European Journal of Medical Genetics 64 (2021) 104194

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

PDX1-MODY: A rare missense mutation as a cause of monogenic diabetes

Gabriella de M. Abreu Miss, MSc. [a,*], Roberta M. Tarantino [b,c], Ana Carolina P. da Fonseca [a,d], Ritiele B. de Souza [a], Camila A.P.D. Soares [a], Pedro H. Cabello [a,e], Melanie Rodacki [b], Lenita Zajdenverg [b], Verônica M. Zembrzuski [a], Mário Campos Junior [a]

[a] Human Genetics Laboratory, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil
[b] Diabetes and Nutrology Section, Internal Medicine Department, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
[c] Ambulatory of Diabetes, State Institute for Diabetes and Endocrinology Luiz Capriglione, Rio de Janeiro, Brazil
[d] Laboratory of Immunopharmacology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil
[e] Laboratory of Genetics, School of Health Science, University of Grande Rio, Rio de Janeiro, Brazil

ARTICLE INFO

Keywords:
Diabetes mellitus
Monogenic diabetes
MODY
PDX1
MODY4
Mutation

ABSTRACT

Maturity-Onset Diabetes of the Young type 4 is a rare form of diabetes mellitus, caused by mutations in the *PDX1* gene. However, only a few mutations in this gene have been associated as a cause of monogenic diabetes up to date. It makes difficult to create a clinical manifestation profile of this disease and, consequently, to improve the therapeutic management for these patients. Here we report a normal weight woman, diagnosed with diabetes mellitus at 27 years old, during her first pregnancy. At the time of the recruitment, she was 40 years old and had a body mass index of 23.9 kg/m^2, glycated hemoglobin level of 9.6%, and fasting plasma glucose (FPG) of 254 mg/dL. She presented no diabetic complications and she was being treated with insulin. She reported a family history of diabetes mellitus characteristic of an autosomal dominant mode of inheritance. Molecular analysis of the *PDX1* gene revealed the missense variant c.532G > A (p.(Glu178Lys)) segregating from the patient to her son, reported as diabetic. It was absent in her healthy daughter. The c.532G > A seems to be a rare variant, absent in human variants databases, and among 86 normoglycemic controls. Eight in silico algorithms classified this variant as probably pathogenic. Additionally, analysis of the evolutionary conservation showed the glutamic acid in the position 178 of PDX-1 protein as conserved among several species. Our findings reinforce the importance of screening rare MODY genes among families with suspicion of monogenic diabetes to help better understand the clinical manifestations of this disease.

1. Introduction

Mutations in genes that express transcription factors that disrupt the insulin metabolism have been recognized as genetic causes of diabetes mellitus (DM) monogenic forms (Firdous et al., 2018). Among these genes, *Pancreatic and Duodenal Homeobox 1* (*PDX1*; OMIM *600733), also known as Maturity-Onset Diabetes of the Young 4 (MODY4; OMIM #606392), encodes PDX-1 protein that is required for normal pancreatic β-cell development and function (Stoffers et al., 1997b).

PDX-1 is a homeodomain transcription factor implicated in the regulation of genes expressed in pancreatic β-cells, such as Glucose Transporter 2 (GLUT2) (Waeber et al., 1996). Studies in mice showed that Pdx1$^{-/-}$ mutants had pancreas agenesis (Jonsson et al., 1994) and Pdx1$^{+/-}$ mice presented normal fasting blood glucose (FBG) (Brissova et al., 2002). However, Pdx1$^{+/-}$ mice also showed impaired glucose tolerance (IGT), reduction in insulin secretion, and lower expression of Pdx1 and Glut2 (Brissova et al., 2002).

To date, PDX1 variants segregating with monogenic diabetes (PDX1-MODY) remains limited to a few families worldwide (Anık et al., 2015; Caetano et al., 2018; Chapla et al., 2015; Deng et al., 2019; Fajans et al., 2010; Schwitzgebel et al., 2003; Stoffers et al., 1997a). Due to its rarity, the clinical characteristics of patients harboring PDX1 mutations remain still unclear. In this context, this study aimed to sequence the coding region of the *PDX1* gene in a sample from Brazil with clinical manifestations of monogenic diabetes.

