Rare *ZMPSTE24* variants increase risk of hypertriglyceridemia and metabolic syndrome.

Lauriane Le Collen[1,2,3], Camille Desgrouas[4], Céline Lukas Croisier[5], Brunot Creugnet[6], Aurélie Dechaume[1,2], Bénédicte Toussaint[1,2], Emmanuel Vaillant[1,2], Souhila Amanzougarene[1,2], Emmanuel Buse Falay[1,2], Mehdi Derhourhi[1,2], Alexandre Lourdelle[7], Brigitte Delemer[5,8], Nathalie Bonello-Palot[4], Martine Vaxillaire[1,2], Catherine Badens[4,9], Philippe Froguel[1,2,10], Amélie Bonnefond[1,2,10]

[1]*Inserm/CNRS UMR 1283/8199, Pasteur Institute of Lille, EGID, Lille, France;*
[2]*University of Lille, Lille University Hospital, Lille, France;*
[3]*Department of Molecular Medicine, Division of Biochemistry, Molecular Biology, Nutrition, and Metabolism, University Hospital of Nancy, F-54000 Nancy, France.*
[4]*Aix Marseille Univ, INSERM, C2VN, Marseille, France;*
[5]*Department of Endocrinology Diabetology, University Hospital Center of Reims, Reims, France;*
[6]*Nouméa, France;*
[7]*Pediatric diabetology Department, American Memorial Hospital, University hospital, Reims, France;*
[8]*CRESTIC EA 3804, UFR Sciences Exactes et Naturelles, Moulin de La Housse, BP 1039, 51687, Reims, France;*
[9]*APHM, Laboratoire de Génétique Moléculaire, Marseille, France*
[10]*Department of Metabolism, Imperial College London, Hammersmith Hospital, London, UK;*

Correspondance to: Lauriane Le Collen (laurianelecollen@wanadoo.fr; ORCID: 0000-0002-5848-3397) and Amélie Bonnefond (amelie.bonnefond@inserm.fr; ORCID: 0000-0001-9976-3005)
Inserm/CNRS UMR 1283/8199- EGID, Pôle Recherche - 1er étage Aile Ouest, 1 place de Verdun, 59019 Lille Cedex, France

Word count (main manuscript): 2685

Abstract

Objective: The global increase in the prevalence of metabolic syndrome represents a significant public health concern. Rare biallelic pathogenic variants in *ZMPSTE24* have been identified as the cause of mandibuloacral dysplasia type B, *i.e.* a lipodystrophy syndrome associated with metabolic complications. The role of monoallelic pathogenic variants in *ZMPSTE24* concerning metabolic syndrome remains uncertain.

Design: Case report and systematic review of literature.

Methods: We investigated a Wallisian family with FPLD and metabolic syndrome via whole-exome sequencing. We performed functional analyses of an identified rare *ZMPSTE24* variant. To broadly assess the effect of heterozygous pathogenic *ZMPSTE24* variants on FPLD-associated phenotypes, and metabolic syndrome, we used the Human Gene Mutation Database [HGMD] and 200K exome data from UK Biobank.

Results: We investigated a Wallisian family where a 40-year-old female with metabolic syndrome was found to carry a rare heterozygous missense variant in *ZMPSTE24*. Functional assays showed a decreased prelamin to lamin A maturation and accelerated senescence. In silico analysis demonstrated that this variant might disrupt the lamin A binding site. We then analyzed the impact of monoallelic pathogenic *ZMPSTE24* variants on metabolic traits using data from the HGMD and the UK Biobank. In HGMD, *ZMPSTE24* variants carriers presented with dyslipidemia and hepatic steatosis. In the UK Biobank, monoallelic pathogenic variants were associated with an increased risk of hypertriglyceridemia, with a trend toward metabolic syndrome.

Conclusions**:** This study underscores the association of *ZMPSTE24* rare variants with metabolic disorders and emphasizes the need for further research to clarify their clinical implications.

Significance statement: We identified a heterozygous likely pathogenic *ZMPSTE24* variant in a Wallisian family with obesity, metabolic syndrome, and suspected familial partial lipodystrophy (FPLD). Functional studies confirmed its deleterious cellular effects, impacting senescence and nuclear morphology. Using large databases, including UK Biobank and the Human Gene Mutation Database, we demonstrated a significant association between heterozygous pathogenic variants in *ZMPSTE24* and an elevated risk of hypertriglyceridemia, along with a trending association with an increased risk of metabolic syndrome. These results suggested that monoallelic *ZMPSTE24* deficiency may represent an oligogenic, rather than a monogenic, form of FPLD. Given the challenges in diagnosing FPLD, we highlight the importance of identifying it in patients with metabolic syndrome, especially those with hypertriglyceridemia and other potential FPLD-related complications.

Introduction

The metabolic syndrome is a constellation of potent cardiovascular risk factors and currently represents a significant major global health concern, with increasing prevalence rates although varying according to the diagnostic criteria employed, gender, and ethnicity (1). The metabolic prevalence is 43% of South Asian American males (2) but “only” 21% of the Australian population (1).

The definition of metabolic syndrome has evolved over time. The International Diabetes Federation's global consensus definition incorporates biological markers glycated hemoglobin A1c (HbA1c), fasting plasma glucose (FPG), triglyceride (TG), and high-density lipoprotein (HDL)-cholesterol, with clinical features waist circumference and blood pressure (1, 3). Metabolic dysfunction-associated fatty liver disease (MAFLD), chronic proinflammatory and prothrombotic states, and sleep apnea, have also been also recognized as integral components of metabolic syndrome (3). The pathogenesis of metabolic syndrome is complex, and insulin resistance and abnormal fat distribution have been identified as primary driving factors (1). Moreover, a genetic predisposition has been identified as a key factor for its development, with a significant heritability of the traits linked to metabolic syndrome, regardless of the subject's sex and age (4, 5). Several monogenic causes of metabolic syndrome associated with obesity have been identified including monoallelic pathogenic variants in *DYRK1B* (6, 7), *MRAP2* (8), and *CELA2A* (9). It has been also demonstrated that lipodystrophy is highly prevalent among patients with metabolic syndrome (10, 11).

