SUPPLEMENTARY DATA

Insights from Molecular Characterization of Adult Patients of Families with Multigenerational Diabetes Mellitus

Serena Pezzilli, Ornella Ludovico, Tommaso Biagini, Luana Mercuri, Federica Alberico, Eleonora Lauricella, Hamza Dallali, Daniele Capocefalo, Massimo Carella, Elide Miccinilli, Pamela Piscitelli, Maria Giovanna Scarale, Tommaso Mazza,
Vincenzo Trischitta, Sabrina Prudente.

SUPPLEMENTARY DATA

SUPPLEMENTARY TABLES

Supplementary Table S1. List of the 27 monogenic diabetes-genes included in the sequencing panel.

GENE	Ref Seq	Diabetes type	OMIM
ABCC8	NM_000352.4	MODY/ND	600509
APPL1	NM_012096.2	MODY	604299
BLK	NM_001715.2	MODY	191305
CEL	NM_001807.4	MODY	114840
EIF2AK3	NM_004836.6	SD	604032
FOXP3	NM_014009.3	SD	300292
GATA4	NM_001308093.1	ND	600576
GATA6	NM_005257.5	ND	601656
GCK	NM_000162.3	MODY/ND	138079
GLIS3	NM_001042413.1	SD	610192
HNF1A	NM_000545.6	MODY	142410
HNF1B	NM_000458.3	MODY/ND	189907
HNF4A	NM_000457.4	MODY	600281
IER3IP1	NM_016097.4	SD	609382
INS	NM_001185098.1	MODY/ND	176730
KCNJ11	NM_000525.3	MODY/ND	600937
KLF11	NM_003597.4	MODY	603301
NEUROD1	NM_002500.4	MODY/ND	601724
NEUROG3	NM_020999.3	ND	604882
PAX4	NM_006193.2	MODY	167413
PDX1	NM_000209.3	MODY/ND	600733
PTF1A	NM_178161.2	ND	607194
RFX6	NM_173560.3	SD	612659
SLC19A2	NM_006996.2	SD	603941
SLC2A2	NM_000340.1	SD	138160
WFS1	NM_001145853.1	SD/ADD	606201
ZFP57	NM_001109809.2	ND	612192

OMIM: Online Mendelian Inheritance in Men (https://www.omim.org); MODY: Maturity Onset Diabetes of the Young; ND: neonatal diabetes. SD: syndromic diabetes; ADD: autosomal dominant diabetes.

SUPPLEMENTARY DATA

Supplementary Table S2. Variant filtering and prioritization in 55 study-samples

Identified variants	Mean	SD
Total variants	186	16.8
High confidence variant calls	159.5	14.1
After exclusion of synonymous and intronic variants	20.5	2.9
After exclusion of variants with MAF >0.00005 (gnomAD- European Non Finnish)	0.42	0.3
Predicted damaging and confirmed by Sanger Sequencing	0.18	0.5

SD: standard deviation; gnomAD: The Genome Aggregation Database (http://gnomad.broadinstitute.org)

SUPPLEMENTARY DATA

Supplementary Table S3. Individual prediction scores for each missense variant presenting a total score >7 when subjected to 13 bioinformatic tools.

PATIENT	GENE	REF SEQ	NUCLEOTIDE CHANGE	AMINOACID CHANGE	PATHOGENICITY PREDICTION SCORES													
					SIFT	POLYPHEN2 HDIV	LRT	MUTATION TASTER	MUTATION ASSESSOR	FATHMM	FATHMM-MKL	PROVEAN	MetaSVM	VEST3	CADD	DANN	MetaLR	TOTAL SCORE
P-1	*HNF1A*	NM_000545.6	Exon 3 c.697 G>C	p.Val233Leu	1	1	1	1	1	1	1	0	1	0	1	1	1	11
P-2	*ABCC8*	NM_000352.4	Exon 20 c.2473 C>T	p.Arg825Trp	1	1	1	1	1	1	1	1	1	0	1	1	1	12
P-3	*BLK*	NM_001715.2	Exon 5 c.338 T>G	p.Val113Gly	1	1	0	1	1	0	1	1	1	0	1	1	0	9
P-4	*PAX4*	NM_006193.2	Exon 5 c.593 C>T	p.Ala198Val	1	1	0	1	0	1	1	0	1	0	1	1	1	9
P-5	*WFS1*	NM_001145853.1	Exon 8 c.2129 C>G	p.Thr710Ser	0	0	1	1	0	1	1	0	1	0	1	1	1	8
P-7	*FOXP3*	NM_014009.3	Exon 4 c.323 C>T	p.Thr108Met	1	1	0	0	0	1	0	0	1	0	1	1	1	7
P-8	*INS*	NM_001185098.1	Exon 2 c.202 C>A	p.Leu68Met	0	1	0	1	1	1	0	0	0	0	1	1	1	7

SUPPLEMENTARY DATA

Supplementary Table S4. Clinical and genetic characteristics of examined family members with available genotypic information.