* Corresponding author. Human Genetics Laboratory, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Leônidas Deane Building, room 615, 21040-360; 4365 Brasil Avenue, Rio de Janeiro, RJ, Brazil.
E-mail address: gabriella.abreu@ioc.fiocruz.br (G.M. Abreu).

https://doi.org/10.1016/j.ejmg.2021.104194
Received 26 August 2020; Received in revised form 4 March 2021; Accepted 14 March 2021
Available online 18 March 2021
1769-7212/

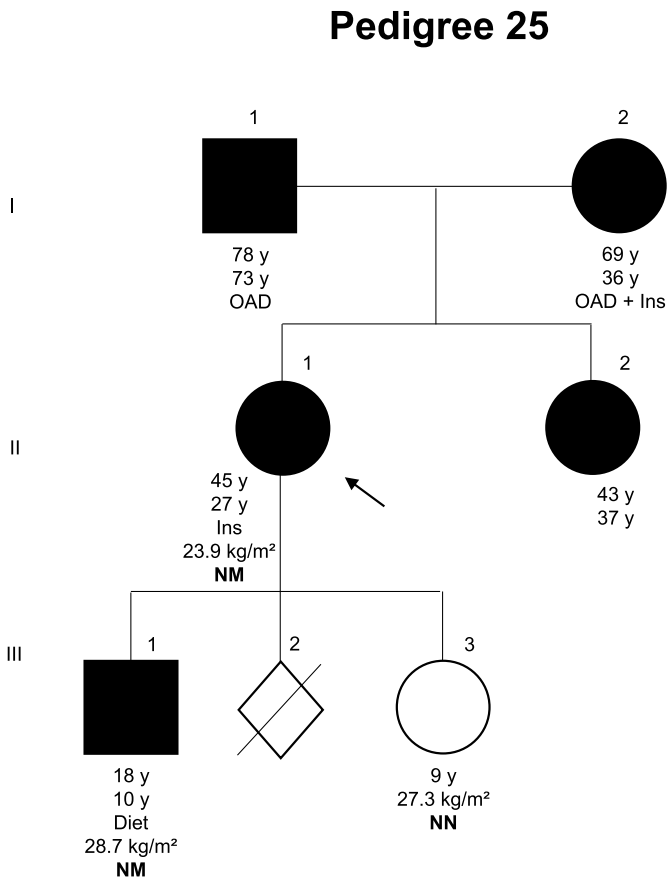

Fig. 1. Family pedigree P25 with PDX1 c.532G > A p.(Glu178Lys) mutation. Filled symbols represent patients with diabetes and empty symbols are non-diabetic individuals. A triangle with a slash extending though symbol indicates neonatal death. The present age of the individuals are shown below the symbols (years), followed by age of diagnosis (years), the most recent treatment, body mass index (kg/m^2) and genotype interpretation. OAD: oral anti-diabetic agents; Ins: insulin; MN: heterozygous for PDX1 c.532G > A; NN: normal for PDX1 c.532G > A; An arrow indicates the index case.

