Heterozygous *RFX6* mutation as a cause of Diabetes Mellitus in a multigenerational family

Zuckerman Levin N [1, 4], Paperna T [2], Hershkovitz T [2, 4], Mory A [2], Kurolap A [2, 4], Mahamid J [3], Baris Feldman H [2, 4], Shehadeh N [1, 4]

[1]Pediatric Diabetes Clinic, Institute of Diabetes, Endocrinology and Metabolism, Rambam Health Care Campus, [2] The Genetics institute, Rambam Health Care Campus, [3] Meuhedet health services, [4] The Ruth and Bruce Rappaport Faculty of Medicine, Technion, Haifa

Background

Monogenic diabetes mellitus (DM) is an early-onset, non- autoimmune diabetes. Genetic diagnosis can personalize patient management and lead to prevention. We describe four generations of DM in one family, caused by a heterozygous mutation in the *RFX6* gene. RFX6 (Regulatory factor X, 6) is essential for the development of the endocrine pancreas. Mutations in *RFX6* can cause neonatal (Mitchell-Riley syndrome) as well as childhood DM, intestinal atresia and hepatobiliary abnormalities. Heterozygous mutations in *RFX-6* were described as associated with MODY with reduced penetrance.

Patients

Transient, stress hyperglycemia was the first clinical presentation of our patient at the age of 3 years. Non-autoimmune DM was diagnosed at 13 years.

Maternal family history revealed great-grandmother, grandmother and a mother, two aunts and one cousin with DM. They were diagnosed as diabetics in adolescence or young adulthood. Only the patient's mother was treated by insulin.

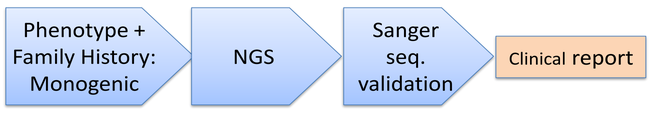
Methods

Next generation sequencing (NGS) panels for genes of monogenic DM, using the Trusight One platform (Illumina), was utilized for genetic analysis of the proband.

MODY - 15 genes panel:
ABCC8, APPL1, BLK, CEL, ***GCK****,* ***HNF1A****,* ***HNF1B****,* ***HNF4A****, INS, KCNJ11, KLF11, NEUROD1, PAX4, PDX1, RFX6*
Expanded panel of 23 additional genes and phenotypic search

Analysis :In house-data relying on pipeline, and local database of >1500 Israeli population samples.

Sanger sequencing was performed to validate the likely- pathogenic finding and for segregation analysis in the family.

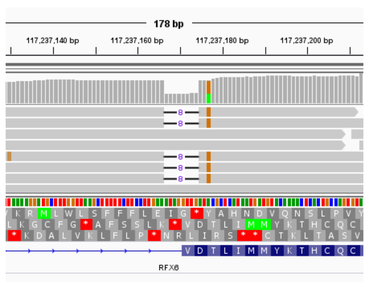


Results

We identified a heterozygous mutation in *RFX6* gene (c.781-2_787delinsG affecting intron7/exon 8) in the proband that co-segregated in five family members with DM, and in the patient's healthy brother and three young cousins. One uncle who carries the mutation has asymptomatic DM.

This mutation was previously reported to cause autosomal recessive neonatal diabetes.

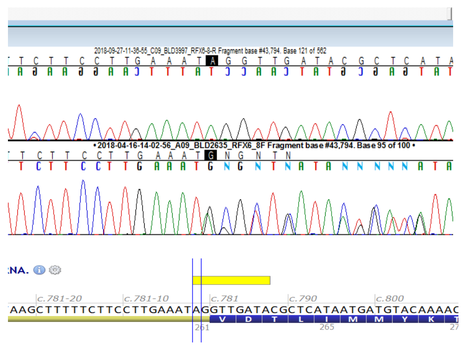
NGS sequencing



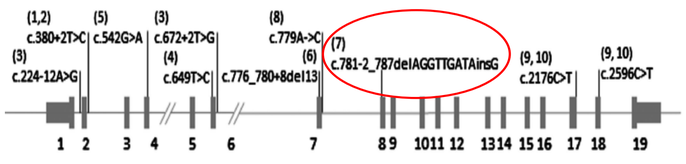
NM_173560.3(RFX6):c.781-2_787delinsG

- Highly likely to affect splicing
- Skip of exon 8 (26 amino acids, conserved) likely

