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VARIATIONS IN MONOGENIC DIABETES AND DIABETES SUSCEPTIBILITY GENES IN PEDIATRIC CASES: SINGLE CENTER EXPERIENCE

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Abstract

Context. Diabetes is a chronic disorder with a complex pathogenetic background including monogenic, polygenic, and environmental causes.

Objective. The aim of the present paper is to share the information related to genetic and clinical data of large pediatric diabetes cohort.

Design. The present study retrospectively analyzes genetic and clinical findings of subjects diagnosed with diabetes under the age of 18 year and are in follow-up in a pediatric diabetes referral center.

Subjects and Methods. Out of 1205 children with diabetes (902 treated with insulin) 246 underwent genetic tests on the basis of clinical selection criteria since 2007.

Results. One hundred and ten variants related to diabetes were found in 89 of them. Age at presentation was 9.5±4.02 years (F/M 44/45). In total 49 pathogenic and likely pathogenic, 11 "hot and warm" of unknown significance variants were found in fourteen MODY and fifteen non-MODY genes according to criteria developed by American College of Medical Genetics. Thirty novel mutations were found. GCK (26.6%) and ABCC8 (10%) were two most frequently affected genes. Antibody testing revealed negative results in 80% of cases.

Conclusions. Genetic interpretation in selected cases is important to understand the nature of the disease better. Improvement in testing opportunity and awareness might increase the prevalence of genetically explained diabetes cases. The distribution of subtypes differs between countries and even regions of the same country.

Keywords: genetics, maturity-onset diabetes of the young, monogenic diabetes, next-generation sequencing, child.

INTRODUCTION

Diabetes mellitus is a chronic disorder presenting with hyperglycemia mainly due to lacking action of insulin, as a result of pathogenic causes of a very wide spectrum. The identification of the underlying pathogenic mechanism is important mainly by two aspects: First, at the individual level, it is important to determine the appropriate management and to predict the course of the disease as well as risks of possible comorbidities in a person diagnosed with diabetes. Second, at global level, knowing the prevalence and mode of presentation of different types of diabetes generally and in various populations is important for better understanding of the disease.

Monogenic diabetes is the title of most heterogeneous subgroup of diabetes presenting with somewhat typical clinical picture and possibility for an exact diagnosis (1). But an exact diagnosis is not always the case because of the overlap of some clinical characteristics between different types of diabetes, the lack of sufficient knowledge about the pathogenic importance of some genetic variations and the existence of still unknown monogenic types. Apart from monogenic causes many diabetes type 1 or type 2 susceptibility genes are defined (2, 3).

MODY-diabetes is the biggest subgroup of monogenic diabetes with to date defined 14 genetic causes. Different case series mostly depict an overrepresentation of the first three MODY types (HNF4A, GCK and HNF1A). The proportion of various types may change between populations (4).

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Increased access to genetic tests, awareness of the heterogeneity of diabetes pathogenesis and existence of atypical presentations of pediatric diabetes patients led us to increasingly search for potential genetic explanations in our cohort. The aim of the present paper is to share the information related to genetic and clinical data of this large pediatric diabetes cohort.

MATERIALS AND METHODS

The present paper describes the genetic findings and general clinical picture of cases collected in a time period of 13 years. All cases with a genetic variation related to diabetes, except for polymorphisms are presented. So-called MODY-X cases with a typical clinical picture but no genetic variation are not included. The study design is retrospective.

The background population and characteristics of the center

The pediatric diabetes center where the study is conducted resides in Northwest Turkey, the West Black Sea Region, to which most of the children diagnosed with diabetes are referred from 8 cities (Duzce, Bolu, Sakarya, Zonguldak, Kastamonu, Karabuk, Bartin, Kocaeli). The center is a SWEET (an initiative aiming to improve quality of pediatric diabetes care and data collection throughout the world) collaborative center since 2017 (5). The population in the geographical region is quite heterogeneous whereas most of them have migrated from North- East, i.e., Circassians, Georgians, and Laz (6).

Routine diagnostic procedures in pediatric diabetes unit

Most of the cases attend with acute metabolic decompensation, i.e., diabetic ketoacidosis or severe hyperglycemia. A minority is diagnosed occasionally during screening with mild or even severe hyperglycemia, impaired fasting glucose, or glucose tolerance. HbA1c, C-peptide, islet and GAD antibodies are performed in all cases along with other routines. Autoantibodies were mostly analyzed in our center, but some patients had already externally performed results, in these patients reference values of the laboratory were accepted. In our center: Anti GAD antibody values above 1 U/mL were accepted as a positive result. Islet cell antibody values were defined as positive or negative results.

Indications for genetic tests

Genetic tests are performed in cases with clinical suspicion of MODY or other forms of monogenic diabetes, after at least one year follow-up with few exceptions. Evidence of slow progression of diabetes, recurrence of diabetes in multiple generations, antibody negativity, persistent C-peptide levels, lack of findings of insulin resistance or ketosis &ketoacidosis at onset, accompanying syndromic features, decreased insulin needs are predominant motivations for genetic testing (6).

In cases suspected for MODY diabetes, up to four consecutive panels comprising of 13 genes are performed (GCK, HNF1A, HNF4A, HNF1B, PDX1, NEUROD1 (ND1), CEL, INS, ABCC8, BLK, KLF11, PAX1 and KCNJ11). Whole exome sequencing and occasionally array CGH are performed on individuals whit a typical MODY clinical picture when no variant could be detected in any of these genes. In syndromic cases targeted gene sequencing is performed.

Genetic testing and interpretation

Total DNA was isolated from peripheral blood samples of the participants and Next Generation Sequencing (MISEQ-Illumina) was performed using primers containing exon / exon intron junctions in the related gene. All disease-causing variants reported in the HGMD®, Illumina BaseSpace Variant Interpreter, InterVar, Franklin, VarSome, ClinVar, OMIM, and Pubmed databases as well as all variants with a minor allele frequency (MAF) of less than 1% in the gnomAD database were considered.

Detected variants were classified as “pathogenic” (P), “likely pathogenic” (LP), “variant of uncertain significance (VUS)”, “likely benign” (LB) or benign according to the international guidelines of the American College of Medical Genetics and Genomics (ACMG) (7). Hot and warm VUS variants were indicated as well (8). All variants were revised just before the paper is written.

Data sampling and presentation

All medical records generated during follow-up in our center or elsewhere are accessible electronically. In addition, the center has a private archive for every child with diabetes including copies of medical records and complementary information when necessary. Every subject with a positive genetic finding is recorded in a separate excel sheet with genetic parameters, i.e., gene number, base pair and amino acid variations, other variations if any, zygosity, pathogenicity, mode of inheritance, exon number, genetic and clinic status of family members if present. At start of the study this excel file and files of cases on the list were reviewed.

Inclusion criteria

A diagnosis of diabetes & prediabetes before the age of 18 years. Testing positive for any variation related to diabetes.

Exclusion criteria

Cases with polymorphisms
Cases with negative genetic results

Ethics

Informed consents were obtained from the patients' parents for genomic analysis. All procedures, which were appropriate to the Declaration of Helsinki, were approved by the Ethics Committee of the Faculty of Medicine at Duzce University (Date February 7, 2022; No. 2022/25).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). Descriptive statistical methods were done. The data were given as mean±SD and percentages.

RESULTS

Since its establishment in 2007, 1205 cases have been recorded in the pediatric diabetes archive of digital files. Nine hundred two of them were apparently type 1 diabetes at onset. In total, 246 of our patients were tested genetically. Thirty-four of them underwent whole exome sequencing following negative panel screenings or as the first option. In two cases array CGH was performed without any positive result. Eighty-nine separate patients were found to have 110 variations which are related to diabetes (Fig. 1). Their age at diagnosis was 9.5±4.02 (0-18) years and sex distribution balanced (Table 1). Variations not related to diabetes are not reported in the present study. In 157 patients, no genetic explanation of diabetes was found with to date conducted genetic tests. The detection rate in our practice is 36%, 24% and 20% when all, P + LP + warm-hot VUS and only P+LP variations are included respectively.

