23 - 24 settembre 2016 - Roma - CONGRESSO AMD – SID LAZIO 2016 NOTIZIE DALLA REGIONE: RICERCA, ASSISTENZA E POLITICHE SANITARIE

Genetica e clinica del diabete familiare dell'età adulta

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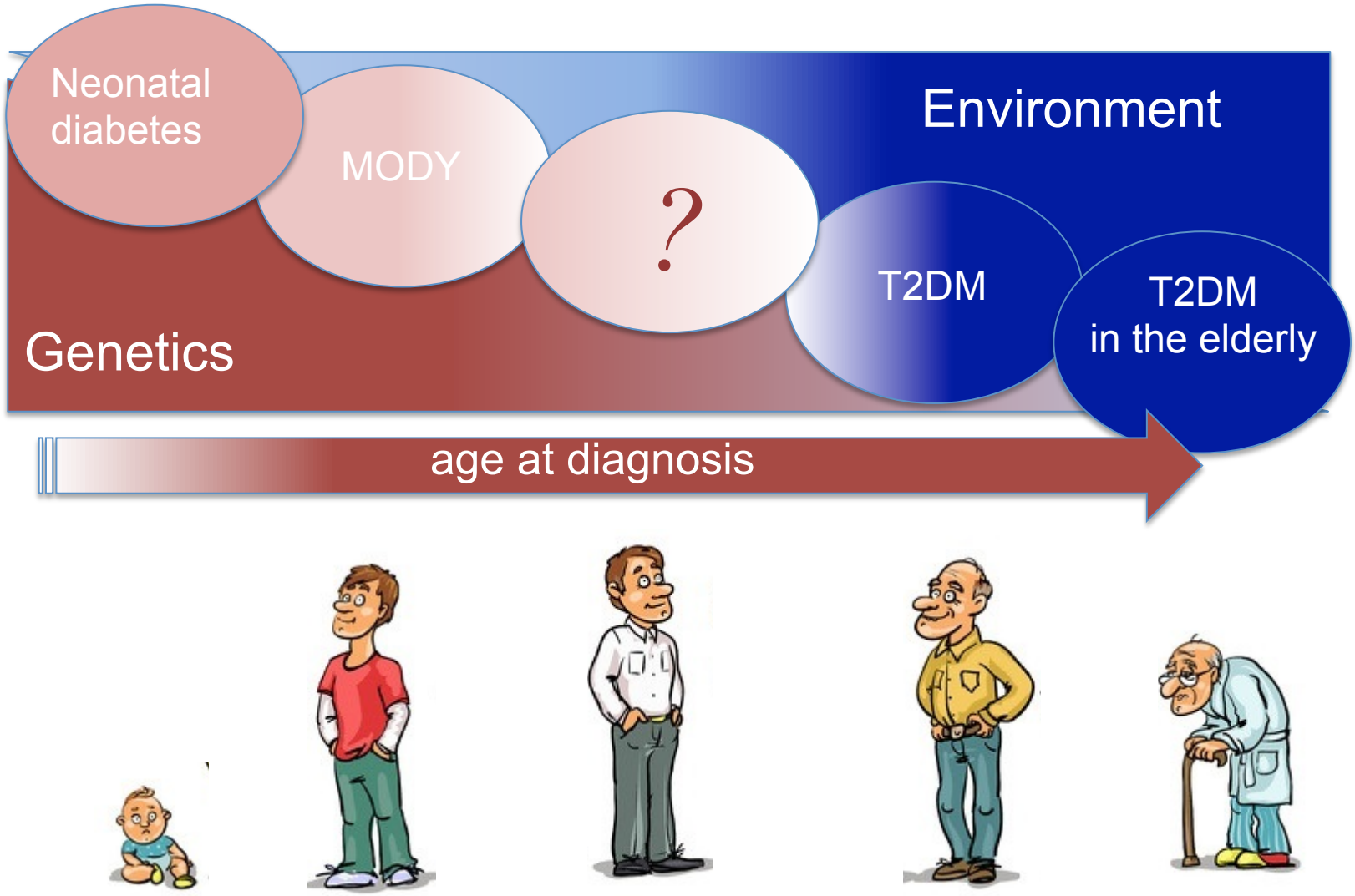
Lo "*Spettro*" del diabete non autoimmune



Esistenza in clinica di pazienti con errata diagnosi di diabete di tipo 2, caratterizzati da un diabete multigenerazionale dell'adulto
(possibile forma intermedia di diabete)

PLOS | ONE 2015, 10:e0135855.

RESEARCH ARTICLE

Identification and Clinical Characterization of Adult Patients with Multigenerational Diabetes Mellitus

Ornella Ludovico[1], Massimo Carella[2], Luigi Bisceglia[2], Giorgio Basile[3,4], Sandra Mastroianno[5], Antonio Palena[1], Salvatore De Cosmo[6], Massimiliano Copetti[7], Sabrina Prudente[3]*, Vincenzo Trischitta[3,4,8]*

FDA
(Familial Diabetes of the Adulthood)

Disegno dello studio

2,583 pazienti con diabete di tipo 2 (DM2)

77 pazienti (3%) presenta una forma di diabete multigenerazionale
Famiglie con diabete in ≥ 3 generazioni

Sequenziamento Sanger: 6 geni responsabili delle forme più comuni di MODY (HNF1A, HNF1B, HNF4A, GCK, NeuroD1 e PDX1) e la mutazione mitocondriale mtDNA (A3243G)

10 pazienti (0.4%): MODY+

67 pazienti (2.6%): ???

