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### ► To cite this version:

Martine Vaxillaire, Amélie Bonnefond, Stavros Liatis, Leila Ben Salem Hachmi, Aleksandra Jotic, et al. Monogenic diabetes characteristics in a transnational multicenter study from Mediterranean countries. *Diabetes Research and Clinical Practice*, 2021, 171, pp.108553 -. 10.1016/j.diabres.2020.108553 . hal-03493952

**HAL Id: hal-03493952**

**<https://hal.science/hal-03493952v1>**

Submitted on 15 Dec 2022

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## **Monogenic diabetes characteristics in a transnational multicenter study from Mediterranean countries**

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## ABSTRACT

**Background.** Diagnosis of monogenic diabetes has important clinical implications for treatment and health expenditure. However, its prevalence remains to be specified in many countries, particularly from South Europe, North Africa and Middle-East, where non-autoimmune diabetes in young adults is increasing dramatically.

**Aims.** To identify cases of monogenic diabetes in young adults from Mediterranean countries and assess the specificities between countries.

**Methods.** We conducted a transnational multicenter study based on exome sequencing in 204 unrelated patients with diabetes (age-at-diagnosis:  $26.1 \pm 9.1$  years). Rare coding variants in 35 targeted genes were evaluated for pathogenicity. Data were analyzed using one-way ANOVA, chi-squared test and factor analysis of mixed data.

**Results.** Forty pathogenic or likely pathogenic variants, 14 of which novel, were identified in 36 patients yielding a genetic diagnosis rate of 17.6%. The majority of cases were due to *GCK*, *HNF1A*, *ABCC8* and *HNF4A* variants. We observed highly variable diagnosis rates according to countries, with association to genetic ancestry. Lower body mass index and HbA1c at study inclusion, and less frequent insulin treatment were hallmarks of pathogenic variant carriers. Treatment changes following genetic diagnosis have been made in several patients.

**Conclusions.** Our data from patients in several Mediterranean countries highlight a broad clinical and genetic spectrum of diabetes, showing the relevance of wide genetic testing for personalized care of early-onset diabetes.

**Keywords:** genetic diagnosis; maturity-onset diabetes of the young (MODY); monogenic diabetes; exome sequencing; pathogenic variant

## 1. Introduction

Maturity-onset diabetes of the young (MODY, OMIM #606391), defined as a dominantly inherited form of early-onset non-autoimmune diabetes, is the most common and widely characterized presentation of monogenic diabetes (MD) [1]. MODY has been estimated to account for two to five percent of young-onset diabetes cases from population-based studies and patient registries [2–5]. MODY can occur at different ages from childhood to early adulthood, while presenting with variable clinical features both at diagnosis and later in life [1,6]. Beyond MODY, the wide clinical spectrum of MD is substantially explained by genetic heterogeneity with various types of genetic defects in more than 30 genes, depending on the consensus of established causal genes, including some involved in rarer forms of early infancy-onset diabetes [1,6,7]. Importantly, a formal genetic diagnosis is the only way to recognize MD from common type 2 diabetes. A diagnosis of MD has important implications for prognosis and choice of treatment regimen, as well as family counseling and health care cost [6,8]. Indeed, the policy of routine genetic screening for MD in pediatric or young adult populations has been shown to be cost-effective and to improve patients' quality of life [9,10]. This is largely due to the possible switch from insulin to sulfonylurea (SU) drugs in patients with a pathogenic variant in *HNF1A*, *HNF4A*, *ABCC8* or *KCNJ11* [10], and also because *GCK*-MODY patients generally do not require treatment unless in pregnancy [1].

Prevalence and genetic subtypes of MD, particularly MODY, are rather well documented in Caucasian populations from Europe, in Australia and in the USA [2-5], and through studies done in South and East Asian populations [11,12], while their estimate in Maghreb and Middle-East remains elusive, mainly because of poor access to genetic testing [13–15]. Yet, in these regions, the prevalence of diabetes is rising steadily, by affecting patients at an increasingly younger age [16]. Actually, most genetic studies from these populations handled only limited sets of candidate genes leading to partial conclusions on the genetic etiology of potentially MD cases [17–20].

Exome sequencing (ES) enables a comprehensive, quick and cost-efficient genetic diagnosis of all currently known MD subtypes, as for other rare genetic disorders [21,22]. ES is also very efficient to thoroughly investigate the relative prevalence of disease genetic subtypes at the population level [23–25]. In this study, we took advantage of this technology to provide diabetes specialists from eleven countries of the Mediterranean area with a comprehensive genetic diagnosis for MD. Our main aim was to assess the genetic subtypes and specificities of diabetes in young adults with a suspicion of MD in Southern European and other Mediterranean countries.

## **2. Subjects and Methods**

### **2.1 Participants and study design**

The Mediterranean Group for the Study of Diabetes (MGSD) has established a transnational multicenter population study of patients with a suspicion of MD from eleven countries of the Mediterranean area including Algeria, Cyprus, Greece, France, Lebanon, Malta, Morocco, Portugal, Serbia, Tunisia and Turkey (**Figure 1**). From these countries, thirty Endocrinology-Diabetology departments or outpatient clinics were involved under the responsibility of one national coordinator per country (online supplementary **Table 1**). Between February 2017 and March 2018, 237 patients were enrolled, considering the following inclusion criteria: i/ an age  $\leq$  40 years at diabetes diagnosis without insulin treatment or low insulin requirements, ii/ complete lack of obesity or lack of significant obesity (body mass index [BMI]  $\leq$  30 kg/m<sup>2</sup>) at diagnosis, iii/ self-reported family history of diabetes consistent with autosomal dominant inheritance, or iv/ atypical presentation of diabetes that may suggest a monogenic form (like onset of diabetes before 45 years with normal BMI, and a family history of diabetes contrasting with type 2 diabetes).