Genetic variations associated with diabetes

Three different variations were detected in four patients and two different variations were detected in thirteen patients (Table 2). One of the 4 patients with three variations and one of the 13 patients carrying two variations in the HNF1A and ABBC8 genes respectively represented codominances. Thus, three patients have triple heterozygosity, two have codominances, 12 have double heterozygosity, remaining 69 carried one variation related to diabetes each (Table 2).

We found 30 novel mutations in 16 genes, 34 times in total (Table 2). The repeated 4 mutations, two in GCK, one in ABCC8 and one in TBC1D4 were found in 4 sib-pairs. Other four were in GCK, three in CEL, three in HNF4A, two times each in HNF1B, ABCC8, INS and WFS1; the remaining in HNF1A, KLF11, PAX4, PDX1, APLL1, PPARɤ and SLC5A2; once in each gene (Table 2).

Forty-nine patients were positive for pathogenic and likely pathogenic variations, of which 37 are in 8 genes related to MODY. Twelve non-MODY variations which are pathogenic or likely athogenic were found, MC4R three times, WFS1 and EIF2AK3 two times and the remaining one time each (Table 3). In two patients with GCK and in one patient with GCK and HNF1A file records were indicating the gene with the variation, but genetic reports were not obtained. Their pathogenicity was classified as "unknown".

Of the 44 variations classified as VUS, 9 were in "hot" and 2 were in "warm" category (Tables 2, 3).

In 56 patients anti-GAD and islet cell antibodies were negative, in four patients anti GAD, in four patients islet cell and in two patients both antibodies were positive. In three patients anti GAD was tested twice and one result was negative and the other weak positive. In 20 patients, antibodies were not tested, mostly because of exclusion of type 1 diabetes via clinical findings such as non-progressive hyperglycemia (Table 1). Pts 30 presented with ketosis, mild acidosis, she is lean, and her insulin dose is <0.5 U/kg/day, there is history of diabetes in four consecutive generations, and she carries three variations related to MODY (Table 1).

We tested sulphonylurea in seven patients. None of them except one was successful. Even in the patient with HbA1c levels ranging between 5.8-7% under glibenclamide monotherapy for the first two years, we started insulin rather than increasing glibenclamide dose during puberty.

GCK mutations

GCK was the predominant gene with 26 pathogenic and likely pathogenic variations. In three subjects with GCK the variation detail was not recorded in the file (Tables 2, 3). Of the 29 patients, 12 are siblings from five different families.

Family 1: Three siblings are affected. Two

older brothers presented with non-progressive hyperglycemia during school-age and the third sibling with neonatal diabetes who is homozygote for the variation (p.Phe419SerfstTer12). The two brothers, mother (gestational diabetes) and father (non-progressive hyperglycemia) were heterozygous.

Family 2: Three siblings are affected, two are males with double heterozygosity (MODY2 and MODY12) and their sister is carrying only the p.Phe419SerfstTer12. GCK variation is probably inherited from the mother who is not tested genetically. The father carried the p.Gln483Arg variation in the

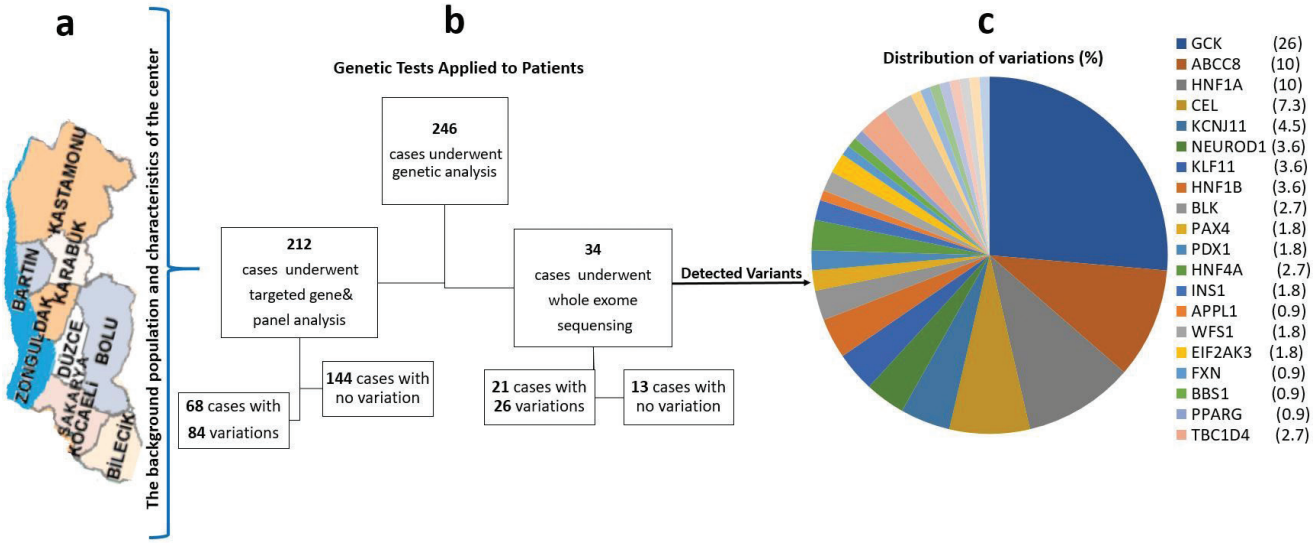

Figure 1. Demonstrative examples of the study. The background population and characteristics of the center (a). Genetic tests type applied to patients and the number of detected variation (b). Distribution of detected variation (c).

Table 1. Clinical characteristics

Sex(F/M)		44/45
Age, decimal years (mean±SD)		9.5±4.02
Presentation at onset	n (%)	20
Ketosis Hyperglycemia By screening		32
*Prediabetes**		37
		5
BMIcentile	n (%)	71
< 85		15
85-95		3
> 95		33
Medication	n (%)	7
None		1
SU(temporary)** MetforminInsulin		55
≤0.5U/kg		38
>0.5 U/kg		17
Follow-upduration	years(min-max)	1-27
Family history	n (%) Atleast3consequetive-	27
generations		35
<3generations None		10
Unknown		17
		4
Autoantibodiesto(n) GAD		3
GADsuspicious***Islet cell		4
Both None		2
Nottested		56
		20

*Included in screening cases. **Included in insulin treatment cases. ***Two tests with one negative and one positive result.

ABCC8 gene, i.e. the boys inherited that variant from the father. Clinically all three siblings shared almost the same degree of hyperglycemia, HbA1c 6.2% and fasting blood glucose 128-130 mg/dL. The father of these siblings had a HbA1c of 7.2% and triglycerides > 300 mg/dL.

Family 3: Two siblings are affected. The p.Phe150del variation in these siblings caused an early diagnosed (the girl at 2 years and the boy at 3 months of age) mild-moderate hyperglycemia with HbA1c changing between 5.5-6.6% during >7 years of follow-up. The early diagnosis was thanks to their mother's similarly progressing diabetes.

Family 4: Two siblings are affected. The elderly brother was diagnosed with diabetes at 6 years of age followed by screening of his 3 years old sister with a similar degree of hyperglycemia. Their mother reported gestational diabetes but neither parent was tested genetically.

Family 5: Two siblings are affected. The older sister was diagnosed by screening at the age of three years and during 5-year follow-up, HbA1c is ranging between 6.4-6.8% without treatment. The younger brother is screened later on at the same age and HbA1c is 6.3-6.5% in last two years.