Sanger



RFX6 gene mutations



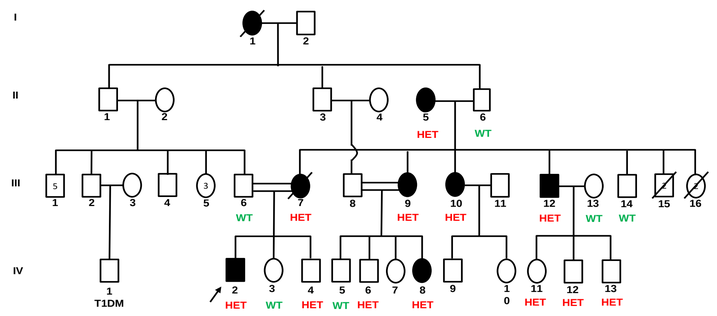
Inframe deletion of 9 nucleotides , insertion of G, across intron 7/ exon 8

Spiegel R et al, Am J Med Genet 2011

Sansbury FH, EJHG 2015

Paperna T ,Mory A, Baris Feldman H 2018

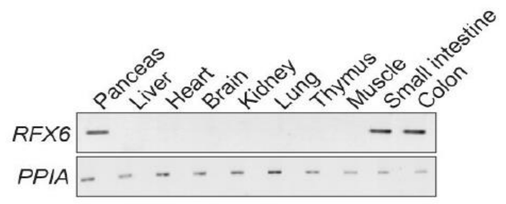
Family pedigree



RFX-6 (NM_173560): c.781-2_787delinsG
(heterozygous state)

RFX6 = Regulatory factor X.6

- A transcription factor that is encoded by the *RFX6* gene located on chromosome 6.
- RFX6 regulates in mammals (mice and human) :
  Development of the endocrine pancreas, islet cell differentiation
- RFX6 is also expressed in small intestine and colon



- Biallelic mutations in *RFX6* (Mitchell- Riley syn.) cause neonatal diabetes due to pancreatic hypoplasia, intestinal atresia and hepatobiliary abnormalities

*Mitchell J, Diabetologia 2004,*Smith SB, Nature 2010, *Spiegel R, Am J Med Genet 2011, *Sansbury FH, EJHG 2015. *Concepcion JP, Pediatr Diab 2015

Clinical characteristics of patients with heterozygous RFX6 -MODY

Characteristic	($n = 27$)
Age at diagnosis (years), median (IQR)	32 (24–46)
Duration of diabetes, median (IQR)	10 (5–22)
Female, *n* (%)	22 (81%)
BMI (kg/m^2), median (IQR)	25.1 (23–28) $n = 22$
Initial treatment, n (%)	
Diet	4 (15%)
Oral hypoglycaemic agents	14 (54%)
Insulin	5 (19%)
Insulin + oral hypoglycaemic agents	3 (12%)
Current treatment, n (%)	
Diet	2 (8%)
Oral hypoglycaemic agents	6 (23%)
Insulin	12 (46%)
Insulin + oral hypoglycaemic agents	6 (23%)
HbA1c at recruitment, mmol/mol, median (IQR)	51 (45–70) $n = 22$
Significant endogenous insulin at recruitment[a], *n* (%)	24 (96%) $n = 25$

BMI, body mass index; IQR, interquartile range; MODY, maturity-onset diabetes of the young
[a]Non-insulin treated or insulin treated with urine/blood random C-peptide > 200 pmol/l at recruitment

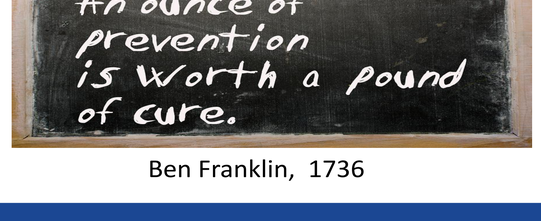
Patel KA Nature Comm.2017

Conclusions

- Heterozygous *RFX6* mutation was diagnosed as the cause of familial DM
- Genetic evaluation of youth with non – autoimmune DM provides accurate diagnosis and identifies subjects at risk.

Take home messages

- Diabetes mellitus prevalence is increasing
- Accurate family history provides clues to diagnosis
- Investigating the genetic etiology of diabetes is important and may pave the way to cure
- Diagnosing the correct type of diabetes has an impact on: Personalized treatment , accurate prediction of diabetes risk in unaffected carriers and education of the families
- Diagnosis of family at risk can lead to prevention !



Ben Franklin, 1736

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