Exclusion criteria included: i/ presence of type 1 diabetes as documented by antibodies to at least three islet cell antibody tests (when searched for) and ii/ diabetes due to diseases of the exocrine pancreas or drug-related diabetes or diabetes related to endocrine diseases. In the present study, we excluded 33 patients as they were relatives of an index case, or the related DNA sample had poor quality and/or quantity (online supplementary **Figure 1**).

Patient information, including demographics, family history of diabetes, age at diagnosis, initial treatment of diabetes, glycated hemoglobin A1c (HbA1c) and treatment of diabetes at inclusion, were provided through a dedicated electronic case report form (eCRF) (**Table 1** and online supplementary **Table 2**). Information on C-peptide levels was not available in many patients of the study. At the inclusion visit, a salivary sample was collected for each participant using the Oragene-DNA self-collection kit (DNA Genotek, Ottawa, ON, Canada). Each participating center transmitted the salivary samples to Lille laboratory (UMR1283/8199, EGID, France) for DNA extraction and further genetic analyses.

This study was conducted according to the principles of the Declaration of Helsinki, and was approved by the Ethics committee of CPP Ile-de-France VI (GH Pitié Salpêtrière). Each national coordinator was responsible for regulatory and ethical issues according to local regulatory requirements. The local/national Ethics committee in each country approved the study protocol, the "Participant information and consent form" document, the list of investigators and the insurance documents. Each study participant provided written informed consent.

## **2.2 Target enrichment preparation, next-generation sequencing and variant detection**

We used the NimbleGen SeqCap EZ MedExome target enrichment kit (Roche, Basel, Switzerland) for Illumina sequencing (San Diego, CA, USA). Briefly, 300 ng of DNA were fragmented through sonication via the Covaris E220 Focused-ultrasonicator (Woburn, MA, USA), end-repaired with adapter ligation and PCR-amplified with individual sample barcodes, then equimolar pools were hybridized to the biotin-labeled SeqCap EZ capture probe pool. The

capture pools were subsequently amplified using the KAPA HiFi HotStart ReadyMix (Wilmington, MA, USA) and quantified for paired-end sequencing using the Illumina NovaSeq system according to the manufacturer's instructions. The sequence reads from FASTQ files were aligned to the human genome (GRCh38/hg38) using Burrows-Wheeler Aligner (version 0.7.15). The variant calling was performed using Genome Analysis ToolKit (GATK; version 3.8.1) [26], for single nucleotide variants, small insertions and deletions. Only variants covered with  $\geq 8$  sequencing reads were kept for variant annotation and analysis. With a mean depth of coverage of 105 ( $\pm 32$ ) reads across the samples, 98.8 ( $\pm 0.8$ ) % of targeted bases were covered with at least 8 reads.

Sanger sequencing was used to confirm the presence of pathogenic or likely pathogenic variants identified by ES in several relatives with diabetes from six families (when DNA sample was available). Primer sequences and PCR conditions are available upon request. Fragments were sequenced in both directions, and subsequently analyzed using the 3730xl DNA Analyzer (Applied Biosystems). Electrophoregram reads were assembled and examined using the SeqScape software (Applied Biosystems).

### **2.3 Variant annotation and selection, interpretation and classification of rare variants**

All variants were annotated using the Ensembl Perl Application Program Interfaces (version 89), which integrate genomic data from dbNSFP 3.4a, including information on functional predictions (*i.e.*, Polyphen-2\_HumDiv, SIFT, MutationTaster), variant allele frequencies from several public databases including the Genome Aggregation Database (gnomAD v2.1.1) and our in-house database (including genetic variants identified in all individuals who have been sequenced at the LIGAN-PM sequencing platform, Lille). We investigated rare coding variants (*i.e.*, initiation codon variants, stop lost and stop gained variants, missense variants, splice donor and acceptor variants, frameshift and inframe variants) with a minor allele frequency (MAF)

below 0.1% in 35 genes involved in MD (online supplementary **Table 3, and information in [1,6,8]**).

To evaluate the pathogenicity of the variants, we applied the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG) established in 2015 (online supplementary **Tables 4 and 5**) [27]. For this purpose, we used the AlamutVisual version 2.11 software (Interactive Biosoftware), including ClinVar information, and the Human Gene Mutation Database Professional 2019.3 (HGMD-Pro, Qiagen). For the moderate pathogenic criterion PM2 and the supporting pathogenic criterion PP2, we used information from the gnomAD browser. The variants were classified independently by two geneticists into three categories: pathogenic (P), likely pathogenic (LP) or variants of uncertain significance (VUS) (online supplementary **Table 5**).

## **2.4 Statistical analysis**

All statistical analyses were performed using the R software (version 3.4.2.) [28].