Among the 29 patients the one with neonatal diabetes is treated with moderate dose insulin via pump, one unrelated patient and two siblings in the fourth family are using low dose basal insulin. The remaining patients did not receive any medication.

ABCC8 mutations

The second biggest subgroup was made of cases with variations in the ABCC8 gene (Table 3). Two of them were pubertal girls with likely pathogenic mutations. They were obese and had persisting C peptide levels. We found 8 variations classified as VUS, their carriers all had features supporting MODY such as positive segregation with parents, low insulin requirements and persisting C peptide levels.

Other variations

Eleven variations in HNF1A genes, 8 in CEL gene, 5 in KCNJ11 gene and 4 in NEUROD1 gene variations were found (Table 3).

Variations in BLK were found in three patients. The first one is a girl with incidentally found hyperglycemia and later dependent on low dose insulin. The c.223C>A variation, which is reported as VUS is co-segregated with his father and uncle with diabetes and was not found in her non-diabetic mother as well as brother. The second case carrying the likely benign c.424A>G variation presented with negative autoantibodies but severe ketoacidosis, diabetes in consequent paternal three generations, a slowly dropping but after 7 years too low c-peptide. Neither his father nor his mother has the same variation. This patient has also one VUS and one benign variation in HNF1A gene which are not tested yet in his parents. The third one is a pubertal, insulin dependent girl who has inherited the VUS from her father who has impaired fasting glucose.

One patient with consanguinity in multiple generations had HNF1A, HNF4A and WFS1 mutations and died at the age of 29 seemingly related to a cardiovascular event. This patient was diagnosed with diabetes at 2 years of age and with optic atrophy later. He had life-long insulin-dependency and late-onset mild ataxia and depression.

Two patients with INS-MODY were diagnosed at very similar ages of 3 year 9 months and 3 year 6 months. Both attended with symptomatic hyperglycemia, the girl with a HbA1c level of 13.5% and ketosis, the boy with a HbA1c level of 7.4% and without ketosis. Both have negative autoantibodies and the same insulin need of 0.47 U/kg/d as well as excellent metabolic control in the fourth and third year of diagnosis.

We identified MC4R mutations in three patients presented with diabetes. One morbid obese boy with a homozygote pathogenic known mutation was typically insulin resistant diagnosed with type 2 diabetes at the age of 11 years. On the other hand a lean boy with postprandial hyperglycemia and HbA1c 13.2%, without ketosis and autoantibodies was initially started and favorably controlled with low dose sulfonylurea for two years. This patient underwent panel investigations, array CGH with no positive finding and WES revealed a likely pathogenic mutation in MC4R, which has extremely low frequency in gnomAD population databases. The third boy carried the WFS1 gene as well homozygous and was slightly overweight. None of the cases with channelopathy had history of neonatal diabetes.

The patient with a VUS variation in MAPK8IP1 gene presented with prediabetes and history of anal atresia, later progressed severe insulin dependence.

There was a very large spectrum of clinical picture at presentation as well as during follow-up even within the same gene group ranging from prediabetes to DKA at presentation and from decreasing insulin needs to very high HbA1c in parallel with high insulin doses during follow-up except for the heterozygote GCK cases who share a very similar and stabile course. Moreover,