Family	Family member	Mutation carrier	Gender	Age (yrs)	Age at diagnosis (yrs)	BMI (Kg/m^2)	Glycemic status	FPG	PG 2h-OGTT	HbA1c % (mmol/mol)	Hyperglycemia treatment	Hypertension	Dyslipidemia	Micro-/Macro-albuminuria
1	II-1 **(P-1)**	yes	M	58	22	22.9	DM	na	na	7.5 (58)	Ins+OADs	no	no	no
	III-8	no	F	33	na	22.5	NG	85	68	na	na	na	na	na
	III-9	no	M	23	na	19.3	NG	85	98	na	na	na	na	na
	III-10	yes	M	30	26	27.1	DM	na	na	6.1 (43)	OADs	no	no	no
	III-11	no	M	25	na	21.9	NG	78	72	na	na	na	na	na
	III-12	yes	M	23	18	24.9	DM	na	na	6.0 (42)	OADs	no	no	no
	III-13	no	M	20	na	22.4	NG	81	62	na	na	na	na	na
	IV-1	no	M	20	na	33.7	NG	86	78	na	na	na	na	na
	IV-2	yes	M	18	18	28.4	PD	112	126	na	na	no	no	no
2	IV-1 **(P-2)**	yes	F	18	18	25.8	DM	na	na	12.3 (111)	Ins+OADs	no	yes	no
	II-4	yes	F	68	55	22.9	DM	na	na	8.1 (65)	OADs	yes	yes	yes
	II-6	yes	F	66	51	30.0	DM	na	na	7.8 (62)	OADs	yes	yes	yes
	II-8	yes	F	72	60	22.8	DM	na	na	7.0 (53)	OADs	yes	yes	yes
	II-10	yes	F	60	u	u	DM	na	na	u	Ins	u	u	u
	III-1	yes	M	49	27	27.1	DM	na	na	11.3 (100)	Ins	no	yes	yes
3	II-2 **(P-3)**	yes	F	44	32	31.8	DM	na	na	7.9 (63)	Ins+OADs	yes	yes	no
	III-6	yes	F	22	22	24.8	DM	na	na	5.8 (40)	OADs	no	yes	yes
4	III-3**(P-4)**	yes	M	45	20	30.2	DM	na	na	8.4 (68)	OADs	yes	yes	yes
	III-4	yes	F	47	39	49.8	DM	na	na	5.6 (38)	Ins+OADs	yes	yes	yes
5	III-2 **(P-5)**	yes	M	52	38	31.9	DM	na	na	8.4 (68)	Ins+OADs	yes	yes	yes
	II-1	yes	M	80	50	30.4	DM	na	na	10.2 (88)	OADs	yes	no	no
	II-2	no	F	77	58	24.6	DM	na	na	9.9 (85)	OADs	yes	yes	no
	III-1	yes	F	42	33	30.0	DM	na	na	11.2 (99)	OADs	no	yes	no