The characteristics of the study participants were compared between the countries of recruitment and the studied groups (*i.e.*, with and without P/LP variants) using one-way ANOVA for continuous variables or chi-squared test for discrete phenotypes. *Post-hoc* tests were performed using Tukey's honestly significant difference (HSD) method. A factor analysis of mixed data (FAMD) taking into account both continuous and categorical variables was performed to explore the dissimilarities between individuals and between the different phenotypes, using the R package *FactoMineR* [29]. Phenotypes with missing values (namely age at diagnosis and family history of type 2 diabetes) were imputed by the mean. A principal component analysis (PCA) was performed using the R package *flashpcaR* [30] to assess the ethnicity of study participants based on the first two genomics components. The PCA was performed on a combined dataset of 1,711 variants with MAF > 1% from ES data of the study participants and data from the 1,000 Genomes [31]. Further analyses were performed to test differences between

European (EUR) and non-EUR individuals. Individuals were defined as of EUR ancestry if they were closest to EUR sub-populations (British in England and Scotland [GBR], Toscani in Italy [TSI], Iberian populations in Spain [IBS], Utah residents with Northern and Western EUR ancestry [CEU] and Finnish in Finland [FIN]) from 1,000 Genomes project on the factorial plane using the first two principal components. Then, the predicted ethnicities were compared between the studied groups (*i.e.*, samples with and without P/LP variants) using a chi-squared test. All results were considered significant when the  $p$ -value was below 0.05.

### 3. Results

#### 3.1 Characteristics of the study participants

The study included 204 unrelated participants with a suspicion of MD from eleven countries of the Mediterranean area (**Figure 1**). The clinical characteristics of the participants are shown per country in **Table 1**. A total of 114 (55.9%) participants were female, the mean age at diagnosis was  $26.1 \pm 9.1$  years, and 173 (84.8 %) participants had diabetes diagnosed at age  $\leq 35$  years, of them 89 (51.4%) were diagnosed at age  $\leq 25$  years; a first degree family history of diabetes was reported by 150 (73.5%) participants, with a significant variability among countries ( $p = 0.003$ ; **Table 1**). At inclusion in the study, the mean age of the participants was  $34.9 \pm 11.4$  years and 169 (82.8%) participants were under glucose-lowering treatment, 89 (43.6%) of whom received insulin injections. Through FAMD analysis to assess the discriminating parameters enabling to stratify patients into phenotypic subgroups, we found that the main contributing variables were the treatment both at diagnosis and at inclusion in the study, and to a lesser extent the HbA1c levels at inclusion and ages at diagnosis and at inclusion (online supplementary **Figure 2A**).

#### 3.2 Genetic diagnosis of MD

From the exome data of 204 unrelated individuals, we assessed the pathogenicity of each rare variant detected in 35 genes involved in MD (online supplementary **Table 3**) using ACMG criteria. We identified forty P/LP heterozygous variants in 36 (17.6%) patients (**Table 2**); of them, fourteen variants were novel, namely they have not been reported in the literature nor listed in gnomAD (**Table 2**).

Among these 36 participants diagnosed as MD cases, most of the P/LP variants were identified in *GCK* (37.5%), *HNF1A* (27.5%), *ABCC8* (12.5%) and *HNF4A* (7.5%), while few other P/LP mutations were found in *CEL*, *HNF1B*, *KCNJ11*, *PAX4*, *PDX1* and *WFS1* (**Table 2** and online supplementary **Figure 3**). Of note, three patients carried P/LP variants in two MD genes

suggesting a likely digenic effect (*HNFI1A/ABCC8*, *GCK/HNF1B*, *ABCC8/ PAX4*), yet no striking phenotypes were observed as they all developed diabetes after 25 years old (online supplementary **Table 6**). In contrast, one patient who developed early-onset diabetes (at 11 years old) carried two novel LP variants in *ABCC8* (p.Phe295Leu and p.His1537Tyr) (**Table 2** and online supplementary **Table 6**).

### **3.3 Clinical parameters of patients carrying a P/LP variant versus non-carriers**

The participants with P/LP variants were significantly leaner (BMI:  $23.4 \pm 3.9$  kg/m<sup>2</sup> vs.  $26.3 \pm 4.7$  kg/m<sup>2</sup>,  $p = 0.001$ ) and their diabetes was better controlled (HbA1c:  $6.8 \pm 1.3\%$  vs.  $7.7 \pm 2.2\%$ ,  $p = 0.016$ ) at the time of inclusion in the study, when compared to non-carriers (**Table 3** and **Figure 2**). It is to note that BMI and HbA1c values were not available at diagnosis. Furthermore, the P/LP variant carriers were less likely to be treated with insulin both at diagnosis (13.9% vs. 35.7%,  $p = 0.019$ ) and at inclusion (22.2% vs. 48.2%,  $p = 0.008$ ), when compared to non-carriers (**Table 3**). In 171 patients with fully available information to calculate the MODY probability score [32], we found that the mean value of this score was significantly higher in P/LP variant carriers when compared to non-carriers ( $56.7 \pm 23.8\%$  vs.  $38.2 \pm 28.7\%$ , respectively,  $p = 0.001$ ; online supplementary **Figure 4**). When we took into account a threshold value of 40% for this score [32], we found that 67.7% of P/LP variant carriers compared to 47.8% of non-carriers had a MODY probability score above this value, showing the limitation of its use in older, non-EUR populations, as in the context of our study.