Table 2. List and assessment of the detected variants

PN	Gene	Transcript Number	Nucleotide Change	AA Change	MAF by gnom AD	Zyg	Variant Location	Variant Type	Clin Var	ACMG Class	ACMGPatCrit	Novelity
P1	***HNF1A***	**NM_000545.8**	**c.1513C>A**	**p.His505Asn**	**0.0000797**	**Het**	**Exon8**	**Mis**	**VUS**	**LP**	**PP2, PP3, BS2**	**rs577078110**
P2	***BLK***	**NM_001715.3**	**c.424A>G**	**p.Ile142Val**	**0.000513**	**Het**	**Exon6**	**Mis**	**Benign**	**VUS**	**BS1, BS2, BP4, BP6**	**rs562247916**
P2	***HNF1A***	**NM_000545.8**	**c.79A>C**	**p.Ile27Leu**	**0.34**	**Het**				**VUS**		**rs1169288**
P2	***HNF1A***	**NM_000545.8**	**c.1460G>A**	**p.Ser487Asn**	**0.32**	**Het**				**Benign**		**rs2464196**
P3	***PAX4***	**NM_ 001366110.1**	**c.421C>T**	**p.Arg141Trp**	**0.00699**	**Het**	**Exon6**	**Mis**	**Benign**	**LB**	**BA1, BS3, BP4**	**rs2233578**
P4	***HNF1B***	**NM_000458.4**	**c.421G>A**	**p.Asp141Asn**	**ND**	**Het**	**Exon2**	**Mis**	**NP**	**LP**	**PM1, PM2, PP2, PP3**	**Novel**
P5	***ABCC8***	**NM_000352.4**	**c.2861G>T**	**p.Gly954Val**	**0.00000398**	**Het**	**Exon24**	**Mis**	**NP**	**LP**	**PM2, PP2**	**rs1285168546**
P6	***HNF1A***	**NM_000545.8**	**c.208T>C**	**p.Ser70Pro**	**ND**	**Het**	**Exon1**	**Mis**	**NP**	**VUS[h]**	**PM2, PP2, PP3**	**Novel**
P7	***GCK***	**NM_000162.5**	**c.214G>A**	**p.Gly72Arg**	**0.00000398**	**Het**	**Exon3**	**Mis**	**P**	**P**	**PM1, PM2, PP5, PS1, PS3, PP3**	**rs193922289**
P8	***NEUROD1***	**NM_002500.4**	**c.-71G>A**	**-**	**0.0000637**	**Het**	**5'UTR**	**Mis**	**NP**	**VUS**	**BP4, PM2**	**rs528005664**
P9	***HNF1B***	**NM_000458.4**	**c.311C>T**	**p.Ala104Val**	**ND**	**Hom**	**Exon1**	**Mis**	**VUS**	**VUS[h]**	**PM1, PM2, PP2**	**Novel**
P10	***CEL***	**NM_001807.6**	**c.1766G>A**	**p.Gly589Glu**	**0.000107**	**Het**	**Exon11**	**Mis**	**NP**	**VUS**	**BS2, BP1, BP4**	**rs565866896**
P11	***KLF11***	**NM_003597.4**	**c.677C>G**	**p.Thr226Ser**	**0.00000398**	**Het**	**Exon3**	**Mis**	**NP**	**VUS**	**PM2, BP1, BP4**	**rs120927416**
P11	***SLC5A2***	**NM_003041.4**	**c.1176dupC**	**p.Ser393 LeufsTer69**	**ND**	**Het**	**Exon10**	**Frms**	**NP**	**LP**	**PVS1, PM2**	**Novel**
P12	***GCK***	**NM_000162.5**	**c.700T>G**	**p.Tyr234Asp**	**ND**	**Het**	**Exon7**	**Mis**	**NP**	**LP**	**PM1, PM2, PP3**	**Novel**
P13	***GCK***	**NM_000162.5**	**c.1256delT**	**p.Phe419 SerfsTer12**	**ND**	**Het**	**Exon10**	**Mis**	**NP**	**LP**	**PVS1, PM2**	**Nors-PF**
P14	***KCNJ11***	**NM_000525.3**	**c.526C>T**	**p.Arg176Cys**	**0.0000241**	**Het**	**Exon1**	**Mis**	**VUS**	**LP**	**PM1, PM2, PP2**	**rs201264306**
P14	***PDX1***	**NM_000209.4**	**c.433_ 435delGAG**	**p.Glu145del**	**0.0000205**	**Het**	**Exon2**	**Ifr**	**NP**	**VUS[h]**	**PM2, PM4**	**rs768856218**
P15	***CEL***	**NM_001807.6**	**c.1341C>A**	**p.Tyr447Ter**	**0.000016**	**Het**	**Exon10**	**Non-S**	**NP**	**LP**	**PVS1, PM2**	**Novel**
P16	***CEL***	**NM_001807.6**	**c.446A>G**	**p.Asp149Gly**	**ND**	**Het**	**Exon4**	**Mis**	**NP**	**VUS**	**PM2, PP3**	**Novel**
P16	***GCK***	**NM_000162.5**	**c.943C>T**	**p.Leu315Phe**	**ND**	**Het**	**Exon8**	**Mis**	**VUS**	**LP**	**PM1, PM2, PM5, PP3**	**Novel**
P17	***BLK***	**NM_001715.3**	**c.1307A>G**	**p.Tyr436Cys**	**0.00000399**	**Het**	**Exon12**	**Mis**	**NP**	**VUS**	**PM2, PP3**	**rs1393176878**
P18	***CEL***	**NM_001807.6**	**c.679-4G>A**	**-**	**0.0000537**	**Het**	**Intron5**	**Mis**	**LB**	**Benign**	**BS2, BP4, BP6**	**rs371303105**
P19	***ABCC8***	**NM_000352.6**	**c.2434G>A**	**p.Asp812Asn**	**0.0000795**	**Het**	**Exon20**	**Mis**	**NP**	**VUS**	**PM2, PP2**	**rs146916682**
P20	***GCK***	**NM_000162.5**	**c.943C>T**	**p.Leu315Phe**	**ND**	**Het**	**Exon8**	**Mis**	**VUS**	**LP**	**PM1, PM2, PM5, PP3**	**rs1583594450**
P21	***HNF1A***	**NM_000545.8**	**c.713+14C>T**	**-**	**0.000121**	**Het**	**Intron3**	**Mis**	**VUS**	**LB**	**BS2, BP4**	**rs1939226601**
P22	***GCK***	**NM_000162.5**	**c.449_451del**	**p.Phe150del**	**ND**	**Het**	**Exon4**	**Ifr**	**NP**	**LP**	**PM1, PM2, PM4, PM5**	**Novel**
P23	***GCK***	**NM_000162.5**	**c.617C>T**	**p.Thr206Met**	**0.00000398**	**Het**	**Exon6**	**Mis**	**P**	**P**	**PM1, PM2, PM5, PP3, PP5**	**rs1441649062**
P24	***GCK***	**Not provided**										
P25	***ABCC8***	**NM_000352.6**	**c.3393C>T**	**p.Ile1131Ile**	**0.000183**	**Het**	**Exon27**	**Mis**	**Benign**	**LB**	**BP4, BP6, BP7**	**rs776975807**
P25	***ABCC8***	**NM_000352.6**	**c.1943G>A**	**p.Arg648His**	**0.000012**	**Het**	**Exon14**	**Mis**	**VUS**	**VUS[h]**	**PM2, PP2**	**rs773168707**
P26	***NEUROD1***	**NM_002500.4**	**c.-81C>T**	**-**	**ND**	**Het**	**5'UTR**	**Mis**	**VUS**	**VUS**	**BP4, PM2**	**rs567688779**
P27	***KCNJ11***	**NM_000525.4**	**c.67A>G**	**p.Lys23Glu**	**0.64**	**Het**	**Exon1**	**Mis**	**LB**	**VUS**	**BA1, BS3, BP4, BP6**	**rs5219**
P28	***GCK***	**NM_000162.5**	**c.449_ 451delTCT**	**p.Phe150del**	**ND**	**Het**	**Exon4**	**Ifr**	**NP**	**LP**	**PM1, PM2, PM4, PM5**	**Novel**
P29	***HNF1B***	**NM_000458.4**	**c.1414G>A**	**p.Val472Ile**	**0.00000624**	**Het**	**exon7**	**Mis**	**VUS**	**VUS**	**PM2, PP2**	**rs762841746**
P30	***NEUROD1***	**NM_002500.4**	**c.590C>A**	**p.Pro197His**	**0.0193**	**Het**	**Exon2**	**Mis**	**Benign**	**Benign**	**BS1, BS2, BP4**	**rs8192556**
P30	***HNF1A***	**NM_000545.8**	**c.79A>C**	**p.Ile27Leu**	**0.34**	**Het**				**VUS**		**rs1169288**
P30	***HNF1A***	**NM_000545.8**	**c.1460G>A**	**p.Ser487Asn**		**Het**				**Benign**		**rs2464196**
P31	***ABCC8***	**NM_000352.6**	**c.1616A>G**	**p.Tyr539Cys**	**0.00000398**	**Het**	**Exon10**	**Mis**	**VUS**	**VUS**	**PM1, PM2, PP3, PP5**	**rs193922397**
P32	***KCNJ11***	**NM_000525.4**	**c.57G>A**	**p.Glu19Glu**	**0.00000399**	**Het**	**Exon1**	**Mis**	**LB**	**LB**	**BP4, BP6, BP7, PM2**	**rs748791717**
P33	***GCK***	**Notprovided**										
P34	***GCK***	**Notprovided**										
P34	***HNF1A***	**Notprovided**										
P35	***GCK***	**NM_000162.5**	**c.1256delT**	**p.Phe419Serf-stTer12**	**ND**	**Het**	**Exon10**	**Frms**	**NP**	**LP**	**PVS1, PM2**	**Nors-PF**
P36	***CEL***	**NM_001807.6**	**c.268A>C**	**p.Thr90Pro**	**0.000112**	**Het**	**Exon3**	**Mis**	**NP**	**VUS**	**BS2, BP1, BP4**	**rs756184460**
P37	***GCK***	**NM_000162.5**	**c.1328A>C**	**p.Glu443Ala**	**ND**	**Het**	**Exon10**	**Mis**	**NP**	**LP**	**PM1, PM2, PM5, PP3**	**Novel**
P38	***HNF1A***	**NM_000545.8**	**c.862G>A**	**p.Gly288Arg**	**0.0000461**	**Het**	**Exon4**	**Mis**	**VUS**	**VUS[h]**	**PM2, PP2, PP3, BS2**	**rs539507291**