The most prevalent monogenic form of lipodystrophy is familial partial lipodystrophy, Dunnigan type (FPLD2), an autosomal dominant disorder caused by heterozygous pathogenic variants in *LMNA*, which encodes Prelamin A. Prelamin A undergoes transformation into mature lamin A through the action of ZMPSTE24, *i.e.* a unique metalloprotease. ZMPSTE24 is known to remove a farnesyl group by cleaving the C-terminal amino acids of prelamin A. Billalelic pathogenic variants in *ZMPSTE24* cause dysfunctional metalloprotease production, resulting in a total lipoatrophy with accumulation of prelamin A (12, 13, 14) (#OMIM * 606480). The question of whether heterozygous pathogenic *ZMPSTE24* variants contribute to a subtype of FPLD associated with severe metabolic syndrome remains elusive (10, 15).

We explored a Wallis island family with Polynesian origin affected by metabolic disorders with some traits of FPLD. We identified three carriers of a heterozygous *ZMPSTE24* variant that was found likely pathogenic according to *in vitro* assays. We then conducted a large-scale genomic study, focusing on rare, pathogenic or likely pathogenic (P/LP) *ZMPSTE24* variants in the literature and UK Biobank, to unravel their influence on various metabolic traits.

Materials and Methods

Study participants. We collected clinical data from the medical records of a Polynesian family, with a focus on diabetes and its treatment, hypertriglyceridemia, body mass index (BMI), adipose tissue distribution, and cardiovascular history. In accordance with French laws, we obtained consent from both patients and their relatives. Prior to the collection and use of patient photographs for research and publication purposes, written informed consent was obtained in accordance with ethical guidelines and the Declaration of Helsinki.

We also conducted a thorough literature review and collected clinical and genetic details from the Human Gene Mutation Database (HGMD Pro 2022 v4) for individuals carrying a heterozygous, P/LP *ZMPSTE24* variant.

Subsequently, we extracted metabolic syndrome-associated traits, encompassing abdominal obesity, hyperglycemia, elevated triglycerides, and reduced high-density lipoprotein (HDL)-cholesterol levels, from 200K participants of UK Biobank. Additionally, we collected information on overweight or obesity status, cardiovascular disorders, and hepatic steatosis. UK Biobank has approval from the North West Multi-centre Research Ethics Committee (MREC) as a Research Tissue Bank (RTB) approval. This analysis was conducted through research application #67575.

In accordance with guidelines established by the centers for disease control and prevention, obesity was characterized by a BMI equal to or exceeding 30 kg/m², whereas overweight was defined by a BMI ranging from 25 kg/m² to 30 kg/m² for adults.

The reference values for the lipid profile are as follows: TG < 1.70 mmol/L; for Low-Density Lipoprotein (LDL)- cholesterol, thresholds were adjusted according to cardiovascular risk based on the ESC September 2019 guidelines: low risk < 3 mmol/L, moderate risk < 2.6 mmol/L, high risk < 1.80 mmol/L, very high risk < 1.40 mmol/L, and < 1 mmol/L if atherosclerosis was present. We applied metabolic syndrome criteria to define abdominal obesity, hypertriglyceridemia, low HDL, and high blood pressure (3). Metabolic syndrome was characterized by abdominal obesity accompanied by two or more of the following: hypertriglyceridemia, low HDL, hyperglycemia, and high systolic and/or diastolic blood pressure (3). According to the international diabetes federation consensus worldwide definition, abdominal obesity in males corresponded to a waist circumference ≥ 94 cm (except ≥ 90 cm for individuals of South-East Asian, South Asian, Japanese, and ethnic South and Central American origin), and in females, a waist circumference ≥ 80 cm (1, 3, 16). Diabetes was indicated by HbA1c ≥ 48 mmol/L or a random glucose measurement ≥ 11.1 mmol/L (17). The American Diabetes Association (ADA) categorizes pre-diabetes with HbA1c levels ranging from 39 to 46 mmol/mol or FPG levels between 5.6 mmol/L and 6.9 mmol/L (17). High triglycerides were defined by a triglyceride value ≥ 1.70 mmol/L (1, 3). Low HDL levels were defined as < 1.03 mmol/L for men or < 1.29 mmol/L for women (1, 3). High blood pressure was characterized by systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg (3).

Exome sequencing and analysis of siblings. We performed whole-exome sequencing in the index case of the Wallisian family using Twist Bioscience in combination with Illumina sequencing on the NovaSeq6000 System. Reads were aligned to the human genome (GRCh38). The index case's initial analysis involved 69 genes responsible for monogenic diabetes, 128 genes for monogenic obesity, 28 genes for monogenic lipodystrophy and 73 genes for lipid disorders (**Supplementary Table 1**). We then performed Sanger sequencing targeting the variant encoding p.(Leu438Phe) in *ZMPSTE24* (NM_005857.5) across the extended family, including both parents, sister, and son. Primer sequences and PCR conditions are available upon request.

Functional *in vitro* analysis. The functional analyses were conducted as previously described, encompassing cellular culture and transfection, immunofluorescence imaging and analysis, senescence-associated beta-galactosidase assay, and cellular BrdU labeling (10, 15). Further details can be found in the **Supplementary Methods**.

Analyses of variants' pathogenicity. To assess the pathogenicity of the variants throughout the manuscript, we used the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG) (18).

Association between monoallelic P/LP *ZMPSTE24* variants and metabolic traits in UK Biobank. We utilized exome data (200K) in pVCF format to identify monoallelic, P/LP *ZMPSTE24* variants. As detailed in a previous study (19), we employed the MiST method (20) to comprehensively evaluate the impact of monoallelic P/LP *ZMPSTE24* variants on multiple metabolic traits. These traits included obesity, high systolic or diastolic blood pressure, hyperglycemia, hypertriglyceridemia, low HDL-cholesterol and metabolic syndrome. In brief, the monoallelic, P/LP *ZMPSTE24* variants detected in UK Biobank were treated as a singular cluster for analysis. We did not identify biallelic, P/LP *ZMPSTE24* variants in UK Biobank. Via the MiST method, we generated $S(\pi)$ to quantify the mean effect (π) of the variant cluster. We only showed the p-values of the mean effects (π) in the present study. These analyses were adjusted for age, sex, and ancestry, represented by the first five genetic principal components available.