2. Patient's data

The patient P25 is a normal weight woman of 46 years old from the Northeast region of Brazil. She was diagnosed with DM at 27 years old during her first pregnancy. She was treated with metformin and presented negative anti-GAD (Glutamic Acid Decarboxylase) and anti-IA-2 (Islet Antigen-2). During her second pregnancy, at 33 years old, her baby died at delivery. She reported that hyperglycemia was measured at that time and she initiates insulin therapy. At her third pregnancy, she was 34 years old, and gave birth to a girl of 2.795 kg by cesarean section delivery at term. During this pregnancy, she showed a glycated hemoglobin (HbA1c) level of 6.2%, fasting plasma glucose (FPG) of 110 mg/dL and she was managed with insulin 0.4 U/kg/d (total dose of NPH insulin 24 U/d and total regular fast-acting insulin of 2 U/d). At the time of recruitment for this study, she was 40 years old, her body mass index (BMI) of 23.9 kg/m^2, HbA1c level of 9.6%, FPG of 254 mg/dL, thyroid-stimulating hormone (TSH) of 0.625 mIU/L, free thyroxine 4 (Ft4) of 1.23 ng/dL and thyroid anti-peroxidase (anti-TPO) negative (14.5 IU/mL). She was on 0.6 U/kg/d basal bolus insulin treatment (total dose of NPH insulin 18 U/d and total regular fast-acting insulin of 22 U/d). By the time of her last medical evaluation, she was 46 years old; she presented an HbA1c level of 9.9%. She was managed with 1.6 U/kg/d basal bolus insulin treatment (total dose of NPH insulin 64 U/d and total regular fast-acting insulin of 30 U/d). The evolution of HbA1c versus the treatment with insulin over the years is shown in the Supplemental Graphic S1. After 19 years since her diagnosis, she presented no diabetic microvascular complications, and normal fundoscopy. Analysis of the proband's pedigree suggest an autosomal dominant inheritance of diabetes (Fig. 1). The patient reported both parents with diabetes; her mother was diagnosed at 36 years old and has been treated with insulin and metformin, while her father was diagnosed at 73 years old, and he has been managed with metformin. The proband also reported her older child, diagnosed with diabetes at 10 years old and one sister diagnosed at 37 years old. Her 18-year-old son is overweight (BMI: 28.6 kg/m^2). At the age of 16 years, on his last clinical evaluation, he showed negative anti-GAD and anti-IA-2, C-peptide of 0.8 ng/mL, HbA1c ranging from 5.3% to 5.8%, FPG ranging from 105 to 113 mg/dL, and postprandial glucose (PPG) of 140 mg/dL. He has been managing the hyperglycemia with nutritional therapy; however, he reported to have abandoned treatment and medical care for the past two years. At the admission on this study, his random capillary blood glucose (RCBG) was 211 mg/dL. At that moment, it was reported that the overweight proband daughter did not present hyperglycemia (BMI: 27.3 kg/m^2; postprandial capillary glucose [PCG]: 138 mg/dL).

3. Ethics statement

This study protocol was approved by The Ethics and Research Committee of the Clementino Fraga Filho University Hospital (CAAE n° 04232512.4.0000.5257) and by the State Institute for Diabetes and Endocrinology Luiz Capriglione (CAAE n° 04232512.4.3001.5266). The participants were informed about the aim of this study and provided verbal and written consent.

4. Methods

4.1. Patient's recruitment

In the present cross-sectional observational study, we recruited 43 unrelated Brazilian patients with clinical characteristics of monogenic diabetes (18 males [41.9%] and 25 females [58.1%]; average age of diagnosis [AOD]: 21 ± 10.3 years) from the Clementino Fraga Filho University Hospital and from the State Institute for Diabetes and Endocrinology Luiz Capriglione. Patients who fulfilled all inclusion criteria were: 1) age at onset ≤ 40 years; 2) positive family history of diabetes in at least two generations; and 3) negative β-cells anti-GAD and anti-IA-2 autoantibodies. Patients with type 1 DM, obesity (BMI ≥ 30 kg/m^2 or ≥ 95th percentile for age at diagnosis), history of diabetic ketoacidosis at diabetes onset, clinical signs of insulin resistance and the presence of secondary causes of diabetes were excluded. All patients were previously screened for *GCK* or *HNF1A* (based on the clinical phenotype), *HNF4A* and HNF1B, and no mutations were observed. Family members of mutation carriers were recruited to this study for segregation analysis. Eighty-six controls (42 males and 44 females; average age: 32 ± 9 years; BMI average: 22.6 ± 1.7 kg/m^2) without DM were screened to investigate the possible recurrence of the observed mutations in healthy individuals. Inclusion criteria for the control subject were as follows: 1) FPG < 100 mg/dL and HbA1c < 5.7%; 2) BMI ≤ 24.9 kg/m^2; and 3) individuals without a family history of DM.