FDA : Familial Diabetes of the Adulthood

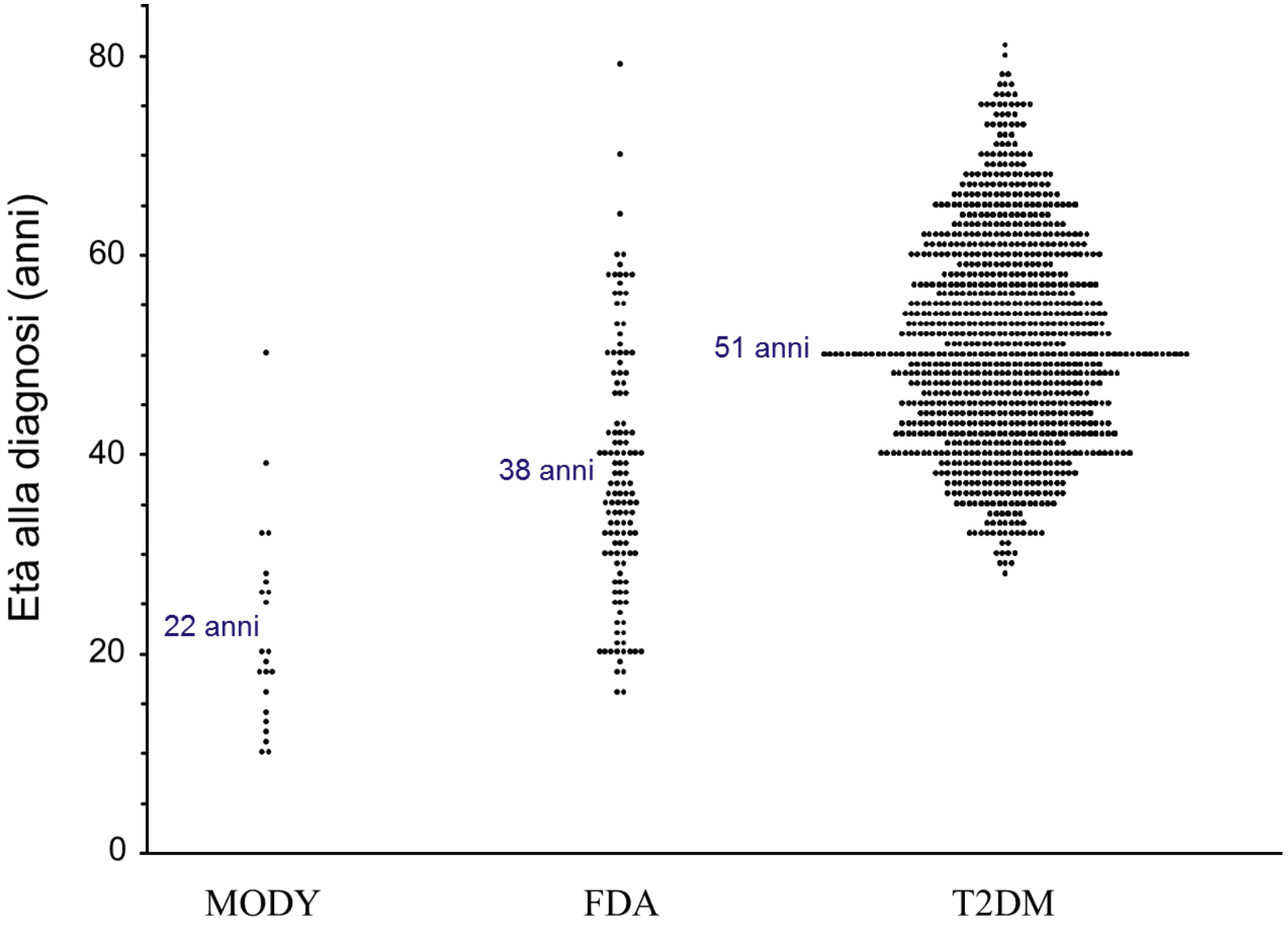
Ludovico et al, PLoS ONE 2015

Identification and Clinical Characterization of Adult Patients with Multigenerational Diabetes Mellitus.

Ludovico O. *et al.;* PLoS One. 2015 Aug 19;10(8

	MODY (10 families, n=22)	FDA (67 families, n=130)	T2DM (n=1,028)
Males (%)	36.4	53.1	50.0
Age (yrs)	40.4±15.3	53.3±13.7*§	62.1±9.7
Age at diagnosis (yrs)	22.0±10.1	37.7±12.5*§	51.2±10.5
BMI (Kg/m^2)	24.9±3.4	30.1±6.3§	31.1±5.8
Waist circumference (cm)	87.7±12.2	97.5±13.6*§	102.4±13.4
HbA1c (%) (mmol/mol)	7.1±2.0 (54.0±21.9)	8.7±2.0 (72.0±21.9)	8.7±2.0 (72.0±21.9)
Anti-hyperglicemic treatment			
Diet (%)	45.5	12.3	15.8
OADs (%)	31.8	42.3	42.5
Insulin ± OADs (%)	22.7	45.4§	41.7
Obesity (%)	13.6	44.4§	53.1
Hypertension (%)	13.6	48.8*§	84.7
Dyslipidemia (%)	63.6	79.8	86.7
Micro-macro-albuminuria (%)	9.5	29.6	30.7

Ludovico et al, PLoS ONE 2015



Ludovico et al, PLoS ONE 2015


FDA
What else….

FDA: what else?