SUPPLEMENTARY DATA

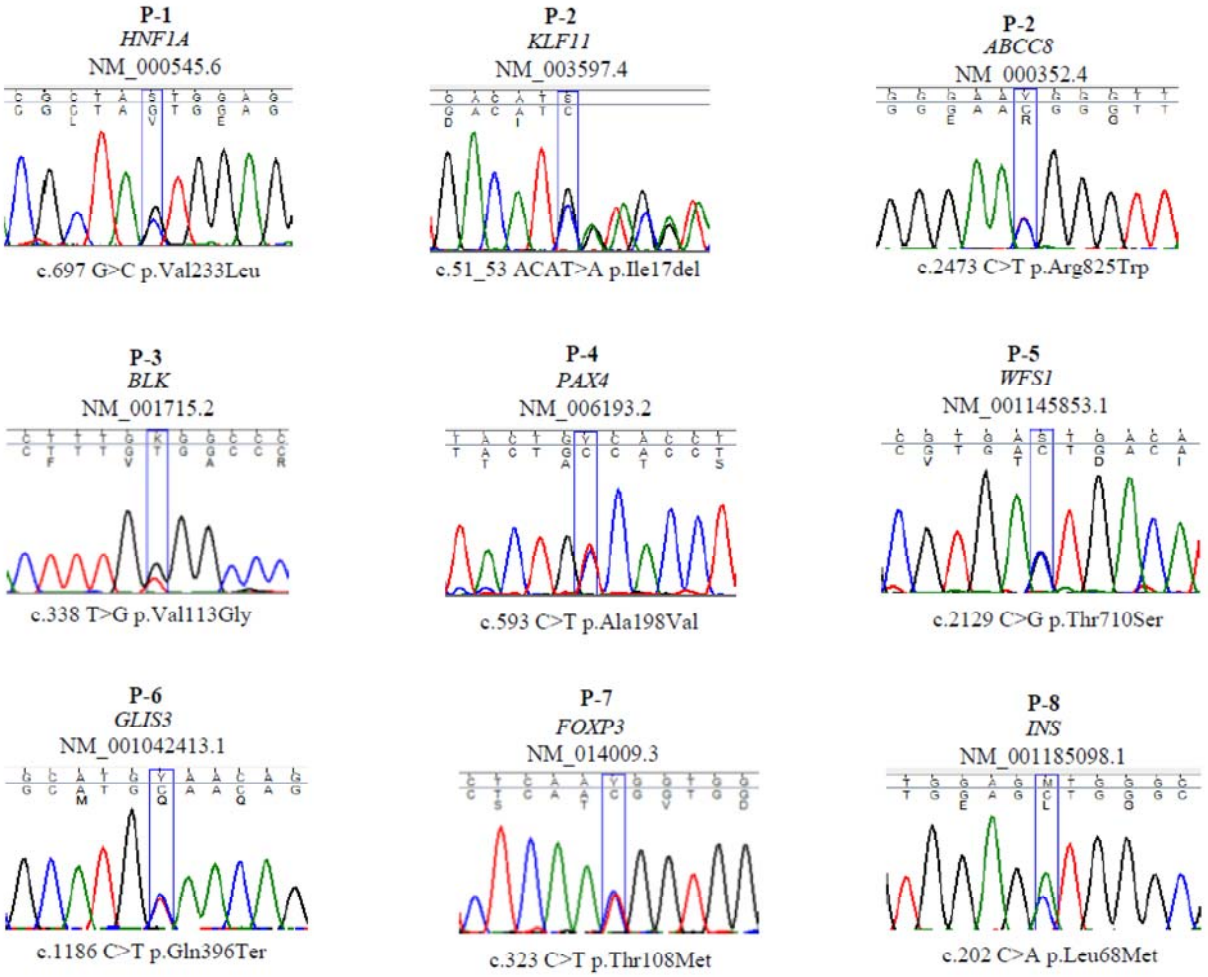
Family	Family member	Mutation carrier	Gender	Age (yrs)	Age at diagnosis (yrs)	BMI (Kg/m^2)	Glycemic status	FPG	PG 2h-OGTT	HbA1c (%) mmol/mol	Hyperglycemia treatment	Hypertension	Dyslipidemia	Micro-/Macro-albuminuria
6	IV-3 **(P-6)**	yes	M	48	28	35.5	DM	na	na	11.0 (97)	Ins+OADs	yes	yes	yes
	III-8	no	M	79	44	34.3	DM	na	na	8.4 (68)	Ins+OADs	yes	yes	yes
	III-9	no	F	77	50	37.1	DM	na	na	7.9 (63)	Ins+OADs	yes	yes	yes
	IV-2	no	M	53	53	28.0	PD	na	na	5.8 (40)	na	no	yes	yes
	IV-4	no	F	45	35	35.9	DM	na	na	7.0 (53)	diet	no	yes	no
	IV-5	no	F	42	42	43.8	PD	na	na	6.6 (49)	na	no	yes	yes
	IV-6	no	M	41	41	34.3	PD	na	na	6.0 (42)	na	yes	yes	yes
7	IV-3 **(P-7)**	yes	F	64	40	30,4	DM	na	na	9.2 (77)	Ins+OADs	yes	yes	yes
	IV-1	yes	F	41	26	21,5	DM	na	na	5.6 (38)	OADs	no	no	yes
8	III-1 **(P-8)**	yes	F	24	20	46,1	DM	na	na	6.7 (50)	OADs	no	yes	yes

BMI: body mass index; NG: normoglycemic; DM: diabetes mellitus; PD: pre-diabetes (as defined, according to ADA 2017 criteria, when presenting impaired fasting glucose or impaired
glucose tolerance or HbA1c level > 5.7%); OGTT: oral glucose tolerance test; FPG: fasting plasma glucose; PG: plasma glucose; HbA1c: glycated haemoglobin; Ins: insulin; OAD: oral
antidiabetes drugs; nt: not assessed; u: unavailable.
Hypertension was diagnosed if individuals were currently receiving anti-hypertensive drugs or if their systolic or diastolic blood pressure was >130/85 mmHg.
Dyslipidemia was diagnosed if individuals were currently receiving anti-lipid agents or if they had total cholesterol >200 mg/dl, or triglycerides >150 mg/dl, or HDL-cholesterol <40 mg/dl
for male and <50 mg/dl for female.
Micro- and macro-albuminuria was diagnosed if morning urinary albumin creatinine ratio (ACR; mg/mmol) was >2.5/3.5 in males and female and >30 in both sexes, respectively.

