign|Benign
305	chr16	88870369	88870369	exonic	CDT1	nonsynonymous SNV	CDT1:NM_030928:exon1:c.136A>C:p.T44P	C	A	het	AR	251.77	15	AC=1;AF=0.;	.	.	rs587798306	0.0001876	0.0006	0.0373	0	0	0	0	0	0	0.43	0	0.000001	-6.38	.	Uncertain significance
306	chr16	88872222	88872222	splicing	CDT1	nonsynonymous SNV	CDT1:NM_030928:exon3:c.404C>T:p.A135V	T	C	het	AR	1455.77	161	AC=1;AF=0.;	.	.	rs3218725	0.0012742	0.0013	0.0124	0.001	0	0	0.0002	0.0037	0	0.28	0.162	0.616516	2.96	.	Benign
313	chr4	104096063	104096063	splicing	CENPE	.	NM_001813:exon16:c.1489-3T>C	G	A	het	AR	105.77	10	AC=1;AF=0.;	.	.	rs60151877	0.0157307	0.0359	0.2658	0.0248	0	0	0.0024	0.0155	0.0008	.	.	.	.	0.0001	.
315	chr4	104117412	104117412	splicing	CENPE	.	NM_001813:exon3:c.149-3T>GTTT	AAAC	A	het	AR	859.73	67	AC=1;AF=0.;	Score=444;cod=85	.	rs111297674	0.0004592	0.0215	0.2178	0.0132	0.0028	0.0002	0.0027	0.0108	0.0016	.	.	.	.	.	.
319	chr13	25467010	25467010	splicing	CENPJ	.	NM_018451:exon10:c.2992-5G>TG	CA	C	het	AR	398.73	71	AC=1;AF=0.;	.	.	rs565804928	0.0002652	0.381	.	.	.	.	.	.	.	.	.	.	.	.	.
320	chr13	25486877	25486877	exonic	CENPJ	nonsynonymous SNV	CENPJ:NM_018451:exon22:c.2674A>G:p.I892R	C	T	het	AR	2154.77	274	AC=1;AF=0.;	.	.	rs61730263	0.003771	0.004	0.0395	0.0031	0	0	0.0005	0.0011	0.0001	0.89	0.003	0.005184	-2.88	.	Benign
322	chr4	61655009	61655009	exonic	CHD7	nonsynonymous SNV	CHD7:NM_017780:exon2:c.1018A>G:p.M340V	G	A	het	AD	2473.77	217	AC=1;AF=0.;	Score=406;cod=2521	.	rs41305525	0.0006118	0.0042	0.0018	0.0027	0	0.0018	0.0063	0.0011	0.0013	0.18	0	0.10665	.	.	other
325	chr7	147926795	147926795	exonic	CNTNAP2	nonsynonymous SNV	CNTNAP2:NM_014141:exon20:c.3385T>C:p.V1102	C	T	het	AR	1992.77	194	AC=1;AF=0.;	.	.	rs111599875	0.0006339	0.0007	0.0073	8.65E-05	0	0	3.00E-05	0	0	0.69	0.324	0.196797	2.11	.	Likely benign
353	chr7	107204371	107204371	exonic	COG5	nonsynonymous SNV	COG5:NM_001161520:exon1:c.64G>A:p.A22T,COG	T	C	het	AR	979.77	87	AC=1;AF=0.;	.	.	rs73419464	0.007626	0.0094	0.1041	0.0063	0	0	0.0007	0.0071	0.0002	0.3	0.028	0.000157	0.0287	.	.
354	chr8	68102977	68102977	exonic	CSPP1	nonsynonymous SNV	CSPP1:NM_024790:exon27:c.3298T>C:p.W1100R	C	T	het	AR	1189.77	131	AC=1;AF=0.;	.	.	rs1808148	0.0192882	0.0217	0.0355	0.0079	0.0146	0.101	0.0089	0.0401	0.0459	0.37	0.054	0.006358	-3.83	.	.
429	chr12	31244809	31244809	splicing	DDX11	.	NM_001257145:exon16:c.1164-4T>C,NM_004399:e	C	T	het	AR	389.77	221	AC=1;AF=0.;	Score=0.978888	.	rs2111769	6.50E-06	.	.	.	.	.	.	.	.	.	.	.	.	0	Uncertain significance