Interestingly, through FAMD analysis relying on individual phenotypic features, including both continuous and categorical variables (online supplementary **Figure 2A**), we looked for characteristics that could contribute to the stratification of patients in the entire study population. As shown in online supplementary **Figure 2B**, the projection of all patients on the first plan and additional marking of the P/LP variant carriers compared to the non-carriers pointed that patients with P/LP variants were not discriminated at all from non-carriers; this implies the

impossibility of distinguishing clinically these two groups of patients without a precise genetic diagnosis.

### 3.4 Specificities of MD subtypes per country

The distribution of MD subtypes per country is depicted in online supplementary **Figure 3**. Importantly, we observed highly variable genetic diagnosis rates according to the country of recruitment, ranging from 0% in Morocco to 40% in Greece (**Figure 1** and **Table 1**). Furthermore, we found that P/LP variants in *GCK* were more frequent in patients from France and Malta, and that P/LP variants in *HNF1A* were more frequent in patients from Greece and Serbia (**Table 2** and online supplementary **Figure 3**). In particular, the nonsense *GCK*-p.Gln26\* variant identified in three Maltese patients (**Table 2**) has already been reported in seven Italian families living in the Veneto region of North-Eastern Italy with a suspected founder effect [33]. In addition, in two Algerian and Greek patients, we identified a previously reported loss-of-function *GCK* variant (p.Gly72Arg; **Table 2**), according to decreased enzyme activity and loss of stabilizing interaction with glucokinase regulatory protein [34].

Furthermore, using targeted Sanger sequencing, we confirmed the presence of several P/LP variants in *GCK*, *HNF1A* or *HNF4A* in affected relatives in six independent families, which further substantiated a monogenic cause of diabetes in these families (online supplementary **Figure 5**). Notably, a complete co-segregation between the *HNF4A*-p.Arg231Gln variant and diabetes was found in six relatives over two generations in a family from Cyprus (including three cases diagnosed with diabetes at ages 16, 18 and 19 years) (online supplementary **Figure 5**). The functional causality of this missense *HNF4A* variant has previously been reported in a Japanese family [35].

Taking advantage of the individual genomic information from ES, we evaluated the ethnic components of each individual using the 1,000 Genomes project (online supplementary **Table 7**). As expected, specific ethnic diversity was observed according to the country of recruitment

(online supplementary **Table 7**). Importantly, we found that the genetic diagnosis of MD was significantly higher in EUR participants when compared to non-EUR cases: among the carriers of a P/LP variant, 86.1% were defined as EUR while 13.9% were non-EUR, and among the non-carriers, 67.3% were defined as EUR while 32.7% were non-EUR ( $p = 0.040$ ). We then assessed whether some clinical features might differ between the EUR and non-EUR participants from the entire study, which would explain this difference in genetic diagnosis. We found that EUR participants were older at diagnosis (27.0 [ $\pm 8.9$ ] vs. 23.8 [ $\pm 9.1$ ] years,  $p = 0.029$ ) and less often treated for diabetes at diagnosis, particularly by insulin (23.6% vs. 51.7%,  $p < 0.001$ ), and their HbA1c values at study inclusion were lower (7.3% [ $\pm 2.1$ ] vs. 8.0% [ $\pm 2.0$ ],  $p = 0.040$ ).

### **3.5 Impact of genetic diagnosis of MD on diabetes treatment**

We also assessed the information on possible treatment adaptation following the genetic diagnosis. Eleven out of fourteen (78.6%) patients with P/LP variants in *HNF1A* or *HNF4A* were treated by oral hypoglycemic agent (OHA) at inclusion in the study, and three patients received insulin injections. Six of those who were treated with OHA before genetic testing took SU or dipeptidyl peptidase-IV (DPP-IV) inhibitor or glucagon like peptide-1 (GLP1) agonist drugs, which have proven to be very effective in patients with mutations of *HNF1A* or *HNF4A*. Importantly, two patients carrying a *HNF1A* mutation experienced a change in treatment after the genetic diagnosis with good metabolic control: patient ID-521000401 switched from insulin, metformin and DPP-IV inhibitor (treatment at study inclusion) to DPP-IV inhibitor and gliclazide (SU drug) with last HbA1c  $< 7\%$  (current age 50 years old); patient ID-420003301 switched from metformin to gliclazide only with last HbA1c of 6.2% compared to 6.5% before switching treatment (current age 36 years old) (**Table 2**). Furthermore, patient ID-850000601 (**Table 2**) with a *HNF4A* mutation who was treated with metformin switched to gliclazide upon genetic diagnosis.

Seven out of fifteen (46.7%) patients with P/LP variants in *GCK* were treated by OHA (in six patients) or insulin (in one patient); after genetic diagnosis, drug therapy was stopped in two *GCK-MODY* patients, who were put on diet only and exercise with optimal metabolic control: patient ID-324000301 showed a HbA1c value of 6.1% at last examination (compared to 6.4% at inclusion visit; current age 32 years old), and patient ID- 420000801 had a HbA1c of 5.8% at last examination (HbA1c < 6% at inclusion visit; current age 30 years old).

One patient bearing a LP variant in *ABCC8* was on insulin treatment before genetic testing, which was identified as candidate for treatment switch to oral SU drug. In three participants, we found three variants, *KCNJ11*-p.Arg27Cys, *ABCC8*-p.Asp310Asn and *ABCC8*-p.Glu1506Lys, previously reported with experimentally validated (or presumed) loss-of-function on K-ATP channel activity (*i.e.*, leading to channel closure and increased insulin secretion). Consequently, oral SU treatment might not be recommended for these patients.