Results

Clinical features in a family harboring a P/LP *ZMPSTE24* variant

We studied a female patient (referred to as the proband, Patient III.1; **Table 1 and Figure 1**) and her four siblings from a nonconsanguineous family originating from Wallis. Three of them presented with obesity and nonautoimmune diabetes (**Table 1**). Three family members, including the proband (Patients III.1, II.13, and II.14; **Table 1**), fulfilled the criteria for metabolic syndrome. The proband, *i.e.* a 40-year-old female (Patient III.1; **Table 1, Supplementary Table 2 and Supplementary Figure 1**), was further evaluated for metabolic traits. Abdominal ultrasound revealed hepatic steatosis. She was initially treated with metformin and a glucagon-like peptide-1 receptor agonist (GLP1-RA), but later required basal insulin due to poor glycemic control (**Table 1** and **Supplementary Table 2**). Dyslipidemia was managed with 40 mg/day of statin. Physical examination revealed facial and cervical fat accumulation without subcutaneous fat lipoatrophy in the abdominal or leg areas (**Table 1, Supplementary Table 2 and Supplentary Figure 1**). The patient presented with a metabolic syndrome characterized by early-onset insulin resistance beginning at the age of 15, without associated obesity at that time. Following a marked increase in HbA1c to 155.8 mmol/mol at age 42, due to discontinuation of her diabetes treatment, further assessments were conducted. Adiponectin and leptin levels were low (**Supplementary Table 2**). Due to her low leptin levels and lack of satiety, recombinant leptin therapy was recommended but not prescribed yet.

In this index case (Patient III.1), we identified a heterozygous variant in *ZMPSTE24* (NM_00587.5: c.1312C>T / p.(Leu438Phe)) through whole-exome sequencing. No other putative variants of interest were identified in genes causing monogenic forms of diabetes, obesity, lipodystrophy or lipid disorders (**Supplementary Table 1)**. Sanger sequencing subsequently confirmed the presence of the *ZMPSTE24* variant in the index case and revealed that both her father (Patient II.13) and one of her sisters (Patient III.6) were also carriers (**Table 1 and Figure 1**). Each carrier displayed insulin resistance, nonautoimmune diabetes, obesity and metabolic syndrome (**Table 1 and Figure 1**). The adipose marker measurements could not be conducted. Furthermore, genetic testing could not be performed for all family members.

This *ZMPSTE24* variant encoding p.(Leu438Phe) has previously been suggested to be disease-causing in a male patient from Wallis who presented with a similar phenotype, including severe metabolic syndrome and ectopic fat accumulation (10). We employed a combination of functional tests to analyze the *in vitro* effect of the variant in the patient's fibroblasts (10, 15) (**Supplementary Figure 2**). Cellular senescence, assessed by measuring beta-galactosidase levels in fibroblasts from index case III.1, was significantly higher than in controls (**Figure 2A**). These results were comparable to those found in a patient from the previous study (10), who carried the same *ZMPSTE24* variant and who also presented with metabolic syndrome (**Figure 2A**). Moreover, fibroblast cells from the proband exhibited reduced replication capacity when compared to controls (**Figure 2B**). Using siRNAs targeting prelamin A, we also found a significant reduction in prelamin A accumulation in the index case (**Figure 2C**), resulting in marked decrease in nuclear abnormalities compared to the patient's cells transfected with siRNA negative control (**Figure 2D**). Interestingly, the deleterious impact of the *ZMPSTE24* variant was also suggested by *in silico* software programs (REVEL score of 0.87) (21). Indeed, this variant was located within the peptidase M48 and CAAX prenyl protease 1 protein domains, involved in a lamin A binding site, in a conserved region (data not shown). Therefore, using the ACMG criteria, the *ZMPSTE24* variant was characterized as likely pathogenic.

Impact of monoallelic P/LP *ZMPSTE24* variants on metabolic traits in different studies

We analyzed the impact of heterozygous, P/LP *ZMPSTE24* variants on the development of impaired metabolic traits in the literature and UK Biobank. In HGMD (HGMD Pro 2022 v4), we identified 37 P/LP *ZMPSTE24* variants, including four variants only in a heterozygous state (**Supplementary Table 3**). Heterozygous carriers of c.1085dup / p.Leu362PhefsTer19, c.1204-1G>A, c.1204-5_1210del or c.1312C>T / p.(Leu438Phe) (all defined as P/LP variant) had dyslipidemia (10, 22, 23). Additionally, the heterozygous variant encoding p.(Leu438Phe) was identified in two twins with MAFLD (24).

Among the 200,585 exome samples of UK Biobank, we identified 16 heterozygous P/LP *ZMPSTE24* variants carried by 34 individuals (**Supplementary Table 4**). When applying the MiST method adjusted for age, sex, BMI, and ancestry, we found an association between monoallelic, P/LP *ZMPSTE24* variants and a higher risk of hypertriglyceridemia (36% in non-carriers *vs.* 59% in carriers; OR = 2.3 [1.1; 4.7]; $p = 0.017$) with a trend for metabolic syndrome (32% in non-carriers *vs.* 47% in carriers; OR = 1.8 [0.89; 3.5]; $p = 0.097$) (**Figure 3**). Additionally, we also observed a similar trend for metabolic disorders (including high blood pressure, hyperglycemia, low HDL-cholesterol, obesity) in carriers of monoallelic P/LP *ZMPSTE24* variants compared to non-carriers (**Figure 3**).