PN	Gene	Transcript Number	Nucleotide Change	AA Change	MAF by gnom AD	Zyg	Variant Location	Variant Type	Clin Var	ACMG Class	ACMG Pat Crit	Novelity
P39	*CEL*	NM_001807.6	c.670-1G>A	-	ND	Het	Intron5	Mis	NP	LP	PVS1, PM2	rs1323338882
P40	*GCK*	NM_000162.5	c.534delG	p.Asn179Thrf-sTer25	ND	Het	Exon5	Frms	NP	LP	PVS1,PM2	Novel
P41	*GCK*	NM_000162.5	c.571C>T	p.Arg191Trp	0.00000796	Het	Exon5	Mis	P	P	PM1, PM2, PM5, PP3, PP5	rs1085307455
P42	*GCK*	NM_000162.5	c.1256delT	p.Phe419Serf-stTer12	ND	Het	Exon10	Frms	NP	LP	PVS1, PM2	Nors-PF
P43	*GCK*	NM_000162.5	c.943C>T	p.Leu315Phe	ND	Het	Exon8	Mis	VUS	LP	PM1, PM2, PM5, PP3	rs1583594350
P44	*GCK*	NM_000162.5	c.1256delT	p.Phe-419Serfs*12	ND	Hom	Exon10	Frms	NP	LP	PVS1, PM2	Nors-PF
P45	*ABCC8*	NM_000352.6	c.4732C>T	p.Arg1578Cys	0.00000796	Het	Exon39	Mis	NP	VUS[h]	PM1, PM2	Novel
P45	*G6PC2*	NM_021176.3	c.782G>C	p.Arg261Thr	0.0000199	Het	Exon5	Mis	NP	VUS[w]	BP1, BP4, PM2	rs748589053
P46	*HNF4A*	NM_000457.4	c.1255G>A	p.Glu419Lys	ND	Het	Exon9	Mis	NP	VUS	PM2, BP4, PP2	Novel
P47	*CEL*	NM_001807.5	c.907C>T	p.His303Tyr	ND	Het	Exon8	Mis	NP	VUS	PM2, BP4	Novel
P48	*GCK*	NM_000162.5	c.1256delT	p.Phe419Serf-stTer12	ND	Het	Exon10	Frms	NP	LP	PVS1, PM2	Nors-PF
P48	*ABCC8*	NM_000352.6	c.1448A>G	p.Gln483Arg	ND	Het	Exon9	Mis	NP	VUS	PM2, PP2	Novel
P49	*NEUROD1*	NM_002500.4	c.590C>A	p.Pro197His	0.0193	Het	Exon2	Mis	Benign	Benign	BS1, BS2, BP4	rs8192556
P50	*INS*	NM_000207.2	c.84_86delACA	p.Gln28del	ND	Het	Exon2	Ifr	NP	LP	PM1, PM2, PM4	Novel
P51	*KCNJ11*	NM_000525.4	c.67A>G	p.Lys23Glu	0.64	Het	Exon1	Mis	LB	VUS	BA1, BS3, BP4, BP6	rs5219
P52	*ABCC8*	NM_000352.6	c.3613G>A	p.Glu1205Lys	0.0000358	Het	Exon29	Mis	VUS	LP	PM1, PM2, PP3	rs768448830
P52	*ABCC8*	NM_000352.6	c.458A>G	p.Lys153Arg	ND	Het	Exon4	Mis	NP	VUS	PM2, PP2	rs1186544807
P53	*BLK*	NM_001715.3	c.223C>A	p.Arg75=	0.00000398	Het	Exon4	Sil	NP	VUS	BP4, BP7, PM2	rs149393791
P54	*GCK*	NM_000162.5	c.943C>T	p.Leu315Phe	ND	Het	Exon8	Mis	VUS	LP	PM1, PM2, PM5, PP3	rs1583594450
P55	*GCK*	NM_000162.5	c.1256delT	p.Phe419Serf-stTer12	ND	Het	Exon10	Frms	NP	LP	PVS1, PM2	Nors-PF
P56	*ABCC8*	NM_000352.6	c.1448A>G	p.Gln483Arg	ND	Het	Exon9	Mis	NP	VUS	PM2, PP2	Novel
P56	*GCK*	NM_000162.5	c.1256delT	p.Phe419Serf-stTer12	ND	Het	Exon10	Frms	NP	LP	PVS1, PM2	Nors-PF
P57	*KLF11*	NM_003597.4	c.1151C>A	p.Pro384His	ND	Het	Exon3	Mis	NP	VUS	PM2	Novel
P58	*CEL*	NM_001807.6	c.2026G>T	p.Ala676Ser	0.00014	Het	Exon11	Mis	LB	LB	BP4, BP6	rs529652004
P58	*EIF2AK3*	NM_004836.7	c.1392_1396del	p.Tyr464fs		Hom				LP		Novel
P58	*AKT2*	NM_001626.6	c.1126G>A	p.Glu376Lys	0.000007096	Het				VUS		rs1466949645
P59	*KLF11*	NM_003597.4	c.1382G>A	p.Arg461Gln	0.000497	Het	Exon4	Mis	NP	LB	BS1, BS2, BP4	rs199770737
P60	*ABCC8*	NM_000352.4	c.1126T>A	p.Ser376Thr	ND	Hom	Exon7	Mis	NP	VUS	PM1, PM2, PP3	Novel
P61	*HNF1A*	NM_000545.8	c.1386C>T	p.Val462=	0.000176	Het	Exon7	Sil	LB	LB	BS1, BS2, BP4, BP6, BP7	rs143015301
P61	*HNF4A*	NM_000457.5	c.149C>T	p.Ala50Val	0.0000199	Het	Exon2	Mis	VUS	VUS[h]	PM2, PP2	Novel
P61	*WFS1*	NM_006005.3	c.2168T>C	p.Leu723Pro		Hom	Exon8			LP		Novel
P62	*GCK*	NM_000162.5	c.766G>A	p.Glu257Lys	ND	Het	Exon7	Mis	LP	P	PM1, PM2, PM5, PP3, PP5	rs769268803
P63	*GCK*	NM_000162.5	c.1256delT	p.Phe419Serf-stTer12	ND	Het	Exon10	Frms	NP	LP	PVS1, PM2	Nors-PF
P64	*GCK*	NM_000162.5	c.950A>C	p.His317Pro	ND	Het	Exon8	Mis	NP	LP	PM1, PM2, PM5	Novel
P65	*GCK*	NM_000162.5	c.769G>A	p.Glu257Lys	0.00000398	Het	Exon7	Mis	P	P	PM1, PM2, PM5, PP2, PP3, PP5	rs769268803
P66	*GCK*	NM_000162.5	c.1256delT	p.Phe-419Serfs*12	ND	Het	Exon10	Frms	NP	LP	PVS1, PM2	Nors-PF
P67	*KCNJ11*	NM_000525.4	c.-54C>T	-	0.0000958	Het	5'UTR	Mis	VUS	VUS	PM2, BP7	rs1016780684
P68	*KLF11*	NM_003597.5	c.1018A>T	p.Met340Leu	0.00000803	Het	Exon3	Mis	NP	VUS	PM2	rs7472156138
P68	*PAX4*	NM_001366110.1	c.818C>G	p.Pro273Arg	ND	Het	Exon9	Mis	NP	VUS	PM2	Novel
P69	*PDX1*	NM_000209.4	c.402G>A	p.Trp134Ter	0.00000408	Het	Exon1	Non-S	NP	LP	PVS1, PM2	Novel
P70	*LPL*	NM_000237.3	c.89-1G>A	-	ND	Hom	Intron1	Mis	LP	LP	PVS1, PM2, PP5	1494261
P71	*MAP-K8IP1*	NM_005456.4	c.1864G>A	p.Val622Ile	0.000119	Het	Exon9	Mis	NP	VUS	PM2,BP4	rs549861347
P72	*MC4R*	NM_00591.3	c.791A>G	p.His264Arg	ND	Het	Exon1	Mis	NP	LP	PM1, PM2, PP2, PP3	rs191533901 1?novel?