#### **4. Discussion**

Our study provided a genetic etiology of MD in 36 patients with young-onset to middle-age diabetes from geographically more diverse populations than in previous studies on MD. In addition to reporting data on several novel variants of interest in MD genes, the (likely) pathogenic variants having been classified according to strict ACMG criteria, our findings do likely reflect real-world practice for genetic diagnosis. However, we must acknowledge the small number of cases in each country overall, which makes it difficult to accurately infer much about the entire population prevalence of MD in the participating countries.

From the entire study population, a genetic diagnosis of MD was revealed in 17.6% of the participants. Diagnosis rates differed widely by country of inclusion, with the highest rates observed in Greece (40%), Algeria (30%) and France (27.8%), and the lowest in Tunisia (8%) and Turkey (12%) while no variant of interest were found in the Moroccan patients. By

analyzing individual's ancestry background using exome data, the study participants were stratified according to whether or not they belonged to five EUR sub-populations, which allowed us to show a significant association between EUR ancestry and the presence of P/LP variants. This result strengthens previous observations that a lower genetic diagnosis of MD prevails in people from non-EUR origins, such as in South and East Asians or North Africans [12,13,25,36–38], underpinning yet unknown genetic and pathophysiological mechanisms for MD in these regions.

In our study, the average BMI of non-EUR participants did not significantly differ from the one in EUR participants; while ages at onset of diabetes in non-EUR cases were earlier with more severe presentations. Similarly, a recent study of 109 non-type 1 diabetes patients of Korean origin, using targeted gene panel sequencing, found that only 23 (21.1%) patients harbored P/LP variants (representing 1.1% of cases from the overall non-type 1 diabetes clinical cohort), which also had a lower BMI and lower C-peptide levels compared to those without pathogenic variants [25]. On the whole, our data still support the hypothesis that cases of non-EUR ancestry may have other forms of diabetes than the classical MODY or other known MD subtypes. It is therefore important to strengthen genetic studies in non-EUR populations (particularly in North-Africa and Middle-East people).

Of note, the referring physicians in each country may have influenced the observed differences in diagnosis rates in addition to the country of origin and ethnic differences. Another limitation is the heterogeneity of the population study, which is most probably related to the broadly defined inclusion criteria. Among the 40 P/LP variants identified in the study participants, 29 (72.5%) variants were found in the major MD genes (*i.e.*, *GCK*, *HNF1A*, *HNF4A*); as it is very common in EUR populations [36,37], and also more recently reported in Han Chinese and Russian patients [23,24]. Five different *ABCC8* variants have also been identified in four patients (age at diagnosis: 11-40 years), which emphasizes the broad phenotypic spectrum of this genetic subtype. We also observed a variable pattern of MD subtypes between the countries,

namely *GCK* variants are more prevalent in patients from France and Malta, and *HNF1A* variants more prevalent in patients from Greece and Serbia. Noteworthy, in four out of the six Greek patients carrying a variant of interest, we identified three *HNF1A* variants supporting a relatively high proportion of *HNF1A*-MODY in this population [39]. Furthermore, one of our findings was able to confirm a pathogenic effect of the *HNF4A*-p.R231Q variant dominantly inherited in a two-generation family from Cyprus; this variant had only been previously reported with reduced transcriptional activity in a single small family of Japanese origin [35].

When analyzing the clinical phenotype-genotype correlations, we found that patients diagnosed with MD (i.e., those who are carriers of a P/LP variant) presented with a discrete clinical profile, that is mainly associated with a lower BMI and lower HbA1c values suggesting a less severe metabolic disease; as it was recently reported by Donath et al. from a multiethnic series of patients with adolescence or adult-onset diabetes, which have been subjected to targeted genetic screening with a pick-up rate of MD of 16.2% (representing 23.2% among EuroCaucasian patients) [37]. Interestingly, some differences in the treatment modalities were also observed between the P/LP variant carriers and non-carriers, especially the carrier ones are less often treated by insulin (both at diagnosis and after years at the time of study inclusion); this disparity may be attributed to distinctive genetic subtypes requiring distinct diabetes management (notably owing to the metabolic defects due to *GCK* and *HNF1A* variants).

A strong point of our study is that it reflects real world clinical practice in the participating countries and as such it may express the true probability of yielding a positive genetic diagnosis among cases clinically suspected with monogenic diabetes or considered to have atypical diabetes. A secondary advantage of this study was to provide clinicians in different countries with a reliable and easy-to-use tool for genetic diagnosis in order to identify diabetes cases which may benefit from switching to simpler and equally or even more effective treatment regimens.

In conclusion, by looking at monogenic diabetes features in **mostly adult patients from** eleven Mediterranean countries, our findings highlight a broad clinical and genetic spectrum of young-onset to adulthood diabetes, partly explained by ancestry. In addition, a relevant aspect of our study is to illustrate how a comprehensive genetic analysis may provide a better understanding of diabetes subtypes and improved long-term patient monitoring even in adulthood.

**Acknowledgments.** We are grateful to all patients from eleven countries, who participated in the study cohort, and to the medical staffs involved in their recruitment and clinical follow-up. We thank Iandry Rabearivelo and Franck De Graeve (both at UMR1283/8199 research unit in Lille, France) for their contribution to computer analyses.