430	chrX	31462708	31462708	exonic	DMD	nonsynonymous SNV	DMD:NM_004014:exon6:c.787G>A:p.V263M,DMD	T	C	hom	XL	2586.77	99	AC=2;AF=1.;	Score=497;cod=139	.	rs201691420	0.0003105	0.0003	0.0004	0.0001	0	0	0.0005	0.0016	9.91E-05	0.19	0.999	.	4.41	.	Uncertain significance
431	chrX	32613922	32613922	exonic	DMD	nonsynonymous SNV	DMD:NM_000109:exon13:c.1530T>A:p.D510E,DMI	T	A	hom	XL	2372.77	83	AC=2;AF=1.;	Score=598;cod=358	.	rs61733587	0.0030401	0.0038	0.0364	0.0012	0	0	2.21E-05	0	0	0.3	0.998	0	1.54	.	Benign
437	chr19	11347525	11347525	exonic	DOCK6	nonsynonymous SNV	DOCK6:NM_020812:exon19:c.2104G>A:p.G702S	T	C	het	AR	1800.77	213	AC=1;AF=0.;	Score=608;cod=365	.	rs199838752	0.0014553	0.0062	0.0009	0.0047	0	0.0192	0.0028	0.0053	0.012	0.02	0.211	0.324058	.	.	.
441	chr6	56482923	56482923	exonic	DST	nonsynonymous SNV	DST:NM_001723:exon21:c.5909T>C:p.F1970S	G	A	het	AR	2790.77	254	AC=1;AF=0.;	Score=446;cod=87	.	rs141573097	0.0045278	0.0048	0.0005	0.0014	0	0.0024	0.0056	0.0044	0.0103	0.65	0.858	0.000013	5.06	.	.
450	chr22	41545039	41545039	splicing	EP300	.	NM_001429:exon13:c.2242-3C>A	A	C	het	AD	38.77	66	AC=1;AF=0.;	Score=549;cod=227	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.8232	.
453	chr18	43445730	43445730	splicing	EPG5	.	.	C	CAA	het	AR	493.73	65	AC=1;AF=0.;	.	.	.	0.0003299	0.0429	.	.	.	.	.	.	.	.	.	.	.	.	.
523	chr16	14041591	14041591	exonic	ERCC4	nonsynonymous SNV	ERCC4:NM_005236:exon11:c.2138T>C:p.V713A	C	T	het	AR	1837.77	195	AC=1;AF=0.;	Score=369;cod=42	.	.	6.50E-06	8.24E-06	0	0	0	0	1.50E-05	0	0	0	0.996	0.999658	5.14	.	.
524	chr10	5066702	5066702	exonic	ERCC6	nonsynonymous SNV	ERCC6:NM_000124:exon21:c.4322C>T:p.T1441I	A	G	het	AD/AR	1432.77	181	AC=1;AF=0.;	Score=488;cod=128	.	rs4253230	0.0068175	0.0068	0.074	0.0022	0	0	0.0004	0.0033	0	0.58	0.001	.	3.27	.	.
532	chr9	98638341	98638341	exonic	ERCC6L2	nonsynonymous SNV	ERCC6L2:NM_001010895:exon1:c.54G>C:p.Q181	C	G	het	AR	2519.77	242	AC=1;AF=0.;	.	.	rs139286981	0.0011966	0.0023	0.026	0.0026	0	0	0	0	0.0001	0.12	0	0.009819	-2.49	.	.
533	chr8	27657082	27657082	exonic	ESCO2	nonsynonymous SNV	ESCO2:NM_001017420:exon10:c.1522A>G:p.I508V	G	A	het	AR	747.77	108	AC=1;AF=0.;	.	.	rs114956994	0.0062612	0.0064	0.0428	0.0039	0.0267	0.0026	0.0004	0.0055	0.0006	0.36	0.001	0.000001	-3.27	.	.