4.2. Molecular genetics

Genomic DNA from the probands and normoglycemic controls were isolated from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Genomic DNA from the proband's family members were collected and extracted from buccal epithelial cells as previously described (Aidar and Line, 2007). The entire coding region of the *PDX1* gene was sequenced by Sanger sequencing.

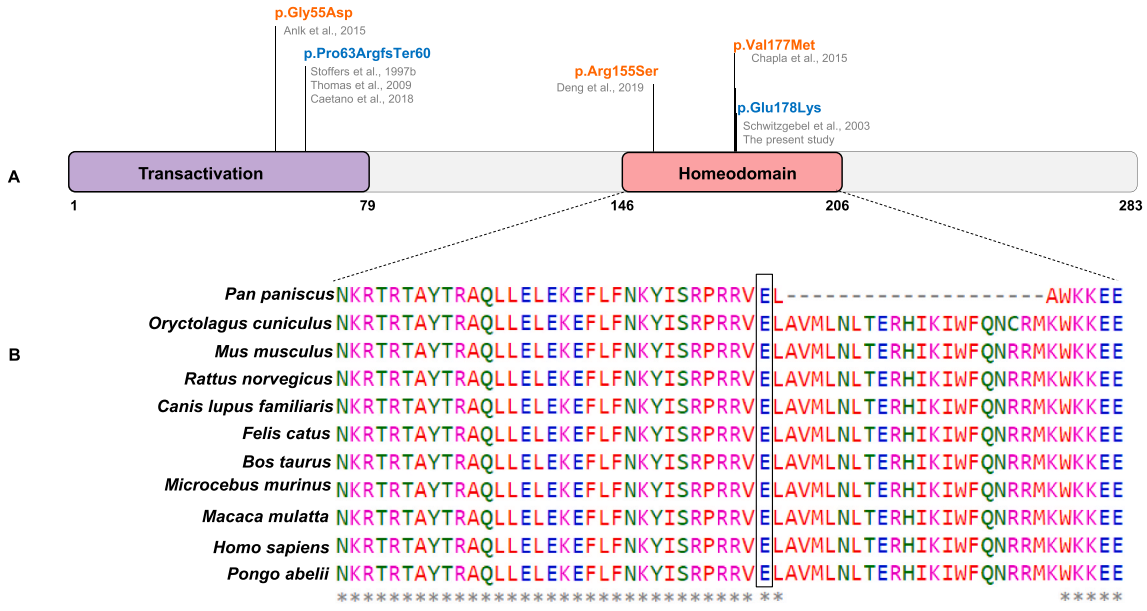

Fig. 2. Schematic representation of PDX-1 protein and location of the mutations as reported in the literature associated to MODY (orange) and MODY and Neonatal Diabetes (blue) (A). Alignment of PDX-1 homeodomain across species by Clustal Omega. Black dashed indicated the amino acid position of PDX1 p.(Glu178Lys) identified in the patient P25. *Pan paniscus* (ENSPPAP00000019094); *Oryctolagus cuniculus* (ENSOCUP00000004229); *Mus musculus* (ENSMUSP00000082729); *Rattus norvegicus* (ENSRNOP00000066935); *Canis lupus* familiaris (ENSCAFP00030032499); *Felis catus* (ENSFCAP00000029882); Bos Taurus (ENSBTAP00000014141); *Microcebus murinus* (ENSMICP00000041397); *Macaca mulatta* (ENSMMUP00000078156); *Homo sapiens* (ENSP00000370421); Pongo abelii (ENSPPYP00000005967) (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4.3. Bioinformatics analysis