IPOTESI I:
- Mutazioni nei geni per le forme non comuni di MODY che non erano stati valutati nel nostro primo screening
- Oppure mutazioni nei 6 geni per le forme più comuni di MODY già screenate e precedentemente sfuggite al sequenziamento.

IPOTESI II:
- Mutazioni nei geni del Diabete Neonatale.

IPOTESI III
- Mutazioni in geni non ancora conosciuti coinvolti nell'omeostasi del glucosio.

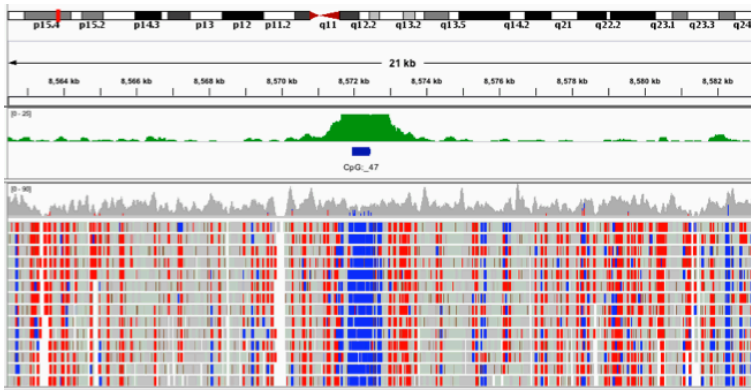
OBIETTIVO: Verificare la presenza di mutazioni nei geni MODY, e del Diabete Neonatale

LO STUDIO:

52 Pazienti FDA

ANALISI *NGS*
(Next Generation Sequencing) su 52 DNA

9 *mediante WES (Whole Exome Sequencing)*
Sequenziamento dell'intera regione codificante del genoma

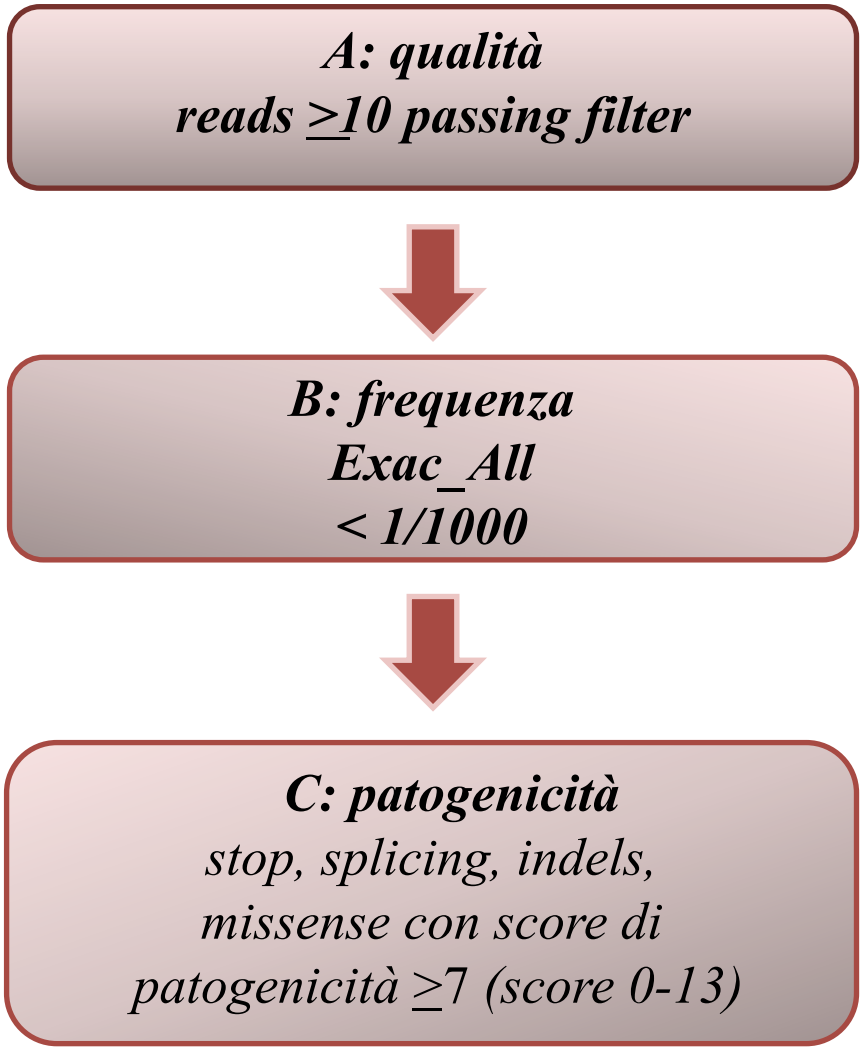


SOLID 5550 XL, Applied Biosystems (Italia)