SUPPLEMENTARY DATA

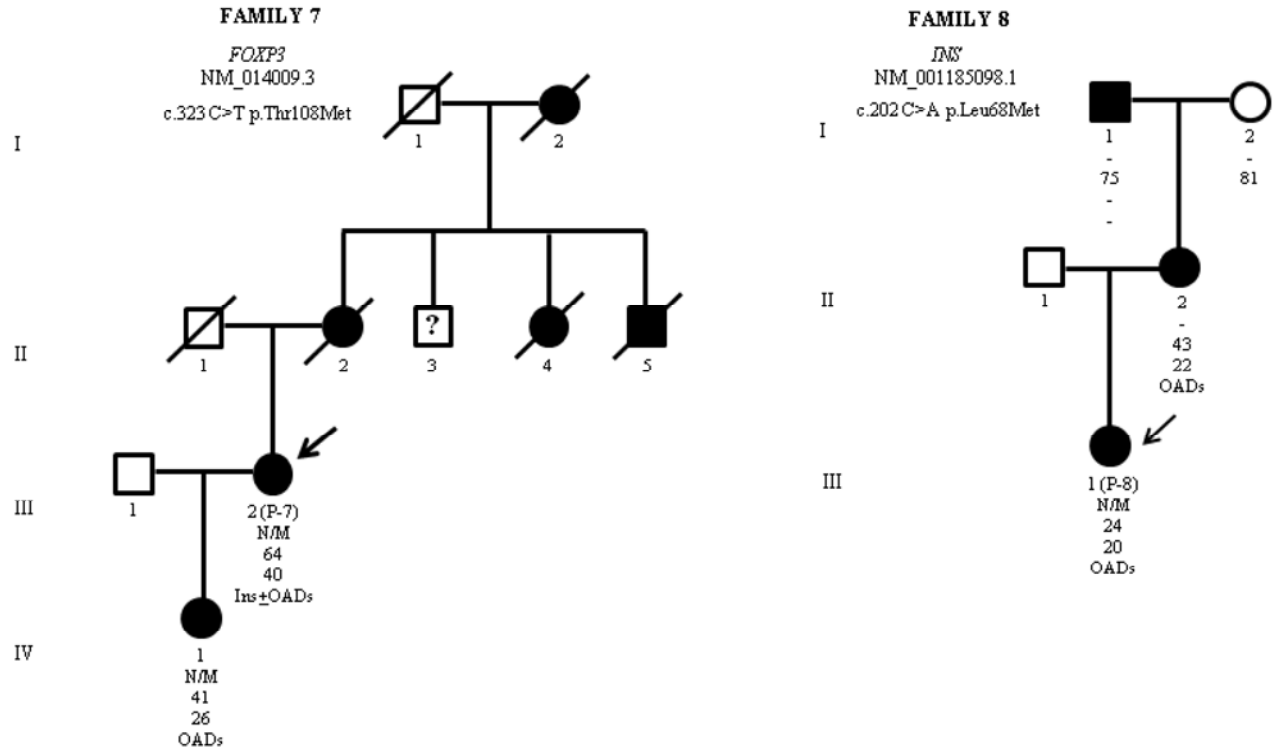
SUPPLEMENTARY FIGURES

Supplementary Figure S1. Sanger Sequencing confirmation of the 9 variants, which survived to filtering/prioritization pipeline.



SUPPLEMENTARY DATA

Supplementary Figure S2. Partial pedigrees of the 2 proband's families carrying VUS.



Filled symbols represent patients with diabetes. The genotype is shown underneath each symbol. N/M denotes mutation, while N/N denotes no mutation.
Glycemic status of individuals with unavailable genotype were reported by the family probands.
Below the genotype are: age at observation, age at diabetes diagnoses and the specific anti-hyperglycemia treatment. Arrow indicates the study proband.
VUS: variant of uncertain significance; Ins: insulin treatment; OADs: oral antidiabetes drugs.