PN	Gene	Transcript Number	Nucleotide Change	AA Change	MAF by gnom AD	Zyg	Variant Location	Variant Type	Clin Var	ACMG Class	ACMG Pat Crit	Novelity
P71	*MAPK8IP1*	NM_005456.4	c.1864G>A	p.Val622Ile	0.000119	Het	Exon9	Mis	NP	VUS	PM2, BP4	rs549861347
P72	*MC4R*	NM_00591.3	c.791A>G	p.His264Arg	ND	Het	Exon1	Mis	NP	LP	PM1, PM2, PP2, PP3	rs191533901 1?novel?
P73	*MC4R*	NM_00591.3	c.493C>T	p.Arg165Trp	0.0000199	Hom	Exon1	Mis	LP	P	PM1, PM2, PM5, PP2, PP5	rs13447332
P74	*TBC1D4*	NM_014832.4	c.1687G>A	p.Ala563Thr	ND	Het	Exon8	Mis	NP	VUS	PM2	Novel
P75	*TBC1D4*	NM_014832.4	c.1687G>A	p.Ala563Thr	ND	Het	Exon8	Mis	NP	VUS	PM2	Novel
P76	*APLL1*	NM_012096.3	c.1274C>T	p.Thr425Ile	0.00000399	Het	Exon15	Mis	NP	VUS	PM2, BP4	Novel
P76	*CCR5*	NM_000579.3	c.164T>A	p.Leu55Gln	0.0136	Het	Exon3	Mis	NP	Benign	BS1, BS2	rs1799863
P77	*PPARG*	NM_015869.5	c.614A>G	p.His205Arg	0.00000399	Het	Exon4	Mis	NP	VUS^{h}	PM2, PP2, PP3	Novel
P77	*TBC1D4*	NM_014832.5	c.3353A>G	p.Lys1118Arg	0.0000319	Het	Exon19	Mis	NP	VUS	PM2	rs755146398
P78	*BBS1*	NM_024649.5	c.752delT	p.Leu251 GlnfsTer25	ND	Hom	Exon9	Frms	P	P	PVS1, PM2, PP5	rs1299581
P79	*FXN1*		GAArepeat>66				Intron1					
P80	*LMNA*	NM_170707.4	c.1444C>T	p.Arg482Trp	0.000004	Het	Exon8	Mis	P	P	PM1, PM2, PP2	rs57920071
P81	*EIF2AK3*	Notprovided										
P82	*GCK*	NM_033508.3	c.1253delT	p.Phe-418Serfs*12	ND	Het	Exon10	Frms	NP	LP	PVS1, PM2	Nors-PF
P83	*HNF4A*	NM_000457.5	c.176T>C	p.Leu59Pro	ND	Het		Non-Syn		VUS^{h}		Novel
P84	*HNF1B*	NM_000458.4	c.1126A>C	p.Thr376Pro		Het		Mis		VUS		rs771937539
P85	*WFS1*	NM_ 001145853,1	c.1395delC	p.Gly466fs		Hom		Frms		LP		Novel
P85	*MC4R*	NM_005912,3	c.380C>T	p.Ser127Leu	0.0001627	Het		Non-Syn		P		rs13447331
P86	*HNF1A*	NM_ 001306179.2	c.1513C>A	p.His505Asn	<0.01	Het		Non-Syn		LP		rs577078110
P87	*GPD2*	NM_000408.5	c.431G>A	p.Arg144His		Het	Non-Syn	VUS^{w}	rs201559833			
P88	*GCK*	NM_033507.3	c.1328A>C	p.Glu443Ala	ND	Het	Non-Syn	LP	Novel			
P89	*INS*	NM_000207.2	c.322T>A	p.Tyr108Asn		Het		LP	Novel			

P: Patient numberAA: Amino AcidMAF: Minor Allele FrequencyZyg: Zygosity ACMG Class: The American College of Medical Genetics and Genomics Classification. Pat Crit: Pathogenicity CriteriaHet: HeterozygousHom: HomozygousDel: Deletion. Frms: Frameshift. Mis: MissenseIfr:In frame Sil:SilentSplc: Splicing Non-S:NonsenseNon-Syn: Non-Synonymous NP: Not ProvidedP: PathogenicLP: Likely Pathogenic. VUS:VariantofUncertain Significance. VUSh:Hot VUS. VUSw:warm VUSLB: Likely benignB: BenignNo rs-PF: rs number unavailable but reporting publication found.

Table 3. The distribution of the variations according to ACMG classification

	Pathogenic	Likely pathogenic	VUS (VUS^{h})*	Likely benign	Benign	Unknown	Total
GCK	5	21	-	-	-	3	29
ABCC8	-	2	8(2)	1	-	-	11
HNF1A	-	2	5(2)	2	2	1	11
CEL		2	4	1	1	-	8
KCNJ11	-	1	3	1	-	-	5
NEUROD1	-	-	2	-	2	-	4
KLF11	-	-	3	1	-	-	4
HNF1B	-	1	3(1)	-	-	-	4
BLK	-	-	3	-		-	3
PAX4	-	-	1	1		-	2
PDX1		1	1(1)	-	-	-	2
HNF4A	-	-	3(2)	-	-	-	3
INS1	-	2	-	-	-	-	2
APPL1	-	-	1	-	-	-	1
WFS1	-	2	-	-	-	-	2
EIF2AK3	2	-	-	-	-	-	2
FXN	1	-	-	-	-	-	1
BBS1	-	1	-	-	-	-	1
PPARG	-	-	1(1)	-	-	-	1
TBC1D4	-	-	3	-	-	-	3
MC4R	1	2	-	-	-	-	3
LMNA	1	-	-	-	-	-	1
AKT2	-	-	1	-	-	-	1
LPL	-	1	-	-	-	-	1
SLC5A2	-	1	-	-	-	-	1
MAPK8IP1	-	-	1	-	-	-	1
CCR5	-	-	-	-	1	-	1
G6PC2	-	-	1(VUS^{w})**	-	-	-	1
GPD2	-	-	1(VUS^{w})**	-	-	-	1
Total	10	39	44	7	6	4	110

*hot VUS. **warm VUS.

the clinical course was changing intra-individually, i.e., some cases with severe hyperglycemia at presentation could stop insulin with time whereas some with incidental impaired fasting glucose progressed to insulin dependency. A considerable number of patients were diagnosed as type 1 diabetes at first admission.

DISCUSSION

Diabetes is a complicated metabolic disorder affecting a striking number of people throughout the world (10). In Turkey, the prevalence of T1D in children under 18 years was reported to be 0.75/1,000 (11). Regarding epidemiological studies, Turkey is a country with an intermediate incidence rate compared to the rest of the world (12). With the advancing scientific knowledge about the disease, classification of diabetes types becomes complicated as well (13). Type 1 and type 2 diabetes are still the leading subgroups, whereas in parallel to the development of genetic techniques, monogenic diabetes, becomes more frequently diagnosed in new cases as well as in cases formerly diagnosed as type 1 or type 2 diabetes (14). A subgroup of monogenic diabetes, MODY, is a category to which new genetic types are added by the time. There are many more diabetes genes, some of them well defined some not, for which the impact or mechanism of action on the development the disease is not sufficiently understood yet. The genes beyond the categories MODY, neonatal diabetes and syndromic diabetes should be interpreted with caution. There are risk scores proposed to interpret T1D or T2D susceptibility genes. Gene to gene and gene-environment interactions may affect the clinical picture in a wider spectrum in comparison to cases accepted as monogenic diabetes (15, 16).

Monogenic diabetes represents essentially the unique subgroup of diabetes with a possibility to make the diagnosis via genetic tests. But still many cases of monogenic diabetes remain diagnosed as type 1 or type 2 diabetes. The reasons for this are numerous. First of all, the availability of genetic tests is limited. Therefore, criteria are developed to indicate genetic testing in diabetic cases (2). In addition, the interpretation of the variations found in target genes is not clear-cut yet. Therefore, we presented variations belonging to all pathogenicity classes in this study. New types of monogenic diabetes are added with new research but there is limited knowledge about their clinical pictures and epidemiology. There are many papers related to the most common monogenic diabetes subtypes and their presentation, i.e., MODY 1, 2 and 3, but not to the remaining forms (15). A higher prevalence of monogenic diabetes is reported in recent studies. Islet autoantibody negativity is often used to identify children who could benefit from genetic testing, the screening and testing strategies are variable, with estimates of prevalence up to 2.5%–6.5% (4, 15).

Our study does not depict the prevalence of monogenic diabetes in our cohort since genetic screening is done on a selective basis among clinically suspected cases. But apparently the prevalence would approach 8% roughly which is on the right side of the prevalence spectrum. In 65% of patients genetically tested in our center, no genetic explanation of diabetes was found to date. These patients are type 1or type 2 or MODY X diabetes which is subject to interpretation with prolonged follow-up and/or further genetic studies.