537	chr6	35423886	35423886	exonic	FANCE	nonsynonymous SNV	FANCE:NM_021922:exon2:c.611C>T:p.S204L	T	C	het	AR	3510.77	299	AC=1;AF=0.;	.	.	rs7761870	0.0177617	0.0177	0.0998	0.0095	0	0.0024	0.0144	0.0089	0.0008	0.02	0.001	0.002788	2.19	.	not provided
558	chr4	1805542	1805542	splicing	FGFR3	.	NM_000142:exon6:c.645-3A>G,NM_001163213:exo	G	A	het	AD/AR	1733.77	227	AC=1;AF=0.;	.	.	rs3135868	0.0349607	0.0375	0.31	0.0238	0.0239	0.0005	0.008	0.0217	0.0154	.	.	.	.	0.0003	.
559	chr4	1805568	1805568	splicing	FGFR3	.	NM_000142:exon8:c.1075+5C>T	T	C	het	AD/AR	2377.77	211	AC=1;AF=0.;	Score=710;cod=1028	.	rs3135885	0.0108407	0.0114	0.0346	0.0109	0.0178	0.0005	0.0071	0.0144	0.0149	.	.	.	.	0.0001	.
560	chr13	39266382	39266382	exonic	FREM2	nonsynonymous SNV	FREM2:NM_207361:exon1:c.4901C>T:p.T1634M	T	C	het	AR	4112.77	332	AC=1;AF=0.;	Score=476;cod=108	.	rs74781600	0.0025744	0.0026	0.0272	0.0013	0.0008	0	0.0002	0	0.0001	0.09	0.986	0.999931	5.36	.	.
562	chr5	161529612	161529612	exonic	GABRG2	nonsynonymous SNV	GABRG2:NM_198903:exon6:c.684C>G:p.H228Q	G	C	het	AD	37.77	11	AC=1;AF=0.;	.	.	.	1.94E-05	9.41E-05	0	0	0	0	0.0004	0	0	0.15	0	0.000004	.	.	.
564	chr14	88417995	88417995	splicing	GALC	.	.	G	GA	hom	AR	946.73	38	AC=2;AF=1.;	Score=409;cod=61	.	rs11300320	0.0208535	0.9775	.	.	.	.	.	.	.	.	.	.	.	.	.
565	chr1	155205581	155205581	exonic	GBA	nonsynonymous SNV	GBA:NM_001171811:exon8:c.1018G>A:p.E340K,GI	T	C	het	AR	4077.77	421	AC=1;AF=0.;	Score=366;cod=41	Score=0.953947	rs149171124	0.0001811	0.0002	0	8.69E-05	0	0.0003	0.0003	0	0.0001	0.58	0.121	0.067567	4.69	.	.
600	chr3	49759379	49759379	exonic	GMPPB	frameshift deletion	GMPPB:NM_013334:exon8:c.970_972G	C	CAG	het	AR	83.73	143	AC=1;AF=0.;	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
601	chr3	49759382	49759384	exonic	GMPPB	frameshift deletion	GMPPB:NM_013334:exon8:c.965_967G	C	CAG	het	AR	71.73	154	AC=1;AF=0.;	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
620	chr9	36233958	36233958	exonic	GNE	nonsynonymous SNV	GNE:NM_001190308:exon3:c.61C>T:p.P20L,GNE	A	G	het	AD/AR	1391.77	174	AC=1;AF=0.;	Score=594;cod=345	.	.	.	.	.	.	.	.	.	.	.	0.01	0.986	0.999736	5.18	.	.
623	chr7	65426054	65426054	splicing	GUSB	.	.	C	CA	het	AR	185.73	69	AC=1;AF=0.;	.	.	.	0.000414	0.4379	.	.	.	.	.	.	.	.	.	.	.	.	.
757	chr2	191125911	191125911	exonic	HIBCH	nonsynonymous SNV	HIBCH:NM_014362:exon7:c.488G>C:p.C163S,HIBC	G	C	het	AR	827.77	125	AC=1;AF=0.;	Score=546;cod=220	.	rs74832989	0.0056015	0.0058	0.0616	0.003	0	0	0.0006	0.0001	0.0005	0.17	0	.	4.44	.	.