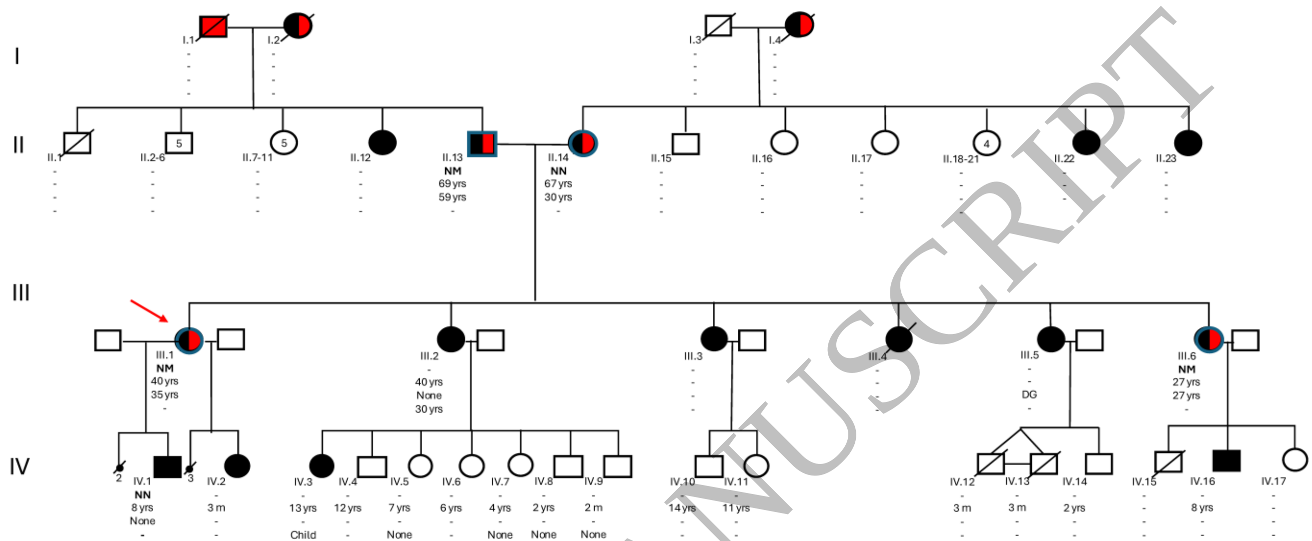
Discussion

We identified in three relatives of a large Polynesian family a heterozygous likely pathogenic variant encoding p.(Leu438Phe) in *ZMPSTE24*. The index case displayed major metabolic syndrome features and abnormal fat distribution, but without overt lipoatrophy. These findings are consistent with a previous report of a patient carrying the same *ZMPSTE24* variant (10). This patient also exhibited metabolic syndrome and ectopic fat accumulation indicative of lipodystrophy, along with commonly associated complications (11, 25, 26). The challenge of assessing adipose tissue distribution is particularly pronounced in populations with a high prevalence of obesity, such as the Polynesian population (70.4% in Wallis and Futuna) (27). The subtlety of fat reduction in FPLD might contribute to its underdiagnosis, as suggested by several studies (28). BMI, which is usually within the normal range, particularly in the most common monogenic form of FPLD, proves to be an unreliable indicator in patients suffering from obesity (11). As mentioned above, certain genes are well-known to be implicated in the development of FPLD when mutated (*e.g., LMNA*, *PPARG*, *AKT2*, and *PLIN1*). However, there are also genes involved in polygenic forms, such as *FPLD1* (Köbberling syndrome) (29). Here, we may question whether heterozygous variants in *ZMPSTE24* could be associated with the emergence of an intermediate, oligogenic form of FPLD (30). Despite severe obesity, one carrier of the p.(Leu438Phe) variant showed low leptin levels, while another exhibited leptin levels in the low-normal range (31, 32); together with the observed magnitude of the odds ratio (OR) for metabolic syndrome in the UK Biobank, these findings would support a potential causal role for heterozygous P/LP *ZMPSTE24* variants in oligogenic FPLD.

Indeed, leptin deficiency has been a hallmark of certain forms of lipodystrophy, where recombinant leptin therapy was shown to alleviate complications (33). The atypical fat distribution, combined with metabolic syndrome and low leptin levels, strengthens the case for considering these patients as having an oligogenic form of FPLD (10, 30). Supporting this hypothesis, P/LP variants in *LMNA* and disruptions in prelamin-A processing are known to alter nuclear envelope proteins, leading to lipodystrophic syndromes (34). In the two monoallelic carriers of the *ZMPSTE24* variant, abnormalities in nuclear architecture were observed in skin fibroblasts (13, 14, 35, 36). The *ZMPSTE24* variant encoding p.(Leu438Phe) has been shown in two studies to reduce enzymatic activity within the zinc metalloprotease domain (22, 37). This reduction likely impairs proper prelamin A cleavage, resulting in the accumulation of farnesylated prelamin A. A previous study examining prelamin A processing through an ELISA-based method revealed a significant reduction (>50%) in mature lamin A production in a patient carrying the p.(Leu438Phe) variant (22). Additionally, a subsequent study using yeast models expressing this variant identified a partial residual metalloprotease activity of 6% (37). This may result from the larger Phe residue altering prelamin A's orientation and hindering its proper cleavage. Our study is limited by the absence of functional analyses on the adipocytes of the carriers. Indeed, proteomic studies on Zmpste24-knockout mice adipose tissue have demonstrated enhanced lipolysis, fatty acid biogenesis, and β-oxidation (38, 39). Furthermore, a slight reduction in adipose tissue storage has been observed in adipocyte-specific Zmpste24-knockout mice (38, 39). Therefore, functional studies on patient's adipocytes would provide valuable insights into the influence of this variant on adipocyte differentiation and lipogenesis.

Our investigation of the UK Biobank identified an association between heterozygous P/LP variants in *ZMPSTE24* and hypertriglyceridemia, with a modest impact on the risk of metabolic syndrome. However, the retrospective nature of the UK Biobank data, lacking critical information such as leptin levels and adipose distribution, limited our ability to fully elucidate the role of FPLD in the onset of hypertriglyceridemia and metabolic syndrome across a broader population. In addition, in the UK Biobank, we have access to a range of objective clinical data, including BMI, waist circumference, and extensive biological data (*e.g.*, HbA1c, blood glucose levels, triglycerides, *etc*.). However, the presence of cardiac and hepatic complications and the details of their treatment are recorded at the clinician's discretion, which may not provide consistently accurate information. Therefore, larger studies incorporating more detailed clinical data and comprehensive biological measurements, including leptin levels, are needed to determine whether monoallelic *ZMPSTE24* variants may be implicated in an oligogenic form of partial lipodystrophy at a population level.

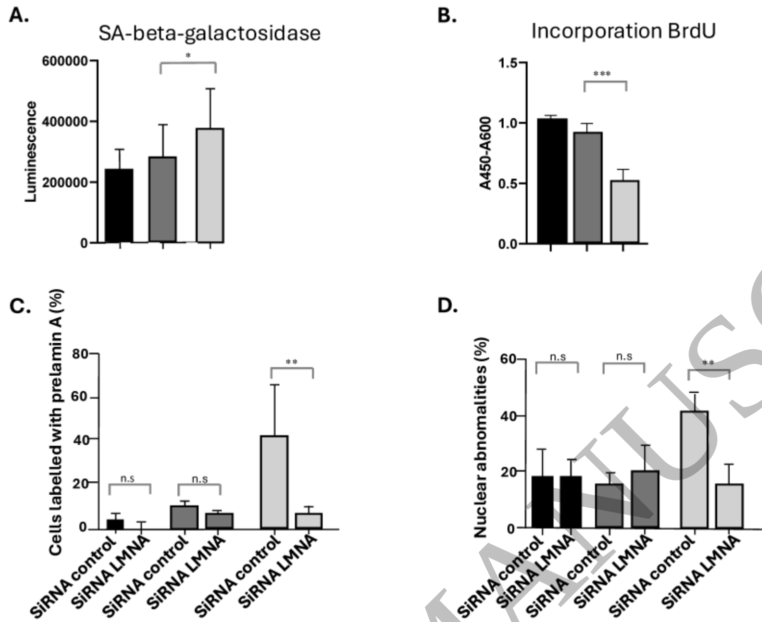
Figure 1. Pedigree of a family with metabolic disorders and segregation of a heterozygous, P/LP *ZMPSTE24* variant