In order to investigate the previous occurrence of PDX1 variants identified in our study, they were checked against the following public databases: PubMed, Clinvar, dbSNP (https://www.ncbi.nlm.nih.gov/), Human Genome Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/), gnomAD (https://gnomad.broadinstitute.org/), and the Online Archive of Brazilian Mutations (ABraOM; http://abraom.ib.usp.br/) (Naslavsky et al., 2017). Missense variants that were absent in the control group and were not classified as likely benign by ClinVar were considered to have a potential impact on the protein and were further tested by in silico predictions. Eight different in silico pathogenicity prediction algorithms were used: 1) Revel (Ioannidis et al., 2016); 2) WS-SNPs&GO (Capriotti et al., 2013); 3) MutPred (Pejaver et al., 2017); 4) SNAP (Bromberg and Rost, 2007); 5) M-CAP (Jagadeesh et al., 2016); 6) CADD (Rentzsch et al., 2019); 7) Align-GVGD (Tavtigian et al., 2008); and 8) PANTHER-PSEP (Tang and Thomas, 2016). The Ensembl *PDX1* transcript ENST00000381033.4 (NM_000209; GRCh37.p13) was used as reference (https://www.ensembl.org/index.html). In addition, to evaluate amino acid conservation we performed a multiple sequence alignment by Clustal Omega (Version 1.2.4) (https://www.ebi.ac.uk/Tools/msa/clustalo/). The Ensembl reference transcript of the *PDX1* gene ENST00000381033.5 (PDX1; NM_000209.4; GRCh38.p13) was used as reference.

5. Results

The p.(Glu178Lys), NG_008183.1 (NM_000209.4): c.532G > A identified in the patient P25 seems to be potentially pathogenic (Supplemental Fig. S1), because it was: 1) absent in ABRaOM and gnomAD databases; 2) classified as pathogenic by ClinVar (Supplemental Table S1); 3) absent in healthy individuals; 4) predicted to be probably pathogenic for all eight in silico algorithms (Supplemental Table S2); 5) the glutamic acid in the position 178 of PDX-1 homeodomain is evolutionary conserved among several species (Fig. 2); 6) and the NM_000209.4: c.532G > A segregates with the disease in the tested individuals (Fig. 1). However, we were not able to recruit and test others relatives with diabetes as the patient's father, mother, and sister (Fig. 1. Individuals I-1, I-2, II-2, respectively).

6. Discussion

Since its first report (Stoffers et al., 1997b), variants in the *PDX1* gene have been associated to multiples types of DM, including monogenic forms of the disease (Fig. 2). The PDX1 c.532G > A identified in the present study was firstly found in a compound heterozygous girl (PDX1 c.492G > T [p.Glu164Asp] and c.532G > A [p.Glu178Lys]) diagnosed with Neonatal DM (NDM). She presented intrauterine growth retardation and pancreatic agenesis. She was treated with insulin and replacement of pancreatic exocrine enzymes. Each mutation was inherited from her non-consanguineous parents, who had high normal fasting blood sugar levels and no glucose intolerance at that moment. Her father (37 years old; BMI: 26 kg/m^2) carried the c.532G > A and presented a family history of type 2 DM (T2-DM) (Schwitzgebel et al., 2003). This mutation seems to decrease PDX-1 half-life, which could prevent the proper PDX1 self-activation and consequently a decrease in protein level (Schwitzgebel et al., 2003).

Nicolino et al. (2010) identified the PDX1 c.533A > G (p.Glu178Gly) missense mutation in the same codon of the variant identified in our patient P25 (p.(Glu178Lys)). It was found in homozygosis in two Moroccan cousins with Permanent Neonatal Diabetes Mellitus (PNDM); both were underweight for the gestational age and presented pancreatic exocrine enzymes insufficiency. The proband had normal pancreas size; however, an ultrasound of his cousin revealed the presence of the pancreas head but was unable to show the body and the tail. The cousins parents carried the c.533A > G in heterozygosis and had normal FPG and normal glucose tolerance; however, they presented low insulin secretory response during oral glucose tolerance test (OGTT) (Nicolino et al., 2010).