Looking at the literature, the distribution of monogenic diabetes subtypes differs between geographic and ethnic backgrounds of the cohorts studied. A large adult study from Qatar investigated variations in 16 MODY related genes in non-diabetic people and in of T1- and T2 diabetes using whole genome sequencing and mutations in HNF1A gene were most frequent (17). A childhood study again from Qatar found the prevalence of monogenic diabetes <2% and GCK as the prominent MODY subtype (18).

In our study as well GCK is the most prevalent subtype of monogenic diabetes. In a multicenter study from Turkey GCK-MODY was the most common as well, but the distribution of remaining subtypes was quite different suggesting the influence of ethnic differences in our study population (19). Turkey is a country with a mixture of many ethnic subgroups, but our center's hinterland is composed of predominantly people migrated from North or Northeast.

Our cases with GCK represent a very large clinical spectrum. A previous paper of us presents their clinic in more detail (20). The most severe one with homozygosity and permanent insulin dependency was previously reported as well (21). Another interesting family in the present study is that with double heterozygous cases of GCK and ABCC8 gene. Clinically all the three siblings shared almost the same degree of hyperglycemia, thus the variation in ABBC8 does not look a game changer, although affected their father in a different degree (see results section). We found seven novel GCK mutations. In fact, the mutations c.1256delT (found in 11 subjects), c.1328A>C (found in 2 subjects) have no rs numbers in databases as well, but we found two publications reporting them, therefore not listed as novel (9, 10).

A case-control study showed the modestly

association of HNF1A gene p.I27L SNP with early-onset, MODY-like diabetes in the Turkish population (22). We found this variation in two patients and listed it as VUS according to ACMG. Many variations in this gene were reported, some of them benign, some of them related to T2D in large cohorts (23, 24).

BLK is a controversial gene as a MODY subtype, suggested in 2009 but recently rejected by Laver *et al.* (25). Our first patient with a VUS variation in BLK gene depicts a well co-segregation in the family and a very typical clinic resembling MODY-diabetes. In MODY-IST study again from Turkey, an apparently benign variation in BLK gene was indicated as a pathogenic one due to segregation findings (26). But the likely benign BLK variation in our second case with triple variation (one BLK, Two HNF1A) is probably not explaining his diabetes.

INS-MODY patients were interestingly diagnosed around the age of 3 years. Their age at diagnosis is unusual compared to the former literature assigning INS mutation as the cause of neonatal diabetes but in accordance with later case presentations broadening the clinical spectrum (27).

We did not discuss clinical picture of every case or genetic subgroup. In general, our detection rate (35%) is quite high. In a study from Turkey with similar number of background diabetes cases, the detection rate was near 10%, still higher than many other cohorts (28). In a previous study we screened 40 selected patients using a 10 gene MODY panel and found 10 P and LP variations, where the diagnostic rate was 25% (29). This might be due to "too" careful selection of cases for genetic testing with concern about cost. Therefore, we are planning to consider further genetic tests in 152 cases with negative results.

All variations in genes known as susceptibility genes (SLC5A2, MAPK8IP1, CCR5, G6PC2, GPD2) are found occasionally during exome sequencing. The patients carrying them represented very atypical clinical patterns compared with type 1 and type 2 diabetes.

MAPK8IP1is suggested as a candidate gene for T2D (30). But yet, we cannot claim that these variations explain their disease.

None of our patients is on successful and permanent SU treatment, except one with transient success. The main reason is our hesitation to stop insulin in this critical age group. The period of growth and puberty, school-life facts, difficulty of evaluating the compliance, fear for treatment failure are factors influencing this attitude.

Limitations and strengths of the study

This study is retrospective, and data are obtained from daily clinical practice spread over a quite long period of time. Even the quality and interpretation of genetic tests changed during this time. The study cannot depict the prevalence of monogenic diabetes.

On the other hand, the clinic where the study was elaborated is in very close contact with families of children with diabetes and both physical and tele-visits are very much facilitated. It is the unique reference center for at least eight close provinces. This bears the advantage for close follow-up and clinical data collection. Therefore, our findings are superior to multi-center or national pooled data analysis studies in describing real life data of monogenic diabetes.

In conclusion, the present study highlights many aspects of childhood monogenic diabetes, i.e., its probability, the distribution of its subgroups, the details and heterogeneity of clinical picture and so on. Future studies will further define the clinical presentation and long-term follow-up data of subgroups in pediatric diabetes cohorts in which variations in different genes associated with the disease have been detected. This kind of observations might change the attitudes and judgements of clinicians in classifying pediatric diabetes cases. More importantly, determined treatment methods need to be studied in subgroups where variations in different genes associated with the disease have been detected in the long term.

Future studies have to include information about close follow-up, detailed and confirmed genetic analysis and prognostic features of monogenic diabetes subgroups.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Schober E, Rami B, Grabert M, Thon A, Kapellen T, Reinehr T, Holl RW; DPV-Wiss Initiative of the German Working Group for Paediatric Diabetology and. Phenotypical aspects of maturity-onset diabetes of the young (MODY diabetes) in comparison with Type 2 diabetes mellitus (T2DM) in children and adolescents: experience from a large multicentre database. Diabet Med. 2009;26(5):466- 473.
2. Robertson CC, Rich SS. Genetics of type 1 diabetes. Curr Opin Genet Dev. 2018;50:7-16.
3. Ndiaye FK, Ortalli A, Canouil M, Huyvaert M, Salazar-Cardozo C, Lecoeur C, Verbanck M, Pawlowski V, Boutry R, Durand E, Rabearivelo I, Sand O, Marselli L, Kerr-Conte J, Chandra V, Scharfmann R, Poulain-Godefroy O, Marchetti P, Pattou F, Abderrahmani A, Froguel P, Bonnefond A. Expression and functional assessment of candidate type 2 diabetes susceptibility genes identify