The family pedigree illustrates the status of metabolic syndrome-related traits. Under each family member, their genetic status is indicated, followed by their current age. Below, we have listed the age of diabetes onset and, if known or present, the age of obesity onset. Female participants are represented by circles, and male participants by squares. Miscarriages are represented by a filled-in black circle with a diagonal line through it. A diagonal line through the symbol indicates deceased family members. Black symbols denote patients with obesity (BMI ≥ 30 kg/m² in adults, and for children, BMI ≥ 95th percentile as per Centers for Disease Control and Prevention standards). Individuals with diabetes are indicated in red, while those with metabolic syndrome are surrounded by blue. A red arrow points to the index case (Patient III.1).

GD, Gestational diabetes; ***NN***, non-carriers; ***NM***, heterozygous, P/LP variant in *ZMPSTE24* (NM_00587.5: c.1312C>T / p.(Leu438Phe)); ***None***, no reports of obesity or diabetes or pre diabetes; ***m***, months; **-**, unavailable data; ***yrs***, years.

Figure 2. *In vitro* analyses of the variant encoding p.(Leu438Phe) in *ZMPSTE24*

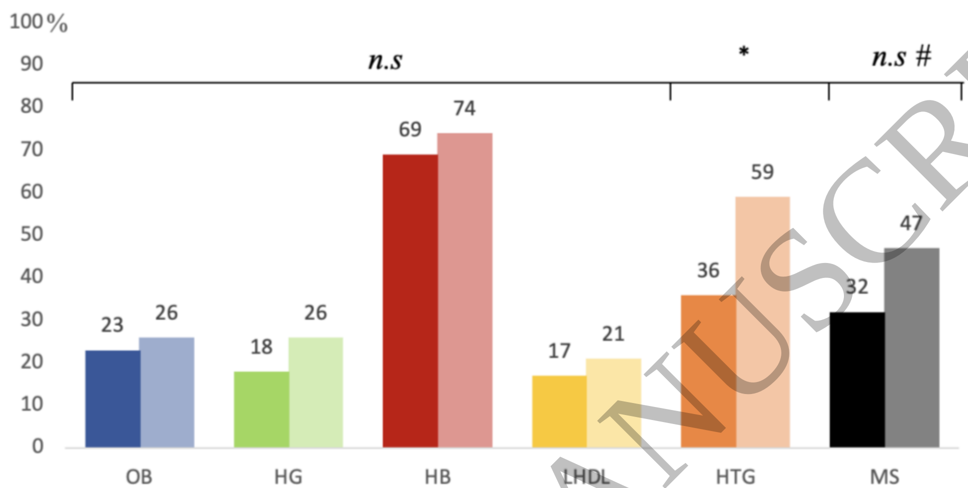


In Figure **2.A-D**, we have depicted the child control in black, the adult control in dark gray, and the index case (III.1) in light gray.

A.The graph presents luminescence signal values proportionate to senescence measurements on the Glomax® platform. The control descriptions are provided in the Supplementary Methods section. Patient III.1 served as the index case for the present family. **B.** The graph depicts absorbance readings corresponding to BrdU incorporation during DNA synthesis in proliferating cells, measured using Glomax®. Non parametric Mann-Whitney tests were executed to compare senescence (**A**) and replication capacity (**B**) between patient' cells and controls. **C.** The graph illustrates the percentage of cells labeled with prelamin A post transfection with siRNAs targeting prelamin A (siRNA *LMNA*) or a negative control (siRNA control). These measurements were captured from nuclei images using an Apotome.2 system (20x magnification). **D.** The graph exhibits the percentage of nuclear abnormalities subsequent to siRNA transfection targeting prelamin A (siRNA *LMNA*) or a negative control (siRNA control).

Statistical analysis of the impact of siRNA treatments and cell lines on nuclear anomalies **(D)** and prelamin A labeling **(C)** was conducted using two-way ANOVA tests, followed by multiple comparisons. The four graphs depict means ± standard deviation (SD) derived from at least three independent experiments, ***n.s***, Not significant, *****, $p < 0.05$; ******, $p < 0.01$; *******, $p < 0.0001$.

Figure 3. Metabolic-related phenotypes in the carriers of heterozygous P/LP *ZMPSTE24* variants versus non-carriers from UK Biobank



Carriers of a heterozygous P/LP *ZMPSTE24* variant are shown in light colors, while non-carriers are depicted in dark colors. The x-axis depicts the presence of obesity, hyperglycemia, high blood pressure, low HDL, hypertriglyceridemia, and metabolic syndrome among non-carriers and carriers of rare, heterozygous pathogenic *ZMPSTE24* variants, with the y-axis indicating their percentages. Colors are used to represent each condition: blue for obesity, green for hyperglycemia, red for high blood pressure, yellow for low HDL, orange for hypertriglyceridemia, and black for metabolic syndrome. Among the 200,550 non-carriers, 45,504 were obese, whereas among the 34 carriers, obesity was present in nine individuals. The prevalence of hyperglycemia was significantly higher among non-carriers (36,536 cases) compared to carriers (9 cases). High blood pressure was observed in 137,599 non-carriers versus 25 carriers. Low HDL levels were found in 35,000 non-carriers compared to seven carriers. Hypertriglyceridemia was identified in 72,408 non-carriers versus 20 carriers overall. A total of 64,260 non-carriers exhibited the presence of metabolic syndrome, compared to 16 carriers.

HB, High blood pressure; ***HG***, hyperglycemia; ***HTG***, hypertriglyceridemia; ***LHDL***, Low HDL-cholesterol; ***MS***, Metabolic syndrome; ***OB***, Obesity.