**Author contributions.** M.V., A.B., S.G., J.V., M.M., and P.F. were involved in the conception and design of this study. M.V. and A.B. interpreted data, and wrote the drafts of the paper. M.V., A.B., M.B., S.G., E.D., E.V., M.D., N.L., F.A. and P.F. provided data and/or analyzed data. S.L., L. B-S-H., A.J., R.M., A.C., A.B., M.C., J-F.R., H.I., D.L., N.L., J.V. and M.M. provided clinical data. M.V., A.B., S.L., M.B., M.C., J.V., M.M., and P.F. critically reviewed and modified the manuscript, and all authors agreed to the final submitted version and to be accountable for the work. M.V., A.B., and P.F. are the guarantors of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**Declaration of Competing Interest.** Prof. M. Marre reports personal fees from Abbott, Intarcia, Eli Lilly, MSD, Novo Nordisk, Sanofi and Servier, grants from Novo Nordisk, Sanofi, Servier, MSD and Novartis, and nonfinancial support from Novo Nordisk. No other potential conflicts of interest relevant to this article were reported.

**Funding.** The study was funded by a clinical research grant from the MGSD board committee with support of Servier Affaires Médicales (Suresnes, France), and by grants from the French National Research Agency (ANR-10-LABX-46 [European Genomics Institute for Diabetes] and ANR-10-EQPX-07-01 [LIGAN-PM], to P.F.), from FEDER (Fonds Européen de DEveloppement Régional/European Regional Development Fund) and the ‘*Région Nord Pas-de-Calais*’ (to P.F.).

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## FIGURE LEGENDS

**Figure 1.** Geographical map of the countries participating in the study with the genetic diagnosis rate per country.

Each of the countries participating in the study is colored according to the genetic diagnosis rate, which is indicated as a percentage below the country name. The number of patients with diabetes studied in each country is indicated in brackets. A full list of the centers participating in the study is given in Table S1.

**Figure 2.** BMI and Hb1Ac levels in patients with and without pathogenic (P) or likely pathogenic (LP) variants in the MD genes.

A) BMI values (in kg/m<sup>2</sup>) at the time of inclusion in the study were compared in the patients with and without pathogenic (P) or likely pathogenic (LP) variants, using one-way ANOVA.

B) Hb1Ac levels (in %), at the time of inclusion in the study, were compared in the patients with and without pathogenic (P) or likely pathogenic (LP) variants, using one-way ANOVA.

**Table 1.** Clinical characteristics of the study participants and genetic diagnosis rate stratified per country

	Algeria	Cyprus	France	Greece	Lebanon	Malta	Morocco	Portugal	Serbia	Tunisia	Turkey	P <sup>†</sup>
<b>N</b>	10	10	18	15	7	33	17	12	32	25	25	
<b>Sex (F/M)</b>	2/8	5/5	8/10	3/12	3/4	28/5	11/6	5/7	19/13	16/9	14/11	0.002
<b>Age at diagnosis, years (± SD)</b>	22.9 (9.7)	26.1 (6.7)	27.8 (10.6)	32.1 (7.7)	17.3 (5.3)	26.8 (7.0)	20.4 (7.2)	27.0 (12.6)	26.0 (9.2)	25.6 (9.3)	27.2 (9.2)	<b>0.022</b>
<b>Treatment at diagnosis (n, %)</b>	9 (90.0)	10 (100.0)	16 (88.9)	10 (66.7)	7 (100.0)	15 (45.5)	16 (94.1)	6 (50.0)	25 (78.1)	19 (76.0)	23 (92.0)	<b>&lt;0.001</b>
<b>Insulin treatment at diagnosis (n, %)</b>	5 (50.0)	3 (30.0)	6 (33.3)	1 (6.7)	3 (42.9)	5 (15.2)	8 (47.1)	2 (16.7)	9 (28.1)	13 (52.0)	10 (40.0)	<b>0.037</b>
<b>OHA treatment at diagnosis (n, %)</b>	4 (40.0)	7 (70.0)	10 (55.6)	9 (60.0)	5 (71.4)	12 (36.4)	8 (47.1)	5 (41.7)	17 (53.1)	10 (40.0)	17 (68.0)	0.339
<b>Age at inclusion visit, years (± SD)</b>	27.2 (11.2)	36.2 (10.8)	37.6 (11.4)	40.5 (8.4)	25.1 (13.3)	35.4 (7.8)	30.8 (8.2)	42.1(16.6)	36.5 (13.1)	28.9 (9.5)	38.0 (10.0)	<b>&lt;0.001</b>
<b>BMI (kg/m<sup>2</sup>)* (± SD)</b>	23.8 (2.9)	24.9 (5.2)	25.8 (5.3)	25.9 (2.9)	26.6 (2.2)	25.6 (6.6)	25.7 (4.6)	26.3 (3.4)	25.1 (5.3)	26.7 (3.8)	27.1 (3.8)	0.814
<b>HbA1c (%)* (± SD)</b>	6.9 (1.6)	8.7 (2.4)	7.4 (1.7)	6.8 (1.0)	7.5 (0.9)	6.4 (2.0)	8.6 (1.8)	8.2 (1.5)	6.63 (0.7)	8.6 (2.7)	8.4 (2.8)	<b>&lt;0.001</b>
<b>Treatment at inclusion visit (n, %)</b>	6 (60.0)	10 (100.0)	16 (88.9)	12 (80.0)	7 (100.0)	14 (42.4)	17 (100.0)	11 (91.7)	29 (90.6)	22 (88.0)	25 (100.0)	<b>&lt;0.001</b>
<b>Insulin treatment at inclusion visit (n, %)</b>	3 (30.0)	6 (60.0)	10 (55.6)	3 (20.0)	2 (28.6)	7 (21.2)	12 (70.6)	9 (75.0)	14 (43.8)	10 (40.0)	13 (52.0)	<b>0.007</b>
<b>OHA treatment at</b>	3 (30.0)	7 (70.0)	7 (38.9)	12 (80.0)	7 (100.0)	7 (21.2)	5 (29.4)	8 (66.7)	20 (62.5)	16 (64.0)	17 (68.0)	<b>&lt;0.001</b>