758	chr4	997829	997829	exonic	IDUA	nonsynonymous SNV	IDUA:NM_000203:exon13:c.1757C>T:p.S586I	T	C	het	AR	3258.77	293	AC=1;AF=0.;	Score=336;cod=31	.	rs187833337	0.0001229	9.10E-05	0.0007	0.0002	0	0	3.02E-05	0	0	0.02	0.862	0.997731	3.2	.	.
763	chr2	27569137	27569137	exonic	IFT172	nonsynonymous SNV	IFT172:NM_015662:exon8:c.4785T>C:p.I1582T	G	A	het	AR	633.77	61	AC=1;AF=0.;	Score=585;cod=319	.	rs61742074	0.0067644	0.0084	0.0018	0.0054	0	0.0024	0.0118	0.0099	0.0077	0.67	0.001	0.138145	-4.21	.	.
764	chr20	10644614	10644614	exonic|splicing	JAG1|JAG1	nonsynonymous SNV	JAG1:NM_000214:exon3:c.436G>A:p.V146I	T	C	het	AD	1352.77	151	AC=1;AF=0.;	.	.	rs6040067	0.0037192	0.0039	0.0425	0.0019	0	0	0.0002	0.0001	0	0.4	0.003	0.000054	-3.11	.	Benign
765	chr9	710966	710966	exonic	KANK1	nonsynonymous SNV	KANK1:NM_015158:exon3:c.200G>A:p.R67Q,KAN	A	G	het	AD	1886.77	159	AC=1;AF=0.;	Score=685;cod=813	.	rs7860464	0.0569721	0.0599	0.5061	0.0379	0.0002	0.0005	0.0077	0.0463	0.061	0.38	0.098	.	2.89	.	.
766	chr10	76786077	76786077	exonic	KAT6B	nonsynonymous SNV	KAT6B:NM_001256468:exon18:c.3946G>A:p.V1314	A	G	het	AD	2367.77	225	AC=1;AF=0.;	.	.	rs3740321	0.0306664	0.0327	0.0567	0.0962	0.0785	0.0027	0.0134	0.0386	0.027	0.2	0.026	0.000003	4.42	.	.
770	chr12	69433883	69433883	exonic	KMT2D	nonsynonymous SNV	KMT2D:NM_003482:exon31:c.7670C>T:p.P2557L	A	G	het	AD	2770.77	216	AC=1;AF=0.;	Score=565;cod=263	.	rs189888707	0.0078848	0.0084	0.0179	0.0088	0.0002	0.0003	0.0072	0.0151	0.0167	0	0.002	.	.	.	Benign
782	chr18	7037639	7037639	exonic	LAMA1	nonsynonymous SNV	LAMA1:NM_005559:exon12:c.1675G>A:p.V559I	T	C	het	AR	2372.77	227	AC=1;AF=0.;	.	.	rs16951079	0.0032212	0.0035	0.0341	0.0045	0	0	8.99E-05	0.0055	6.06E-05	0.37	0.212	0.001119	3.02	.	.
795	chr6	129670438	129670438	splicing	LAMA2	.	NM_000426:exon31:c.4437-5T>A,NM_001079823:ex	A	T	het	AD/AR	2207.77	200	AC=1;AF=0.;	Score=433;cod=77	.	rs61285288	0.0059249	0.0057	0.0021	0.0038	0	0.0026	0.0083	0.0077	0.0033	.	.	.	.	0.8827	Benign
810	chr5	126113229	126113229	exonic	LMNB1	nonsynonymous SNV	LMNB1:NM_005573:exon1:c.29G>T:p.R10L	T	G	het	AD	546.77	36	AC=1;AF=0.;	Score=456;cod=95	.	rs54012367	0.0002005	0.0012	0.0435	0	0	0	0	0	0	0.53	0.004	0.841574	2.79	.	.
813	chr5	126141389	126141389	splicing	LMNB1	.	NM_005573:exon3:c.642-1G>GTAAC,NM_001198	GTAAC	G	het	AD	2040.73	128	AC=1;AF=0.;	Score=488;cod=128	.	rs142214610	0.0008538	0.0009	0.0095	0.0006	0	0	4.51E-05	0	0	.	.	.	.	.	.