Statistical analysis (MiST method): ***n.s,*** *Not significant****; #,*** *p = 0.097****; *, p*** *< 0.05*

Table 1. Clinical and biological characteristics of individuals II.13, II.14, III.1, III.6 and IV.1 from the non-consanguineous, Wallisian family

Patients	**II.13**	**II.14**	**III.1**	**III.6**	**IV.1**
Sex	Male	Female	Female	Female	Male
Heterozygous variant in *ZMPSTE24* (NM_00587.5)	c.1312C>T; p.(Leu438Phe)	NN	c.1312C>T; p.(Leu438Phe)	c.1312C>T; p.(Leu438Phe)	NN
Diabetes Management	Metformin, OHA, GLP1-RA	OHA, GLP1-RA, Insulin	Metformin, GLP1-RA, insulin	Metformin, GLP1-RA, Insulin	None
Age at diabetes diagnosis (years)	59	30	35	27	NA
Current BMI	33.8 kg/m^2 at 68 years	32.7 kg/m^2 at 65 years	31 kg/m^2 at 42 years	48.6 kg/m^2 at 30 years	30 kg/m^2 at 9 years
Current Hba1c (mmol/L)	58.5 at 68 years	97.8 at 65 years	155.8 at 42 years	111 at 30 years	37 at 9 years
Current waist circumference (cm)	112 at 68 years	111 at 65 years	115 at 40 years	125 at 30 years	NA
Insulin resistance: fasting insulin level (Normal: 3-25 µU/mL) **and clinical features**	10.5	7.6	6.3; Acanthosis nigricans	Acanthosis nigricans	None
Metabolic syndrome	Presence	Presence	Presence	Presence	NA
Dyslipidemia	High LDL, low HDL, normal TG	High LDL, high HDL, normal TG	High TG, high LDL, normal HDL	High TG, high LDL, low HDL	NA
Relevant clinical features	None	High blood pressure and Heart disorders	Proteinuria, sleep apnea syndrome, the coronary calcium score indicates a low risk of coronary artery disease, and hepatic steatosis	High blood pressure, no cardiopathy detected (echocardiography and electrocardiography), and hepatic steatosis	None

GLP1-RA, glucagon-like peptide-1 receptor receptor agonist; ***NA***, not applicable; ***NN***, non-carriers; ***OHA***, oral hypoglycemic agent; ***SGLT2I***, sodium-glucose cotransporter 2 inhibitors; ***TG***, triglycerides; ***HDL***: high-density lipoprotein-cholesterol; ***LDL***, low-density lipoprotein-cholesterol; ***HbA1c***, hemoglobin A1c; ***NA***: not applicable.

References

1. Alberti KGMM, Zimmet P, & Shaw J. Metabolic syndrome—a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabetic Medicine* 2006 **23** 469–480. (doi:10.1111/j.1464-5491.2006.01858.x)
2. Palaniappan LP, Wong EC, Shin JJ, Fortmann SP, & Lauderdale DS. Asian Americans have greater prevalence of metabolic syndrome despite lower body mass index. *International Journal of Obesity (2005)* 2011 **35** 393–400. (doi:10.1038/ijo.2010.152)
3. Kassi E, Pervanidou P, Kaltsas G, & Chrousos G. Metabolic syndrome: definitions and controversies. *BMC Medicine* 2011 **9** 48. (doi:10.1186/1741-7015-9-48)
4. Dongen J van, Willemsen G, Chen WM, Geus EJC de, & Boomsma DI. Heritability of metabolic syndrome traits in a large population-based sample. *Journal of Lipid Research* 2013 **54** 2914–2923. (doi:10.1194/jlr.P041673)
5. Rana S, Ali S, Wani HA, Mushtaq QD, Sharma S, & Rehman MU. Metabolic syndrome and underlying genetic determinants-A systematic review. *Journal of Diabetes and Metabolic Disorders* 2022 **21** 1095–1104. (doi:10.1007/s40200-022-01009-z)
6. Keramati AR, Fathzadeh M, Go GW, Singh R, Choi M, Faramarzi S, Mane S, Kasaei M, Sarajzadeh-Fard K, Hwa J, Kidd KK, Babaee Bigi MA, Malekzadeh R, Hosseinian A, Babaei M, Lifton RP, & Mani A. A form of the metabolic syndrome associated with mutations in DYRK1B. *The New England Journal of Medicine* 2014 **370** 1909–1919. (doi:10.1056/NEJMoa1301824)
7. Folon L, Baron M, Scherrer V, Toussaint B, Vaillant E, Loiselle H, Dechaume A, De Pooter F, Boutry R, Boissel M, Diallo A, Ning L, Balkau B, Charpentier G, Franc S, Marre M, Derhourhi M, Froguel P, & Bonnefond A. Pathogenic, Total Loss-of-Function DYRK1B Variants Cause Monogenic Obesity Associated With Type 2 Diabetes. *Diabetes Care* 2024 **47** 444–451. (doi:10.2337/dc23-1851)
8. Baron M, Maillet J, Huyvaert M, Dechaume A, Boutry R, Loiselle H, Durand E, Toussaint B, Vaillant E, Philippe J, Thomas J, Ghulam A, Franc S, Charpentier G, Borys JM, Lévy-Marchal C, Tauber M, Scharfmann R, Weill J, Aubert C, Kerr-Conte J, Pattou F, Roussel R, Balkau B, Marre M, Boissel M, Derhourhi M, Gaget S, Canouil M, … Bonnefond A. Loss-of-function mutations in MRAP2 are pathogenic in hyperphagic obesity with hyperglycemia and hypertension. *Nature Medicine* 2019 **25** 1733–1738. (doi:10.1038/s41591-019-0622-0)
9. Esteghamat F, Broughton JS, Smith E, Cardone R, Tyagi T, Guerra M, Szabó A, Ugwu N, Mani MV, Azari B, Kayingo G, Chung S, Fathzadeh M, Weiss E, Bender J, Mane S, Lifton RP, Adeniran A, Nathanson MH, Gorelick FS, Hwa J, Sahin-Tóth M, Belfort-DeAguiar R, Kibbey RG, & Mani A. CELA2A mutations predispose to early-onset atherosclerosis and metabolic syndrome and affect plasma insulin and platelet activation. *Nature Genetics* 2019 **51** 1233–1243. (doi:10.1038/s41588-019-0470-3)
10. Galant D, Gaborit B, Desgrouas C, Abdesselam I, Bernard M, Levy N, Merono F, Coirault C, Roll P, Lagarde A, Bonello-Palot N, Bourgeois P, Dutour A, & Badens C. A Heterozygous ZMPSTE24 Mutation Associated with Severe Metabolic Syndrome, Ectopic Fat Accumulation, and Dilated Cardiomyopathy. *Cells* 2016 **5** 21. (doi:10.3390/cells5020021)
11. Mosbah H, Donadille B, Vatier C, Janmaat S, Atlan M, Badens C, Barat P, Béliard S, Beltrand J, Ben Yaou R, Bismuth E, Boccara F, Cariou B, Chaouat M, Charriot G, Christin-Maitre S, De Kerdanet M, Delemer B, Disse E, Dubois N, Eymard B, Fève B, Lascols O, Mathurin P, Nobécourt E, Poujol-Robert A, Prevost G, Richard P, Sellam J, … Vigouroux C. Dunnigan lipodystrophy syndrome: French National Diagnosis and Care Protocol (PNDS; Protocole National de Diagnostic et de Soins). *Orphanet Journal of Rare Diseases* 2022 **17** 170.