<b>inclusion visit (n, %)</b>												
<b>1<sup>st</sup> degree history of diabetes (n, %)</b>	6 (75.0)	7 (70.0)	9 (90.0)	14 (93.3)	7 (100.0)	16 (61.5)	14 (87.5)	6 (54.5)	26 (92.9)	21 (91.3)	24 (96.0)	<b>0.003</b>
<b>Genetic diagnosis (%)</b>	<b>30%</b>	<b>20%</b>	<b>27.8%</b>	<b>40%</b>	<b>28.6%</b>	<b>15.2%</b>	<b>0</b>	<b>16.7%</b>	<b>18.8%</b>	<b>8%</b>	<b>12%</b>	

Data are numbers, %, or mean  $\pm$  SD; \* at inclusion in the study. <sup>†</sup>*P*-values are given from  $\chi^2$  test for factorial variables and from ANOVA for continuous traits. *BMI*, body mass index; *F*, female; *HbA1c*, glycated hemoglobin A1c; *M*, male; *OHA*, oral hypoglycemic agent.

**Table 2.** P/LP variants detected by sequencing 35 genes involved in MD from our study including 204 participants

Case ID	Country	Chr*	Position (hg38)	Gene	Nucleotide change	Protein effect	variant type	Novel†	Frequency in gnomAD	Pathogenicity <sup>¶</sup>	References
1355010401	Portugal	11	17460616	<i>ABCC8</i>	c.883T>C	p.Phe295Leu	Missense	Yes	-	LP	-
		11	17393128	<i>ABCC8</i>	c.4609C>T	p.His1537Tyr	Missense	Yes	-	LP	-
718000101	Cyprus	11	17460571	<i>ABCC8</i>	c.928G>A	p.Asp310Asn	Missense	No	0.0000122	LP	41
1355011201	Portugal	11	17397007	<i>ABCC8</i>	c.4028A>G	p.Lys1343Arg	Missense	Yes	-	LP	-
		7	127615032	<i>PAX4</i>	c.184A>T	p.Lys62*	Nonsense	Yes	-	P	-
521000101	Greece	11	17394295	<i>ABCC8</i>	c.4516G>A	p.Glu1506Lys	Missense	No	-	P	42
		12	120993588	<i>HNF1A</i>	c.595G>A	p.Gly199Arg	Missense	Yes	-	LP	-
953000201	Lebanon	9	133071509	<i>CEL</i>	c.2016del	p.Val673Cysfs*34	Frameshift	Yes	-	P	-
223000101	Malta	7	44153433	<i>GCK</i>	c.76C>T	p.Gln26*	Nonsense	No	-	P	33
223000901	Malta	7	44153433	<i>GCK</i>	c.76C>T	p.Gln26*	Nonsense	No	-	P	33
223001001	Malta	7	441534333	<i>GCK</i>	c.76C>T	p.Gln26*	Nonsense	No	-	P	33
		17	37701109	<i>HNF1B</i>	c.1408C>G	p.Gln470Glu	Missense	Yes	-	LP	-
347000301	Turkey	7	44153327	<i>GCK</i>	c.182dup	p.Tyr61*	Nonsense	Yes	-	P	-
638000102	Algeria	7	44152420	<i>GCK</i>	c.214G>A	p.Gly72Arg	Missense	No	0.00000406	P	43
521000901	Greece	7	44152420	<i>GCK</i>	c.214G>A	p.Gly72Arg	Missense	No	0.00000406	P	43
420000801	Serbia	7	44149843	<i>GCK</i>	c.596T>C	p.Val199Ala	Missense	No	-	P	44
535000201	Greece	7	44147732	<i>GCK</i>	c.781G>A	p.Gly261Arg	Missense	No	-	P	45
107000701	France	7	44146619	<i>GCK</i>	c.864 -1G>C	p.?	Splice defect	No	-	P	43