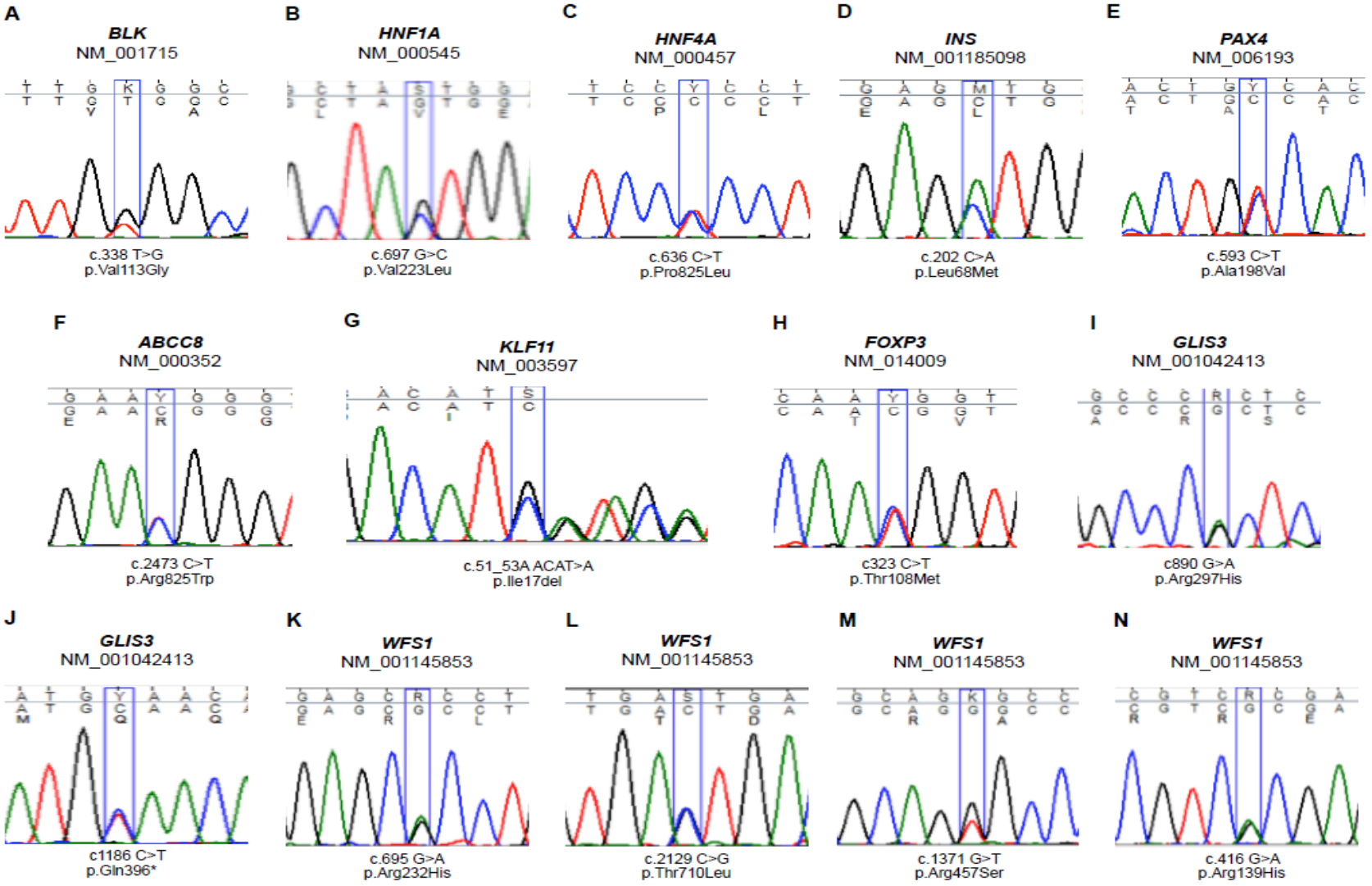
43 *Pazienti sono stati analizzati mediante un Pannello (Targeted Resequencing) di 26 geni responsabili del MODY (n=13) e del Diabete Neonatale (n=13)*

GENE	DM type	GENE	DM type
ABCC8	MODY/Neonatal	*EIF2AK3*	Neonatal
BLK	MODY	*FOXP3*	Neonatal
CEL	MODY	*GATA4*	Neonatal
GCK	MODY/Neonatal	*GATA6*	Neonatal
HNF1A	MODY	*GLIS3*	Neonatal
HNF1B	MODY/Neonatal	*IER3IP1*	Neonatal
HNF4A	MODY	*NEUROG3*	Neonatal
INS	MODY/Neonatal	*PTF1A*	Neonatal
KCNJ11	MODY/Neonatal	*RFX6*	Neonatal
KLF11	MODY	*SLC19A2*	Neonatal
NEUROD1	MODY/Neonatal	*SLC2A2*	Neonatal
PAX4	MODY	*WFS1*	Neonatal
PDX1	MODY/Neonatal	*ZFP57*	Neonatal

Pipeline per filtraggio e prioritizzazione delle varianti in 26 geni



Verifica mediante Sanger Sequencing delle mutazioni filtrate



IPOTESI I:

Mutazioni nei geni MODY a tutt'oggi noti (n=13)

	GENE	REFERENCE SEQUENCE	NUCLEOTIDE CHANGE	AMMINOACID	EFFECT	PATHOGENICITY SCORE (0-13)	COMMENT
1	*BLK*	NM_001715	Exon 5 c.338 T>G	p.Val113Gly	Missense (HET)	9	Novel
2	*HNF1A*	NM_000545	Exon 3 c.697 G>C	p.Val233Leu	Missense (HET)	11	Novel
3	*HNF4A*	NM_000457	c.636 C>T	p.Pro825Leu	Missense (HET)	10	3/ 10 000
4	*INS*	NM_001185098	Exon 5 c.202 C>A	p.Leu68Met	Missense (HET)	7	Edghill EL *et al*; (2007) (rs121908279)
5	*PAX4*	NM_006193	Exon 5 c.593 C>T	p.Ala198Val	Missense (HET)	9	Novel

***5** mutazioni MODY*
2** nei geni responsabili delle forme **comuni
3** nei geni responsabili delle forme **non comuni

IPOTESI II:

Mutazioni nei geni responsabili del Diabete Neonatale

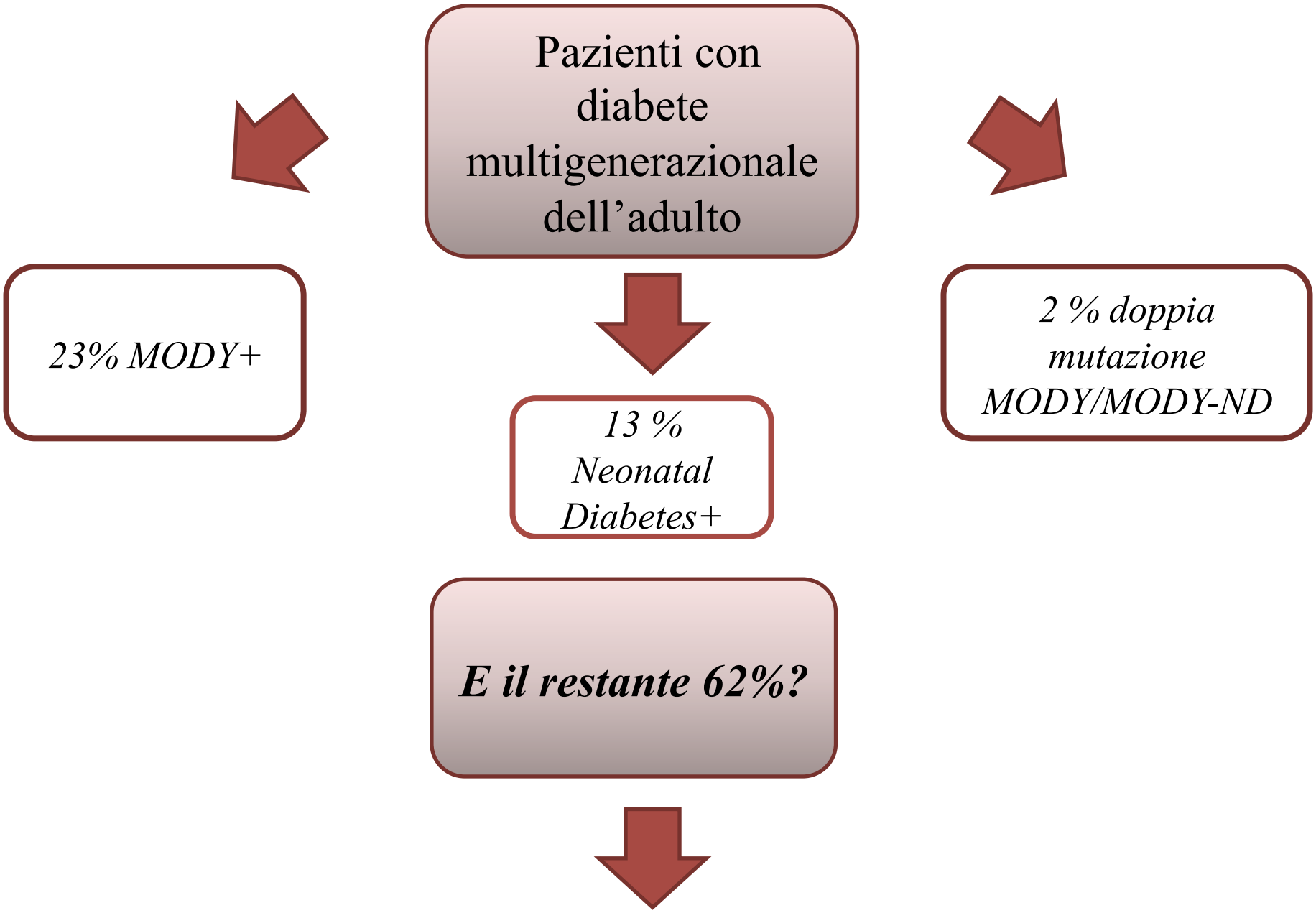
	GENE	REFERENCE SEQUENCE	NUCLEOTIDE CHANGE	AMMINOACID	EFFECT	PATHOGENICITY SCORE (0-13)	COMMENT
6	*FOXP3*	NM_014009	Exon 4 c323 C>T	pThr108Met	Missense (HET)	7	7/1 000 000 (rs374642910)
7	*GLIS3*	NM_001042413	Exon 4 c890 G>A	p.Arg297His	Missense (HET)	7	Novel
8	*GLIS3*	NM_001042413	Exon 4 c1186 C>T	p.Gln396Ter	Stop Gain (HET)	↑↑	Novel
9	*WFS1*	NM_001145853	Exon 6 c695 G>A	p.Arg232His	Missense (HET)	8	2/ 10 000 (rs375904080)
10	*WFS1*	NM_001145853	Exon 8 c. 2129 C>G	p.Thr710Leu	Missense (HET)	8	2,5/ 100 000 (rs200136995)
11	*WFS1*	NM_001145853	Exon 8 c1371 G>T	p.Arg457Ser	Missense (HET)	9	Giuliano F. *et al*; (2005) 2,3/ 10 000 (rs113446173)
12	*WFS1*	NM_001145853	Exon 4 c416 G>A	p.Arg139His	Missense (HET)	10	7/100 000 (rs374642910)

*7 mutazioni in **3** geni del Diabete Neonatale*

	GENE	REFERENCE SEQUENCE	NUCLEOTIDE CHANGE	AMMINOACID	EFFECT	PATHOGENICITY SCORE (0-13)	COMMENT
13	*ABCC8*	NM_000352	Exon 20 c.2473 C>T	p.Arg825Trp	Missense (HET)	12	8/1 000 000 (rs779736828)
	KLF11	NM_003597	Exon 2 c.51_53A ACAT>A	p.Ile17del	Nonframeshift Deletion (HET)	↑↑	8/1 000 000 (rs758083789)

Un paziente presenta
una mutazione nel gene ABCC8
ed una mutazione nel gene KLF11

RIASSUMENDO…….