four new genes contributing to human insulin secretion. Mol Metab. 2017;6(6):459-470.
4. Tosur M, Philipson LH. Precision diabetes: Lessons learned from maturity-onset diabetes of the young (MODY). J Diabetes Investig. 2022;13(9):1465-1471.
5. Gerhardsson P, Schwandt A, Witsch M, Kordonouri O, Svensson J, Forsander G, Battelino T, Veeze H, Danne T. The SWEET Project 10-Year Benchmarking in 19 Countries Worldwide Is Associated with Improved HbA1c and Increased Use of Diabetes Technology in Youth with Type 1 Diabetes. Diabetes Technol Ther. 2021;23(7):491-499.
6. Ellard S, Bellanné-Chantelot C, Hattersley AT; European Molecular Genetics Quality Network (EMQN) MODY group. Best practice guidelines for the molecular genetic diagnosis of maturity-onset diabetes of the young. Diabetologia. 2008;51(4):546-553.
7. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424.
8. Joynt ACM, Axford MM, Chad L, Costain G. Understanding genetic variants of uncertain significance. Paediatr Child Health. 2021;27(1):10-11.
9. Aykut A, Karaca E, Onay H, Gökşen D, Çetinkalp Ş, Eren E, Ersoy B, Çakır EP, Büyükinan M, Kara C, Anık A, Kırel B, Özen S, Atik T, Darcan Ş, Özkınay F. Analysis of the GCK gene in 79 MODY type 2 patients: A multicenter Turkish study, mutation profile and description of twenty novel mutations. Gene. 2018;641:186-189.
10. Magliano DJ, Boyko EJ; IDF Diabetes Atlas 10th edition scientific committee. IDF DIABETES ATLAS. 10th ed. Brussels: International Diabetes Federation; 2021. https://diabetesatlas.org/idfawp/resource - files/2021/07/IDF_Atlas_10th_Edition_2021.pdf
11. Yeşilkaya E, Cinaz P, Andıran N, Bideci A, Hatun Ş, Sarı E, Türker T, Akgül Ö, Saldır M, Kılıçaslan H, Açıkel C, Craig ME. First report on the nationwide incidence and prevalence of Type 1 diabetes among children in Turkey. Diabet Med. 2017;34(3):405-410.
12. Poyrazoğlu Ş, Bundak R, Yavaş Abalı Z, Önal H, Sarıkaya S, Akgün A, Baş S, Abalı S, Bereket A, Eren E, Tarım Ö, Güven A, Yıldız M, Karaman Aksakal D, Yüksel A, Seymen Karabulut G, Hatun Ş, Özgen T, Cesur Y, Azizoğlu M, Dilek E, Tütüncüler F, Papatya Çakır E, Özcabı B, Evliyaoğlu O, Karadeniz S, Dursun F, Bolu S, Arslanoğlu İ, Yeşiltepe Mutlu G, Kırmızıbekmez H, İşgüven P, Üstyol A, Adal E, Uçar A, Cebeci N, Bezen D, Binay Ç, Semiz S, Korkmaz HA, Memioğlu N, Sağsak E, Peltek HN, Yıldız M, Akçay T, Turan S, Güran T, Atay Z, Akcan N, Çizmecioğlu F, Ercan O, Dağdeviren A, Baş F, İşsever H, Darendeliler F. Incidence of Type 1 Diabetes in Children Aged Below 18 Years during 2013-2015 in Northwest Turkey. J Clin Res Pediatr Endocrinol. 2018;10(4):336-342.
13. Deutsch AJ, Ahlqvist E, Udler MS. Phenotypic and genetic classification of diabetes. Diabetologia. 2022;65(11):1758-1769.
14. Nkonge KM, Nkonge DK, Nkonge TN. The epidemiology, molecular pathogenesis, diagnosis, and treatment of maturity-onset diabetes of the young (MODY). Clin Diabetes Endocrinol. 2020;6(1):20.
15. Harsunen M, Kettunen JLT, Härkönen T, Dwivedi O, Lehtovirta M, Vähäsalo P, Veijola R, Ilonen J, Miettinen PJ, Knip M, Tuomi T. Identification of monogenic variants in more than ten per cent of children without type 1 diabetes-related autoantibodies at diagnosis in the Finnish Pediatric Diabetes Register. Diabetologia. 2023;66(3):438-449.
16. Asahara SI, Inoue H, Kido Y. Regulation of Pancreatic β-Cell Mass by Gene-Environment Interaction. Diabetes Metab J. 2022;46(1):38-48.
17. Elashi AA, Toor SM, Diboun I, Al-Sarraj Y, Taheri S, Suhre K, Abou-Samra AB, Albagha OME. The Genetic Spectrum of Maturity-Onset Diabetes of the Young (MODY) in Qatar, a Population-Based Study. Int J Mol Sci. 2022 Dec 21;24(1):130.
18. Haris B, Saraswathi S, Al-Khawaga S, Hasnah R, Saeed A, Mundekkadan S, Hamed N, Afyouni H, Abdel-Karim T, Mohammed S, Khalifa A, Al-Maadheed M, Al-Zyoud M, Shamekh A, Elawwa A, Al-Khalaf F, Boughorbel S, Petrovski G, Hussain K. Epidemiology, genetic landscape and classification of childhood diabetes mellitus in the State of Qatar. J Diabetes Investig. 2021;12(12):2141-2148.
19. Gökşen D, Yeşilkaya E, Özen S, Kor Y, Eren E, Korkmaz Ö, Berberoğlu M, Karagüzel G, Er E, Abacı A, Evliyaoğlu O, Akbaş ED, Ünal E, Bolu S, Nalbantoğlu Ö, Anık A, Tayfun M, Büyükinan M, Abalı S, Can Yılmaz G, Kor D, Söbü E, Şıklar Z, Polat R, Darcan Ş. Molecular Diagnosis of Monogenic Diabetes and Their Clinical/Laboratory Features in Turkish Children. J Clin Res Pediatr Endocrinol. 2021;13(4):433-438.
20. Bolu S, Eroz R, Dogan M, Arslanoglu I, Dundar I. Genotype-Phenotype Characteristics of Turkish Children With Glucokinase Mutations Associated Maturity-Onset Diabetes of the Young. Indian Pediatr. 2020;57(11):1037-1039.
21. Bolu S, Eröz R, Doğan M, Arslanoğlu İ, Uzun H, Timur F. A family with novel homozygous deletion mutation (c.1255delT; p.Phe419Serfs*12) in the glucokinase gene, which is a rare cause of permanent neonatal diabetes mellitus. Turk Pediatri Ars. 2020;55(4):434-437.
22. Beysel S, Eyerci N, Pinarli FA, Kizilgul M, Ozcelik O, Caliskan M, Cakal E. HNF1A gene p.I27L is associated with early-onset, maturity-onset diabetes of the young-like diabetes in Turkey. BMC Endocr Disord. 2019;19(1):51.
23. Kind L, Raasakka A, Molnes J, Aukrust I, Bjørkhaug L, Njølstad PR, Kursula P, Arnesen T. Structural and biophysical characterization of transcription factor HNF-1A as a tool to study MODY3 diabetes variants. J Biol Chem. 2022;298(4):101803.
24. Najmi LA, Aukrust I, Flannick J, Molnes J, Burtt N, Molven A, Groop L, Altshuler D, Johansson S, Bjørkhaug L, Njølstad PR. Functional Investigations of HNF1A Identify Rare Variants as Risk Factors for Type 2 Diabetes in the General Population. Diabetes. 2017;66(2):335-346.
25. Borowiec M, Liew CW, Thompson R, Boonyasrisawat W, Hu J, Mlynarski WM, El Khattabi I, Kim SH, Marselli L, Rich SS, Krolewski AS, Bonner-Weir S, Sharma A, Sale M, Mychaleckyj JC, Kulkarni RN, Doria A. Mutations at the BLK locus linked to maturity onset diabetes of the young and beta-cell dysfunction. Proc Natl Acad Sci U S A. 2009;106(34):14460-14465.
26. Aydogan HY, Gul N, Demirci DK, Mutlu U, Gulfidan G, Arga KY, Ozder A, Camli AA, Tutuncu Y, Ozturk O, Cacina C, Darendeliler F, Poyrazoglu S, Satman I. Precision Diagnosis of Maturity-Onset Diabetes of the Young with Next-Generation Sequencing: Findings from the MODY-IST Study in Adult Patients. OMICS. 2022;26(4):218-235.
27. Piccini B, Artuso R, Lenzi L, Guasti M, Braccesi G, Barni F, Casalini E, Giglio S, Toni S. Clinical and molecular characterization of a novel INS mutation identified in patients with MODY phenotype. Eur J Med Genet. 2016;59(11):590- 595.
28. Karaoglan M, Nacarkahya G. Clinical and laboratory clues of maturity-onset diabetes of the young and determination of association with molecular diagnosis. J Diabetes. 2021;13(2):154-163.
29. Doğan M, Eröz R, Bolu S, Yüce H, Gezdirici A, Arslanoğlu İ, Teralı K. Study of ten causal genes in Turkish patients with clinically suspected maturity-onset diabetes of the young (MODY) using a targeted next-generation sequencing panel. Mol Biol Rep. 2022;49(8):7483-7495.
30. Waeber G, Delplanque J, Bonny C, Mooser V, Steinmann M, Widmann C, Maillard A, Miklossy J, Dina C, Hani EH, Vionnet N, Nicod P, Boutin P, Froguel P. The gene MAPK8IP1, encoding islet-brain-1, is a candidate for type 2 diabetes. Nat Genet. 2000;24(3):291-295.