Variant filtering on biological characteristics

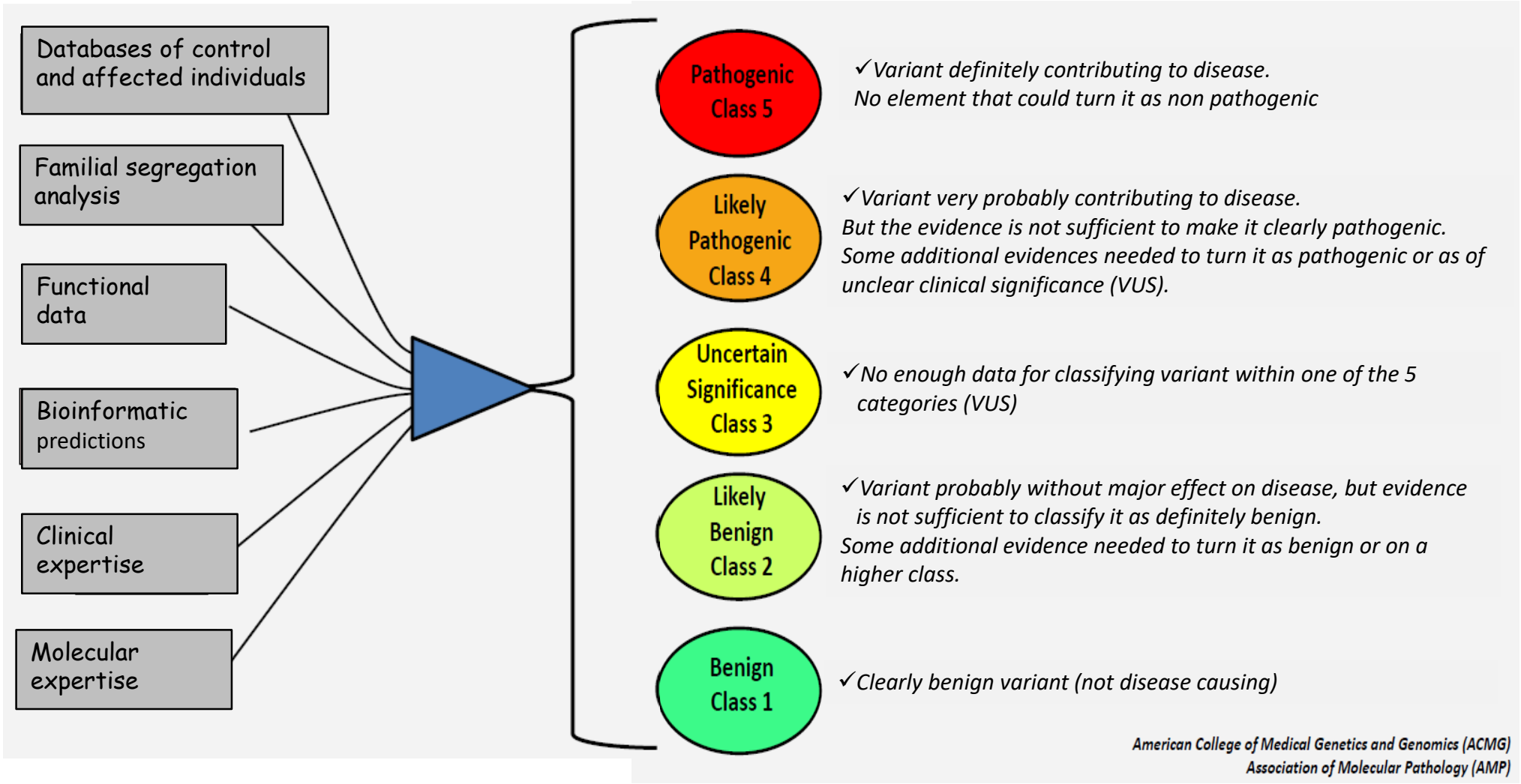
08	89869870	89869870	exonic	POLG	nonsynonymous SNV	POLG:NM_001126131:exon9:c.1685G>A:p.R	T	C	het	AD/AR
57	18907081	18907081	exonic	PRODH	nonsynonymous SNV	PRODH:NM_001195226:exon10:c.810G>A:p.	T	C	het	AR
79	32811752	32811752	exonic	PSMB8	nonsynonymous SNV	PSMB8:NM_148919:exon1:c.22G>A:p.G8R	T	C	het	AR
06	1.18E+08	1.18E+08	exonic	RAD21	nonsynonymous SNV	RAD21:NM_006265:exon7:c.745T>C:p.S249I	G	A	het	AD