349000401	Turkey	7	44146532	<i>GCK</i>	c.950A>C	p.His317Pro	Missense	Yes	-	LP	-
107000601	France	7	44145602	<i>GCK</i>	c.1148C>T	p.Ser383Leu	Missense	No	-	P	46
953000301	Lebanon	7	44145602	<i>GCK</i>	c.1148del	p.Ser383Trpfs*19	Frameshift	No	-	P	45
223001201	Malta	7	44145561	<i>GCK</i>	c.1189C>T	p.Arg397Cys	Missense	No	-	P	45
420002101	Serbia	7	44145273	<i>GCK</i>	c.1261G>T	p.Glu421*	Nonsense	Yes	-	P	-
105000101	France	7	44145189	<i>GCK</i>	c.1345G>A	p.Ala449Thr	Missense	No	-	P	45
105000401	France	12	120978827	<i>HNF1A</i>	c.59G>C	p.Gly20Ala	Missense	No	-	P	47
420001101	Serbia	12	120988879	<i>HNF1A</i>	c.373C>T	p.Gln125*	Nonsense	Yes	-	P	-
420003301	Serbia	12	120988982	<i>HNF1A</i>	c.476G>A	p.Arg159Gln	Missense	No	-	P	47
850001001	Tunisia	12	120988982	<i>HNF1A</i>	c.476G>A	p.Arg159Gln	Missense	No	-	P	47
420003201	Serbia	12	120994238	<i>HNF1A</i>	c.788G>A	p.Arg263His	Missense	No	-	P	47
105000501	France	12	120994265	<i>HNF1A</i>	c.815G>A	p.Arg272His	Missense	No	-	P	47
420001801	Serbia	12	120994322	<i>HNF1A</i>	c.872dupC	p.Gly292Argfs*25	Frameshift	No	0.000332	P	47
521000201	Greece	12	120996610	<i>HNF1A</i>	c.1177delT	p.Ser393Profs*20	Frameshift	No	-	P	39
521000401	Greece	12	120999383	<i>HNF1A</i>	c.1617dup	p.Lys540Glnfs*9	Frameshift	Yes	-	P	-
521000601	Greece	12	120999383	<i>HNF1A</i>	c.1617dup	p.Lys540Glnfs*9	Frameshift	Yes	-	P	-
640123401	Algeria	20	44413799A	<i>HNF4A</i>	c.425A>G	p.Gln142Arg	Missense	Yes	-	P	-
716000101	Cyprus	20	44419742	<i>HNF4A</i>	c.692G>A	p.Arg231Gln	Missense	No	-	P	35,47
850000601	Tunisia	20	44419813	<i>HNF4A</i>	c.763C>T	p.Gln255*	Nonsense	No	-	P	47
342000401	Turkey	11	17388013	<i>KCNJ11</i>	c.79C>T	p.Arg27Cys	Missense	No	0.00000814	LP	RCV000193401.1 <sup>§</sup>
638000301	Algeria	13	27920326	<i>PDX1</i>	c.188dup	p.Asp64Glyfs*161	Frameshift	Yes	-	P	-
223001501	Malta	4	6301111	<i>WFS1</i>	c.1316T>G	p.Phe439Cys	Missense	No	0.0000795	LP	<u>RCV000199551<sup>§</sup></u>

Sequence variants are indicated as nucleotide changes with respect to GenBank cDNA sequences, and protein effects according to Human Genome Variation Society (HGVS) guidelines [39]. The transcript IDs are listed in Table S3.

\*Chr., chromosome; †Fourteen novel variants were not present in gnomAD (v2.1.1), neither reported in the HGMD-Pro (2019.3) or ClinVar databases; all other variants have previously been reported in genetically characterized cases with MD. ¶Pathogenic (P) and likely pathogenic (LP) variants were defined according to ACMG criteria (for details see online supplementary Tables 4 and 5). §ClinVar accession numbers are given for two mutations previously and publicly reported in ClinVar.

**Table 3.** Clinical characteristics of the participants in the entire study and according to the presence or absence of P/LP variants

	Total	With P/LP variants	No variant	P†
<b>Number of patients</b>	204	36	168	
<b>Sex (M/F)</b>	90/114	18/18	72/96	0.550
<b>Age at diagnosis (years ± SD)</b>	26.1 ± 9.1	26.2 ± 9.9	26.1 ± 8.9	0.955
<b>Insulin treatment at diagnosis (n, %)</b>	65 (31.9%)	5 (13.9%)	60 (35.7%)	0.019
<b>OHA treatment at diagnosis (n, %)</b>	104 (51.0 %)	24 (66.7%)	80 (47.6%)	0.059
<b>Age at inclusion visit (years ± SD)</b>	34.9 ± 11.4	36.8 ± 15.5	34.6 ± 10.3	0.277
<b>BMI (kg/m<sup>2</sup>)*</b>	25.8 ± 4.7	23.4 ± 3.9	26.3 ± 4.7	0.001
<b>HbA1c (%)*</b>	7.5 ± 2.1	6.8 ± 1.3	7.7 ± 2.2	0.016
<b>Insulin treatment at inclusion visit (n, %)</b>	89 (43.6 %)	8 (22.2%)	81 (48.2%)	0.008
<b>OHA treatment at inclusion visit (n, %)</b>	109 (53.4 %)	22 (61.1%)	87 (51.8%)	0.404
<b>1<sup>st</sup> degree history of diabetes (n, %)</b>	150 (73.5 %)	24 (85.7%)	126 (83.4%)	0.984

Data are numbers and percent (%), or mean ± standard-deviation (SD); \* at inclusion in the study.

†Statistical significance of comparison between the two groups of patients i/ with pathogenic (P) or likely pathogenic (LP) variants and ii/ without any variant of interest.

P-values are given from chi-squared ( $\chi^2$ ) test for factorial variables and from ANOVA for continuous traits. BMI, body mass index; OHA, oral hypoglycemic agent

Pathogenic (P) and likely pathogenic (LP) variants were classified according to ACMG recommendations (for details see online supplementary Tables 4 and 5). **BMI**, body mass index; **F**, female; **HbA1c**, glycated hemoglobin A1c; **M**, male; **OHA**, oral hypoglycemic agent.