IPOTESI III:

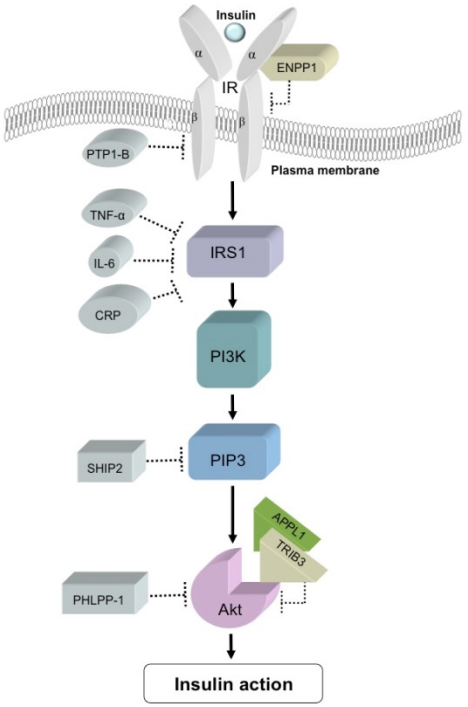
Mutazioni in geni coinvolti nell'omeostasi del glucosio ma non ancora conosciuti.

2015, Pages 177–185 REPORT

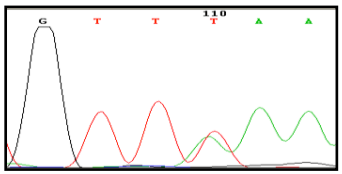
Loss-of-Function Mutations in *APPL1* in Familial Diabetes Mellitus

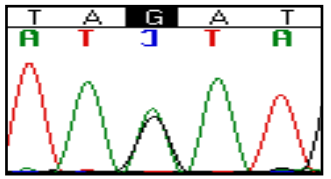
Sabrina Prudente,[1,*] Prapaporn Jungtrakoon,[2,3] Antonella Marucci,[4] Ornella Ludovico,[4] Patinut Buranasupkajorn,[2,3] Tommaso Mazza,[1] Timothy Hastings,[2] Teresa Milano,[5] Eleonora Morini,[4] Luana Mercuri,[1] Diego Bailetti,[1,6] Christine Mendonca,[2] Federica Alberico,[1] Giorgio Basile,[1,6] Marta Romani,[1] Elide Miccinilli,[1] Antonio Pizzuti,[1,6] Massimo Carella,[7] Fabrizio Barbetti,[8,9] Stefano Pascarella,[5] Piero Marchetti,[10] Vincenzo Trischitta,[1,4,6] Rosa Di Paola,[4] and Alessandro Doria[2,3,*]