Until now, at least six probands with clinical manifestations of MODY and two probands with NDM with family members with MODY phenotype were reported harboring variants in the *PDX1* (Anık et al.,

2015; Caetano et al., 2018; Chapla et al., 2015; Deng et al., 2019; Fajans et al., 2010; Mangrum et al., 2015; Stoffers et al., 1997a; Thomas et al., 2009) (Supplemental Table S3). Taken together, the average AOD ranged from birth, in the NDM cases (Fajans et al., 2010; Stoffers et al., 1997a), to 27 years old, in our P25 patient. The BMI average, excluding neonatal cases, was 23.65 ± 1.27 kg/m^2. The majority of the index cases were men (5/8). Among the 42 PDX1 carriers (probands and relatives), 32 (76.19%) presented hyperglycemia and 10 (23.81%) did not show glycemic alteration at the moment of the study or did not reported. Moreover, within these studies, 11 family members of the reported probands presented hyperglycemia but did not carry the PDX1 mutation that segregated in their family. Multifactorial forms of diabetes coexisting with monogenic diabetes could be a possible explanation for this observation (Fajans et al., 2010). Concerning the therapeutic management of the index-cases, with exception of the patient with PDX1 c.463C > A that was reported to manage his hyperglycemic level by exercises only (Deng et al., 2019), the remaining seven patients reported the use of insulin as the main chosen treatment.

Here, we highlight the importance to include the analysis of the *PDX1* gene in the diagnosis of monogenic forms of DM. Heterozygous mutations in *PDX1* may cause PDX1-MODY diabetes and we believe that this is the case of the family reported here. The clinical manifestation of this rare form of diabetes needs to be further elucidated by additional studies; it will be possible to create a more specific profile indicative of PDX1-MODY with more clinical cases reported. The present study has some limitations, we were not able to test the patient's father, mother and sister for the detected mutation, our sample size was small and we did not perform functional studies to evaluate the impact of the PDX1 c.532G > A variant on the protein structure and function.

CRediT authorship contribution statement

Gabriella de M. Abreu: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Project administration. **Roberta M. Tarantino:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration. **Ana Carolina P. da Fonseca:** Resources, Writing – review & editing. **Ritiele B. de Souza:** Software, Formal analysis, Writing – review & editing. **Camila A.P.D. Soares:** Investigation, Writing – review & editing. **Pedro H. Cabello:** Resources, Writing – review & editing, Funding acquisition, Supervision. **Melanie Rodacki:** Resources, Writing – review & editing, Funding acquisition, Supervision. **Lenita Zajdenverg:** Resources, Writing – review & editing, Funding acquisition. **Verônica M. Zembrzuski:** Resources, Writing – review & editing, Funding acquisition. **Mário Campos Junior:** Resources, Writing – review & editing, Supervision, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

The authors are grateful to the patients and their families and to the DNA Sequencing Platform (RPT01A) from Oswaldo Cruz Institute. The National Council for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES), Carlos Chagas Filho Foundation for Research Support of the State of Rio de Janeiro (FAPERJ) and Oswaldo Cruz Institute (IOC) supported this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2021.104194.

References

Aidar, M., Line, S.R.P., 2007. A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. Braz. Dent. J. 18, 148–152. https://doi.org/10.1590/S0103-64402007000200012.

Anık, A., Çatlı, G., Abacı, A., Sarı, E., Yeşilkaya, E., Korkmaz, H.A., Demir, K., Altıncık, A., Tuhan, H.Ü., Kızıldağ, S., Özkan, B., Ceylaner, S., Böber, E., 2015. Molecular diagnosis of maturity-onset diabetes of the young (MODY) in Turkish children by using targeted next-generation sequencing. J. Pediatr. Endocrinol. Metab. 28, 1265–1271. https://doi.org/10.1515/jpem-2014-0430.

Brissova, M., Shiota, M., Nicholson, W.E., Gannon, M., Knobel, S.M., Piston, D.W., Wright, C.V.E., Powers, A.C., 2002. Reduction in pancreatic transcription factor PDX-1 impairs glucose-stimulated insulin secretion. J. Biol. Chem. 277, 11225–11232. https://doi.org/10.1074/jbc.M111272200.