(doi:10.1186/s13023-022-02308-7)
12. Hitzert MM, Crabben SN van der, Baldewsingh G, Amstel HKP van, Wijngaard A van den, Ravenswaaij-Arts CMA van, & Zijlmans CWR. Mandibuloacral dysplasia type B (MADB): a cohort of eight patients from Suriname with a homozygous founder mutation in ZMPSTE24 (FACE1), clinical diagnostic criteria and management guidelines. *Orphanet Journal of Rare Diseases* 2019 **14** 294. (doi:10.1186/s13023-019-1269-0)
13. Cenni V, D'Apice MR, Garagnani P, Columbaro M, Novelli G, Franceschi C, & Lattanzi G. Mandibuloacral dysplasia: A premature ageing disease with aspects of physiological ageing. *Ageing Research Reviews* 2018 **42** 1–13. (doi:10.1016/j.arr.2017.12.001)
14. Agarwal AK, Fryns JP, Auchus RJ, & Garg A. Zinc metalloproteinase, ZMPSTE24, is mutated in mandibuloacral dysplasia. *Human Molecular Genetics* 2003 **12** 1995–2001. (doi:10.1093/hmg/ddg213)
15. Dutour A, Roll P, Gaborit B, Courrier S, Alessi MC, Tregouet DA, Angelis F, Robaglia-Schlupp A, Lesavre N, Cau P, Lévy N, Badens C, & Morange PE. High prevalence of laminopathies among patients with metabolic syndrome. *Human Molecular Genetics* 2011 **20** 3779–3786. (doi:10.1093/hmg/ddr294)
16. Harvard T.H CHAN, Boston, & Ma 02115. Abdominal Obesity Measurement Guidelines for Different Ethnic Groups. *Obesity Prevention Source*2012.
17. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2019. *Diabetes Care* 2019 **42** S13–S28. (doi:10.2337/dc19-S002)
18. 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. *Genetics in Medicine: Official Journal of the American College of Medical Genetics* 2015 **17** 405–424. (doi:10.1038/gim.2015.30)
19. Folon L, Baron M, Toussaint B, Vaillant E, Boissel M, Scherrer V, Loiselle H, Leloire A, Badreddine A, Balkau B, Charpentier G, Franc S, Marre M, Aboulouard S, Salzet M, Canouil M, Derhourhi M, Froguel P, & Bonnefond A. Contribution of heterozygous PCSK1 variants to obesity and implications for precision medicine: a case-control study. *The Lancet. Diabetes & Endocrinology* 2023 **11** 182–190. (doi:10.1016/S2213-8587(22)00392-8)
20. Sun J, Zheng Y, & Hsu L. A unified mixed-effects model for rare-variant association in sequencing studies. *Genetic Epidemiology* 2013 **37** 334–344. (doi:10.1002/gepi.21717)
21. Ioannidis NM, Rothstein JH, Pejaver V, Middha S, McDonnell SK, Baheti S, Musolf A, Li Q, Holzinger E, Karyadi D, Cannon-Albright LA, Teerlink CC, Stanford JL, Isaacs WB, Xu J, Cooney KA, Lange EM, Schleutker J, Carpten JD, Powell IJ, Cussenot O, Cancel-Tassin G, Giles GG, MacInnis RJ, Maier C, Hsieh CL, Wiklund F, Catalona WJ, Foulkes WD, … Sieh W. REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants. *American Journal of Human Genetics* 2016 **99** 877–885. (doi:10.1016/j.ajhg.2016.08.016)
22. Dutour A, Roll P, Gaborit B, Courrier S, Alessi MC, Tregouet DA, Angelis F, Robaglia-Schlupp A, Lesavre N, Cau P, Lévy N, Badens C, & Morange PE. High prevalence of laminopathies among patients with metabolic syndrome. *Human Molecular Genetics* 2011 **20** 3779–3786. (doi:10.1093/hmg/ddr294)
23. Dron JS, Wang J, McIntyre AD, Iacocca MA, Robinson JF, Ban MR, Cao H, & Hegele RA. Six years' experience with LipidSeq: clinical and research learnings from a hybrid, targeted sequencing panel for dyslipidemias. *BMC medical genomics* 2020 **13** 23. (doi:10.1186/s12920-020-0669-2)