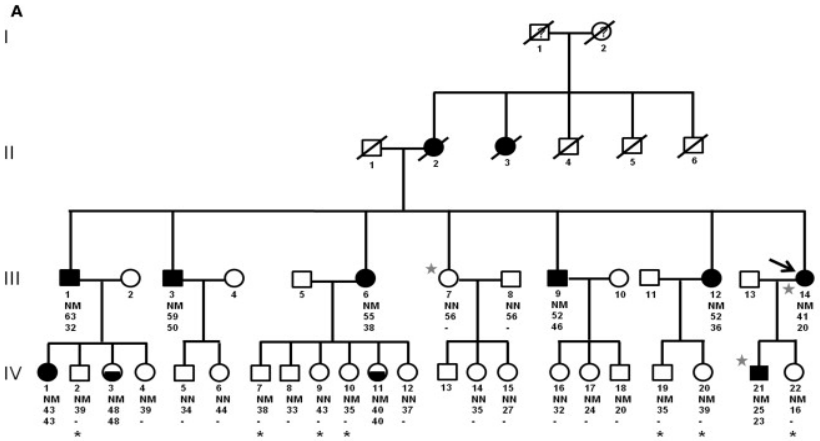
Prudente et al, AJHG 2015

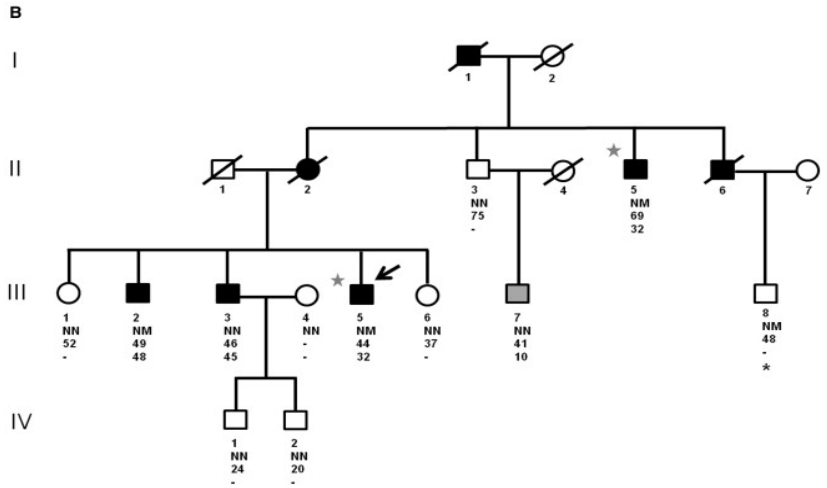


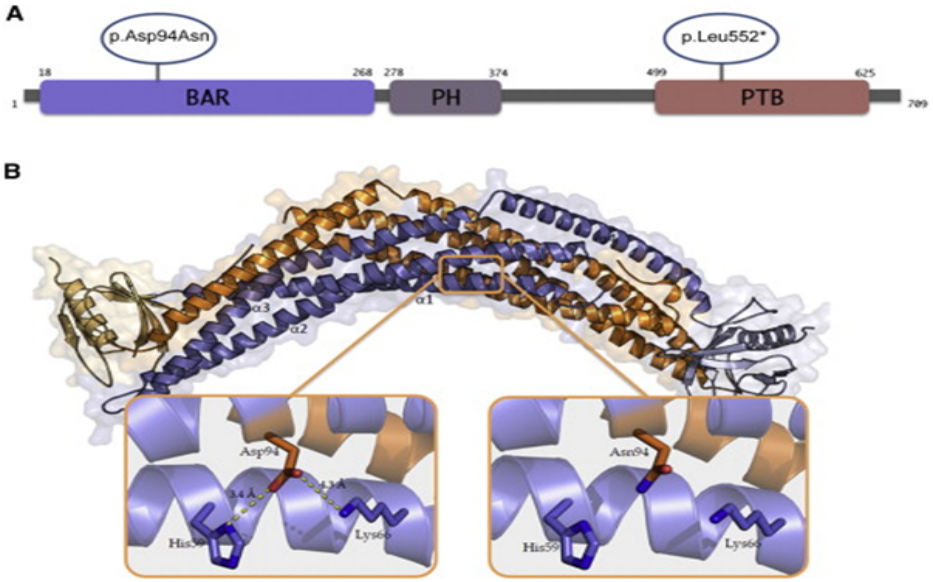
Gene	Mutation	Annotation	Family
APPL1	stopgain SNV	APPL1:NM_012096:exon17:c.T1655A:p.**L552X**	Italian
APPL1	nonsynonymous SNV	APPL1:NM_012096:exon5:c.G280A:p.**D94N**	US