Bromberg, Y., Rost, B., 2007. SNAP: predict effect of non-synonymous polymorphisms on function. Nucleic Acids Res. 35, 3823–3835. https://doi.org/10.1093/nar/gkm238.

Caetano, L.A., Santana, L.S., Costa-Riquetto, A.D., Lerario, A.M., Nery, M., Nogueira, G. F., Ortega, C.D., Rocha, M.S., Jorge, A.A.L., Teles, M.G., 2018. PDX1 -MODY and dorsal pancreatic agenesis: new phenotype of a rare disease. Clin. Genet. 93, 382–386. https://doi.org/10.1111/cge.13044.

Capriotti, E., Calabrese, R., Fariselli, P., Martelli, P., Altman, R.B., Casadio, R., 2013. WS-SNPs&GO: a web server for predicting the deleterious effect of human protein variants using functional annotation. BMC Genom. 14, S6. https://doi.org/10.1186/1471-2164-14-S3-S6.

Chapla, A., Mruthyunjaya, M.D., Asha, H.S., Varghese, D., Varshney, M., Vasan, S.K., Venkatesan, P., Nair, V., Mathai, S., Paul, T.V., Thomas, N., 2015. Maturity onset diabetes of the young in India - a distinctive mutation pattern identified through targeted next-generation sequencing. Clin. Endocrinol. 82, 533–542. https://doi.org/10.1111/cen.12541.

Deng, M., Xiao, X., Zhou, L., Wang, T., 2019. First case report of maturity-onset diabetes of the young type 4 pedigree in a Chinese family. Front. Endocrinol. 10, 1–5. https://doi.org/10.3389/fendo.2019.00406.

Fajans, S.S., Bell, G.I., Paz, V.P., Below, J.E., Cox, N.J., Martin, C., Thomas, I.H., Chen, M., 2010. Obesity and hyperinsulinemia in a family with pancreatic agenesis and MODY caused by the IPF1 mutation Pro63fsX60. Transl. Res. 156, 7–14. https://doi.org/10.1016/j.trsl.2010.03.003.

Firdous, P., Nissar, K., Ali, S., Ganai, B.A., Shabir, U., Hassan, T., Masoodi, S.R., 2018. Genetic testing of maturity-onset diabetes of the young current status and future perspectives. Front. Endocrinol. 9, 1–13. https://doi.org/10.3389/fendo.2018.00253.

Ioannidis, N.M., Rothstein, J.H., Pejaver, V., Middha, S., McDonnell, S.K., Baheti, S., Musolf, A., Li, Q., Holzinger, E., Karyadi, D., Cannon-Albright, L.A., Teerlink, C.C., Stanford, J.L., Isaacs, W.B., Xu, J., Cooney, K.A., Lange, E.M., Schleutker, J., Carpten, J.D., Powell, I.J., Cussenot, O., Cancel-Tassin, G., Giles, G.G., MacInnis, R. J., Maier, C., Hsieh, C.-L., Wiklund, F., Catalona, W.J., Foulkes, W.D., Mandal, D., Eeles, R.A., Kote-Jarai, Z., Bustamante, C.D., Schaid, D.J., Hastie, T., Ostrander, E.A., Bailey-Wilson, J.E., Radivojac, P., Thibodeau, S.N., Whittemore, A.S., Sieh, W., 2016. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. Am. J. Hum. Genet. 99, 877–885. https://doi.org/10.1016/j.ajhg.2016.08.016.

Jagadeesh, K.A., Wenger, A.M., Berger, M.J., Guturu, H., Stenson, P.D., Cooper, D.N., Bernstein, J.A., Bejerano, G., 2016. M-CAP eliminates a majority of variants of uncertain significance in clinical exomes at high sensitivity. Nat. Genet. 48, 1581–1586. https://doi.org/10.1038/ng.3703.

Jonsson, J., Carlsson, L., Edlund, T., Edlund, H., 1994. Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 371, 606–609. https://doi.org/10.1038/371606a0.