24. Brady GF, Kwan R, Ulintz PJ, Nguyen P, Bassirian S, Basrur V, Nesvizhskii AI, Loomba R, & Omary MB. Nuclear lamina genetic variants, including a truncated LAP2, in twins and siblings with nonalcoholic fatty liver disease. *Hepatology (Baltimore, Md.)* 2018 **67** 1710–1725. (doi:10.1002/hep.29522)
25. Patni N & Garg A. Lipodystrophy for the Diabetologist-What to Look For. *Current Diabetes Reports* 2022 **22** 461–470. (doi:10.1007/s11892-022-01485-w)
26. Sollier C, Vatier C, Capel E, Lascols O, Auclair M, Janmaat S, Fève B, Jéru I, & Vigouroux C. Lipodystrophic syndromes: From diagnosis to treatment. *Annales D'endocrinologie* 2020 **81** 51–60. (doi:10.1016/j.ando.2019.10.003)
27. Wallis & Futuna Enquête sur la prévalence des facteurs de risque de maladies non transmissibles STEPS 2019.
28. Rother KI & Brown RJ. Novel forms of lipodystrophy: why should we care? *Diabetes Care* 2013 **36** 2142–2145. (doi:10.2337/dc13-0561)
29. Lotta LA, Gulati P, Day FR, Payne F, Ongen H, Bunt M van de, Gaulton KJ, Eicher JD, Sharp SJ, Luan J, De Lucia Rolfe E, Stewart ID, Wheeler E, Willems SM, Adams C, Yaghootkar H, EPIC-InterAct Consortium, Cambridge FPLD1 Consortium, Forouhi NG, Khaw KT, Johnson AD, Semple RK, Frayling T, Perry JRB, Dermitzakis E, McCarthy MI, Barroso I, Wareham NJ, Savage DB, … Scott RA. Integrative genomic analysis implicates limited peripheral adipose storage capacity in the pathogenesis of human insulin resistance. *Nature Genetics* 2017 **49** 17–26. (doi:10.1038/ng.3714)
30. Le Collen L, Froguel P, & Bonnefond A. Towards the recognition of oligogenic forms of type 2 diabetes. *Trends in endocrinology and metabolism: TEM* 2024 S1043-2760(24)00166-8. (doi:10.1016/j.tem.2024.06.006)
31. Mann JP & Savage DB. What lipodystrophies teach us about the metabolic syndrome. *The Journal of Clinical Investigation* 2019 **129** 4009–4021. (doi:10.1172/JCI129190)
32. Matsubara M, Maruoka S, & Katayose S. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. *European Journal of Endocrinology* 2002 **147** 173–180. (doi:10.1530/eje.0.1470173)
33. Vigouroux C, Mosbah H, & Vatier C. Leptin replacement therapy in the management of lipodystrophy syndromes. *Annales D'endocrinologie* 2024 **85** 201–204. (doi:10.1016/j.ando.2024.05.022)
34. Guénantin AC, Briand N, Bidault G, Afonso P, Béréziat V, Vatier C, Lascols O, Caron-Debarle M, Capeau J, & Vigouroux C. Nuclear envelope-related lipodystrophies. *Seminars in Cell & Developmental Biology* 2014 **29** 148–157. (doi:10.1016/j.semcdb.2013.12.015)
35. Hitzert MM, Crabben SN van der, Baldewsingh G, Amstel HKP van, Wijngaard A van den, Ravenswaaij-Arts CMA van, & Zijlmans CWR. Mandibuloacral dysplasia type B (MADB): a cohort of eight patients from Suriname with a homozygous founder mutation in ZMPSTE24 (FACE1), clinical diagnostic criteria and management guidelines. *Orphanet Journal of Rare Diseases* 2019 **14** 294. (doi:10.1186/s13023-019-1269-0)
36. Simha V, Agarwal AK, Oral EA, Fryns JP, & Garg A. Genetic and phenotypic heterogeneity in patients with mandibuloacral dysplasia-associated lipodystrophy. *The Journal of Clinical Endocrinology and Metabolism* 2003 **88** 2821–2824. (doi:10.1210/jc.2002-021575)
37. Barrowman J, Wiley PA, Hudon-Miller SE, Hrycyna CA, & Michaelis S. Human ZMPSTE24 disease mutations: residual proteolytic activity correlates with disease severity. *Human Molecular Genetics* 2012 **21** 4084–4093. (doi:10.1093/hmg/dds233)
38. Peinado JR, Quirós PM, Pulido MR, Mariño G, Martínez-Chantar ML, Vázquez-Martínez R, Freije JMP, López-Otín C, & Malagón MM. Proteomic profiling of adipose tissue from

Zmpste24-/- mice, a model of lipodystrophy and premature aging, reveals major changes in mitochondrial function and vimentin processing. *Molecular & cellular proteomics: MCP* 2011 **10** M111.008094. (doi:10.1074/mcp.M111.008094)

39. Heizer PJ, Yang Y, Tu Y, Kim PH, Chen NY, Hu Y, Yoshinaga Y, Jong PJ de, Vergnes L, Morales JE, Li RL, Jackson N, Reue K, Young SG, & Fong LG. Deficiency in ZMPSTE24 and resulting farnesyl-prelamin A accumulation only modestly affect mouse adipose tissue stores. *Journal of Lipid Research* 2020 **61** 413–421. (doi:10.1194/jlr.RA119000593)

Statements & Declarations

Funding and Assistance

This study was funded by the French National Research Agency (Agence Nationale de la Recherche [ANR]-10-LABX-46 [European Genomics Institute for Diabetes] and ANR-10-EQPX-07-01 [Lille Integrated Genomics Advanced Network for personalized medicine] to A.B. and P.F.), European Research Council (OpiO 101043671 to A.B.), the European Union's Horizon Europe Research and Innovation Programme (OBELISK grant agreement 101080465 to A.B. and P.F.), and the National Center for Precision Diabetic Medicine (PreciDIAB to A.B. and P.F), which is jointly supported by the French National Agency for Research (ANR-18-IBHU-0001), European Regional Development Fund, Hauts-de-France Regional Council, and the European Metropolis of Lille. We thank the France Génomique consortium (ANR-10-INBS-009). This research has been conducted using the UK Biobank Application #67575.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Acknowledgments

The authors would like to thank the index case and her family for their cooperation..The authors extend their gratitude to the Genetics and Endocrinology team in Angers, with special thanks to Dr. Paul Sibilia, for their valuable assistance in the recent collection of supplementary clinical data.

Author Contributions

A.Bon. and L.L.C. wrote the initial draft of the manuscript. L.L.C., B.D., P.F., and A.Bon. designed the study. M.V., A.D., S.A., M.D., A.Bon, and P.F. contributed to the genetic analyses. L.L.C., C.L.C., A.L. handled clinical data. C.D., N.B.P., and C.B. conducted functional studies. All authors reviewed and edited the manuscript, and they approved the final version. A.Bon., P.F., and L.L.C. are the guarantors of this work and, as such, had full access to all the study data, taking responsibility for its integrity and accuracy.

Data Availability

Some or all datasets analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Ethics approval

Authors attest that the research included in this report was conducted in a manner consistent with the principles of research ethics, such as those described in the Declaration of Helsinki and/or the Belmont Report. Prior to the collection and use of patient photographs for research and publication purposes, written informed consent was obtained in accordance with ethical guidelines and the Declaration of Helsinki. Authors attest that the study was exempt from institutional review/approval.

Consent to participate

Informed consent was obtained from all individual participants included in the study. Written informed consent was obtained from participants (or their parent or legal guardian in the case of children under 16)