Variant filtering on clinical meaning

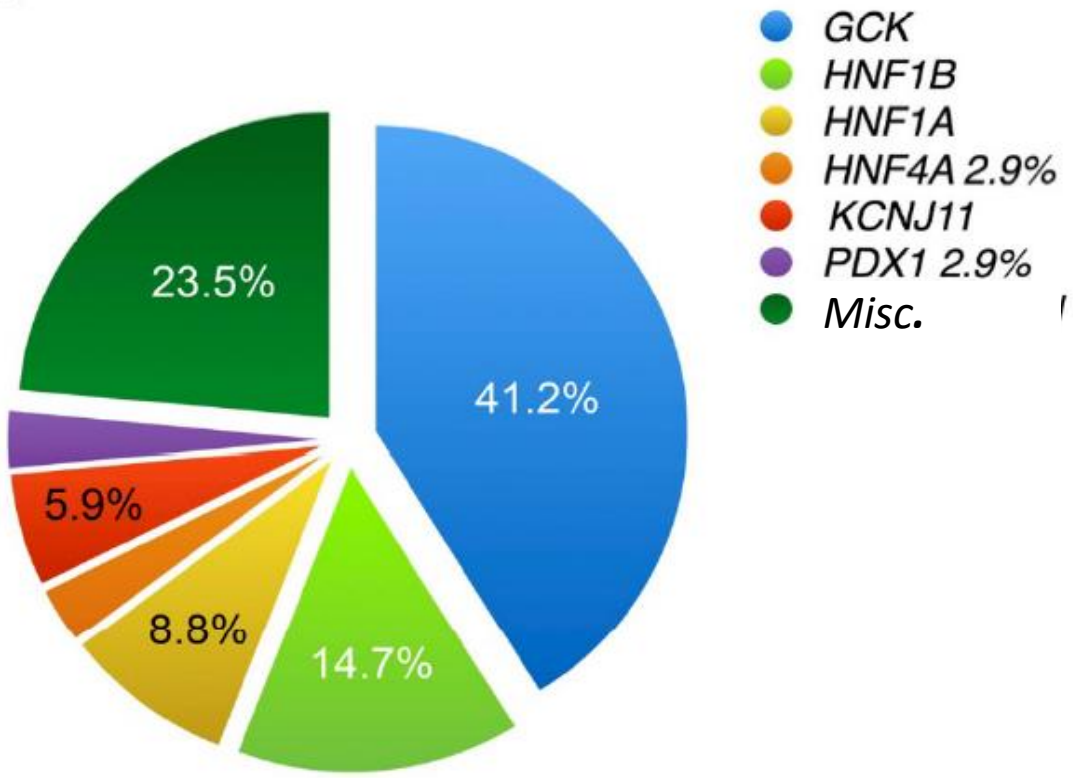
chr15	89869870	89869870	exonic	POLG	nonsynonymous SNV	POLG:NM_001126131:exon9:c.1685G>A:p.R562Q,POLG:I

CLINICAL CLASSIFICATION OF VARIANTS

An important task in variant interpretation is to classify variants according to 5 levels of clinical significance (from pathogenic to benign).



GENE PREVALENCE FROM DIAGNOSTICS



Medical center origins of the patients

Figure 1: Types and proportions of mutations in surveyed patients with a genetically confirmed diagnosis of monogenic diabetes. n=74 patients (44 females/30 males)

For 34 patients, genetic analysis was performed by Sanger sequencing (not by NGS)

CONCLUSIONS

- Childhood onset diabetes are diagnosed in **3 main types** (T1D, Monogenic, other non-genetic causes)

- T1D has complex aetiology including **complex genetic** cause
  - Genetic causes are currently not investigated in routine diagnostics

- Monogenic diabetes is caused by **one gene**, but is genetically **highly heterogeneous**
  - >40 genes can cause the disease: diagnostic **sensitivity is about 50%** today
    → Still unknown genes or genetic causes todate that remain to be identified through research efforts
  - **Genetic diagnostic is available**, and performed routinely in specialized centers
  - Ideally molecular genetic diagnostics should be investigated using next-generation-sequencing (NGS)
  - At least 13 genes for MODY, 22 genes for NIDDM, should be analysed for all coding part
  - Clinical classification of variant is a key step (causative variant, or VUS, or benign)
  - Only the **Pathogenic and Likeky Pathogenic** variants should be reported
  - Variant of unknown Clinical Significance (VUS) or Likely benign/benign should not be reported
  - Knowing the gene dictates the treatment: **personalized medicine** (specific drug administration)

- Perspectives
  - It is expected that T1D and T2D will also be genetically investigated in clinical setting in future
    → **Polygenic Risk Score**: some studies investigating this way allow risk estimation of developing Diabetes
    → Help in life-style choice / decision (specific medical surveillance, food, sport, preventive drug ?, …)

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