Mangrum, C., Rush, E., Shivaswamy, V., 2015. Genetically targeted dipeptidyl peptidase-4 inhibitor use in a patient with a novel mutation of MODY type 4. Clin. Med. Insights Endocrinol. Diabetes 8. https://doi.org/10.4137/CMED.S31926. CMED. S31926.

Naslavsky, M.S., Yamamoto, G.L., de Almeida, T.F., Ezquina, S.A.M., Sunaga, D.Y., Pho, N., Bozoklian, D., Sandberg, T.O.M., Brito, L.A., Lazar, M., Bernardo, D.V., Amaro, E., Duarte, Y.A.O., Lebrão, M.L., Passos-Bueno, M.R., Zatz, M., 2017. Exomic variants of an elderly cohort of Brazilians in the ABraOM database. Hum. Mutat. 38, 751–763. https://doi.org/10.1002/humu.23220.

Nicolino, M., Claiborn, K.C., Senee, V., Boland, A., Stoffers, D.A., Julier, C., 2010. A novel hypomorphic PDX1 mutation responsible for permanent neonatal diabetes with subclinical exocrine deficiency. Diabetes 59, 733–740. https://doi.org/10.2337/db09-1284.

Pejaver, V., Urresti, J., Lugo-Martinez, J., Pagel, K.A., Lin, G.N., Nam, H.-J., Mort, M., Cooper, D.N., Sebat, J., Iakoucheva, L.M., Mooney, S.D., Radivojac, P., 2017. MutPred2: Inferring the Molecular and Phenotypic Impact of Amino Acid Variants, 134981. https://doi.org/10.1101/134981 bioRxiv.

Rentzsch, P., Witten, D., Cooper, G.M., Shendure, J., Kircher, M., 2019. CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res. 47, D886–D894. https://doi.org/10.1093/nar/gky1016.

Schwitzgebel, V.M., Mamin, A., Brun, T., Ritz-Laser, B., Zaiko, M., Maret, A., Jornayvaz, F.R., Theintz, G.E., Michielin, O., Melloul, D., Philippe, J., 2003. Agenesis of human pancreas due to decreased half-life of insulin promoter factor 1. J. Clin. Endocrinol. Metab. 88, 4398–4406. https://doi.org/10.1210/jc.2003-030046.

Stoffers, D.A., Ferrer, J., Clarke, W.L., Habener, J.F., 1997a. Early-onset type-ll diabetes mellitus (MODY4) linked to IPF1. Nat. Genet. 17, 138–139. https://doi.org/10.1038/ng1097-138.

Stoffers, D.A., Zinkin, N.T., Stanojevic, V., Clarke, W.L., Habener, J.F., 1997b. Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. Nat. Genet. 15, 106–110. https://doi.org/10.1038/ng0197-106.

Tang, H., Thomas, P.D., 2016. PANTHER-PSEP: predicting disease-causing genetic variants using position-specific evolutionary preservation. Bioinformatics 32, 2230–2232. https://doi.org/10.1093/bioinformatics/btw222.

Tavtigian, S.V., Byrnes, G.B., Goldgar, D.E., Thomas, A., 2008. Classification of rare missense substitutions, using risk surfaces, with genetic- and molecular-epidemiology applications. Hum. Mutat. 29, 1342–1354. https://doi.org/10.1002/humu.20896.

Thomas, I.H., Saini, N.K., Adhikari, A., Lee, J.M., Kasa-vubu, J.Z., Vazquez, D.M., Menon, R.K., Chen, M., Fajans, S.S., 2009. Neonatal diabetes mellitus with pancreatic agenesis in an infant with homozygous IPF-1 Pro63fsX60 mutation. Pediatr. Diabetes 10, 492–496. https://doi.org/10.1111/j.1399-5448.2009.00526.x.

Waeber, G., Thompson, N., Nicod, P., Bonny, C., 1996. Transcriptional activation of the GLUT2 gene by the IPF-1/STF-1/IDX-1 homeobox factor. Mol. Endocrinol. 10, 1327–1334. https://doi.org/10.1210/mend.10.11.8923459.