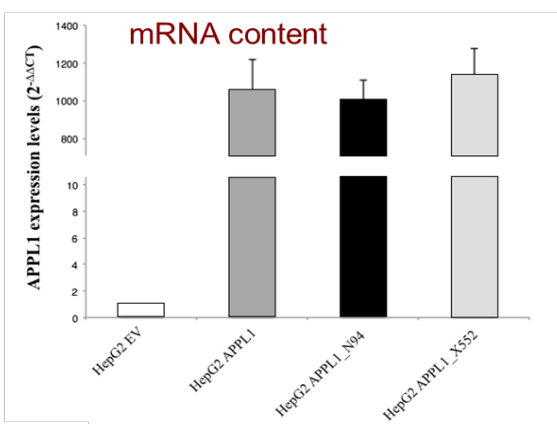


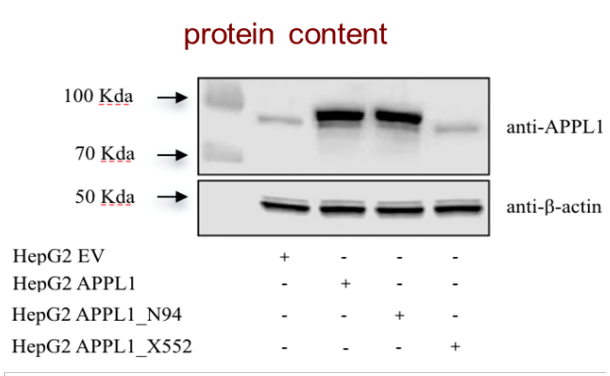




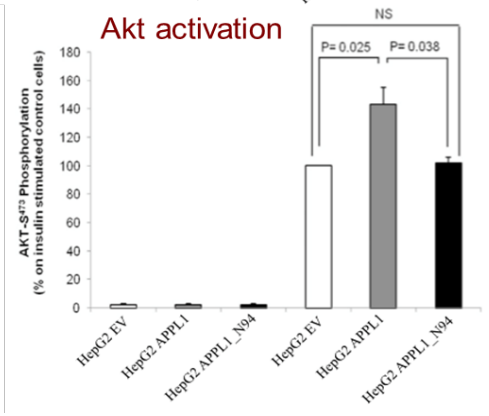


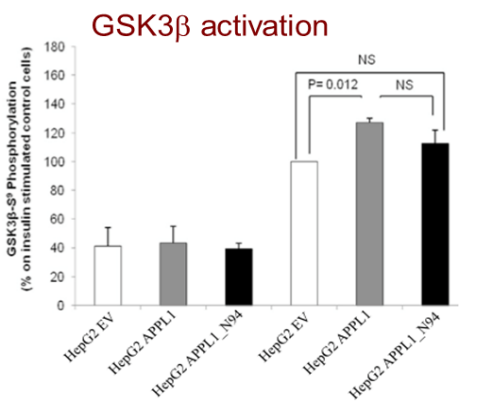
D94N
L552X





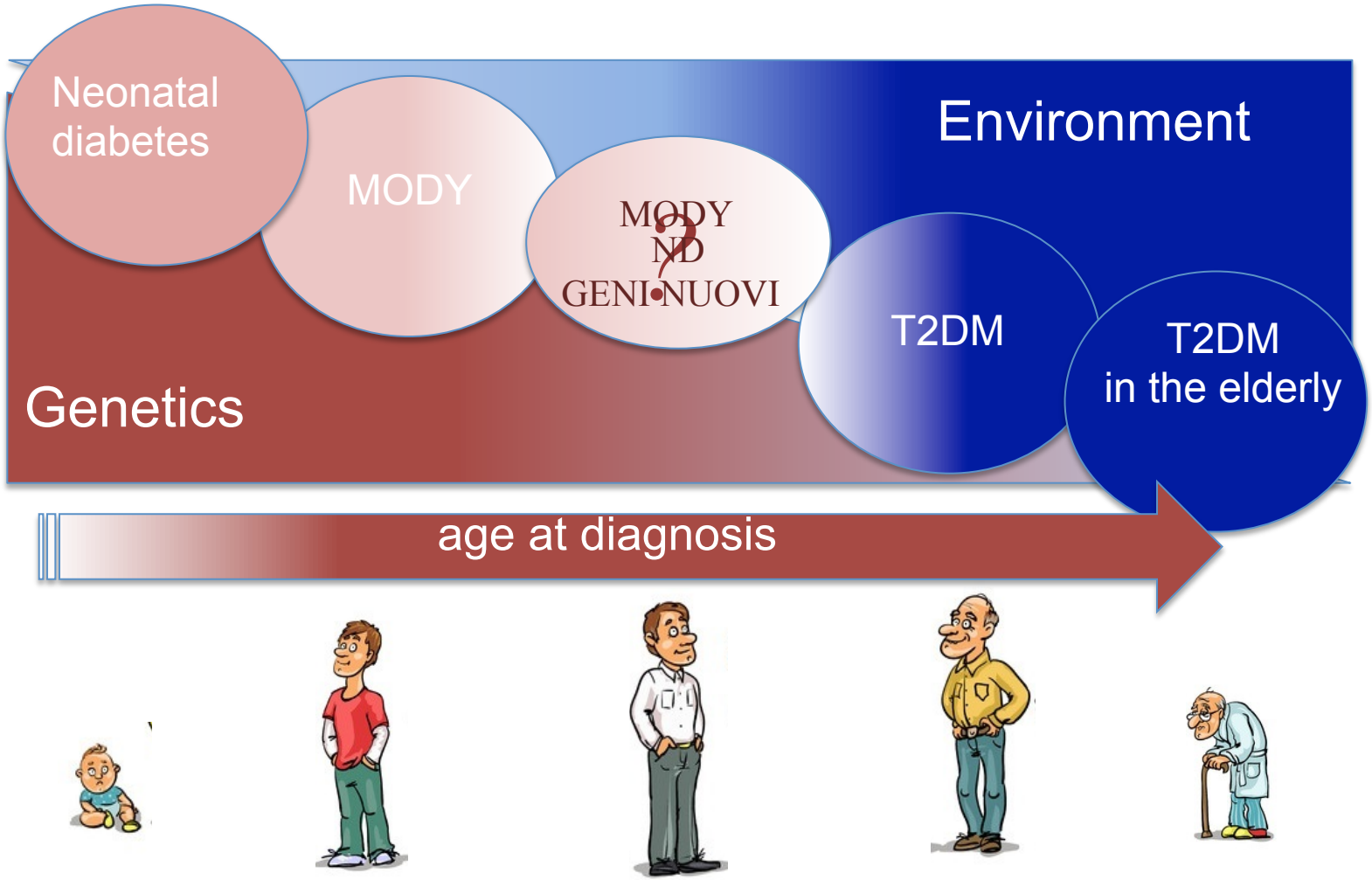
D94N





Prudente et al, AJHG 2015

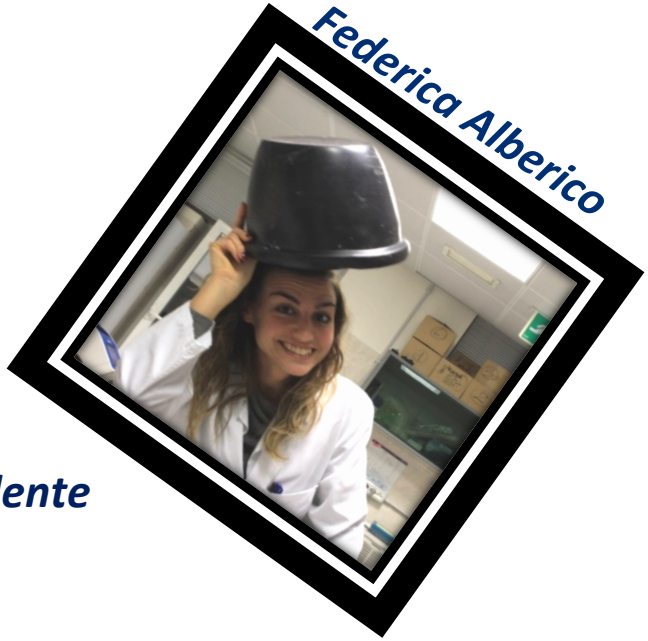
CONCLUDENDO……



Luana Mercuri

Giorgio Basile

Federica Alberico



Vincenzo Trischitta/Sabrina Prudente

Hamza Dallali

Eleonora Lauricella

GRAZIE MILLE PER L’ATTENZIONE