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RESEARCH LETTER

Mutations in *G6PC2* do not contribute to monogenic forms of early infancy diabetes and beta cell dysfunction

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Abbreviations

EID	Early infancy diabetes #
G6PC	Glucose-6-phosphatase catalytic subunit
G6PC2	Glucose-6-phosphatase catalytic subunit 2
HI	Hypoglycaemia of infancy
MODY	Maturity-onset diabetes of the young
SNP	Single nucleotide polymorphism
UTR	Untranslated region

To the Editor: Monogenic forms of beta cell dysfunction in childhood and young adulthood are rare disorders with genetically heterogeneous aetiologies [1, 2]. They include neonatal diabetes mellitus (~1:300,000 newborn infants), maturity onset diabetes of the young (MODY; ~1–2% of non-autoimmune diabetes) and congenital hypoglycaemia of infancy (HI; ~1:50,000 newborn infants) [1, 2]. At present these disorders are known to involve more than ten genes that are highly expressed in pancreatic beta cells [1–3]. Causal mutations resulting in severely impaired beta cell function and inadequate insulin secretion have been implicated in both early onset diabetes and HI, as has been described for *HNF4A* (hepatocyte nuclear factor 4, alpha), *GCK* (glucokinase) or the ATP-sensitive K^+ channel genes (*KCNJ11*, *ABCC8*) [2, 3].

Glucose-6-phosphatase catalytic subunit 2 (G6PC2), also known as islet specific glucose-6-phosphatase related protein (IGRP), is a glycoprotein embedded in the endoplasmic reticulum membrane [4]. G6PC2 belongs to the glucose-6-phosphatase family that includes the glucose-6-phosphatase catalytic subunit (G6PC), which catalyses the hydrolysis of glucose 6-phosphate to release endogenous glucose from the gluconeogenic tissues, mainly the liver [5]. The expression of *G6PC2* is highly specific to pancreatic islets and beta cells

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[6]. Thus, G6PC2 has been proposed to modulate the beta cell glycolytic pathway and glucose-stimulated insulin secretion by antagonising the activity of glucokinase, the major beta cell glucose sensor [6]. Despite 50% amino acid sequence identity with G6PC, G6PC2 shows little or no enzymatic activity on glucose 6-phosphate hydrolysis [4]. However, we have recently shown that a frequent single nucleotide polymorphism (SNP) within the third intron of *G6PC2* is strongly associated with fasting plasma glucose levels and insulin release indices in several independent European populations [6]. Another study independently confirmed our findings and reported several SNPs that had strong effects on fasting plasma glucose levels in the same locus [7]. In a Chinese population, Hu and colleagues identified a novel *G6PC2* SNP rs16856187 that confers risk for type 2 diabetes [8]. These genetic findings in humans are consistent with *G6pc2* invalidation that results in a decrease of approximately 13% in fasting glucose concentrations in mice [9].

Inactivated heterozygous mutations of *GCK* that reduce the glucose input into the glycolytic pathway are responsible for MODY type 2; when homozygous, the mutations are responsible for a few cases of permanent neonatal diabetes mellitus. However, gain-of-function mutations that increase glucose metabolism in the beta cell are associated with a persistent phenotype of HI sensitive to diazoxide [1–3]. In order to evaluate whether gain- or loss-of-function mutations in *G6PC2* may be a cause of monogenic forms of beta cell dysfunction in humans, we screened 88 patients for mutations of *G6PC2*. These patients had genetically unexplained non-autoimmune early infancy diabetes (EID) (either transient [n=12] or permanent [n=32]), or MODY (n=16) or the inverse phenotype of congenital HI (n=28) (Table 1). The patients were recruited by the French Network for the Study of Neonatal Diabetes Mellitus, the CNRS-UMR8090 Unit (Lille, France) and the Department of Metabolic Disorders of Necker Hospital (Paris, France). The study was approved by the local ethics committees, and parents provided written informed consent for the genetic testing of their children. Details of the previous genetic testing in these patients are shown in full in Table 1. *G6PC2* is located on

Table 1 Clinical characteristics and previous gene screening of patients with unexplained permanent or transient EID, MODY or persistent HI

Variable	Permanent EID	Transient EID	MODY	Persistent HI
n	32	12	16	28
Sex ratio (male/female) (*n*)	18/14	5/7	11/5	15/13
Age at diagnosis (*n*)	6 (2–15.75)[a]	2 (1–4.25)[b]	20 (15.5–22.75)[c]	3.75 (0–8)[a]
Neonates (≤1 month of age)	6	10	0	11
Infants (1 month to 18 months of age)	19	2	0	15
Children (18 months to 6 years old)	7	0	1	2
Current treatment (%)				
Insulin/OHA/diet/none/not known	94/3/0/3/0	0/0/17/83/0	38/25/6/12/19	–
Diazoxide	–	–	–	100
Birthweight (g)	3,079±784	2,018±652	NA	3,598±544
Below 3th percentile (%)	15.4	66.7	NA	0
Below 10th percentile (%)	23.1	75	NA	4.2
Below median (%)	73.1	100	NA	33.3
Above 97th percentile (%)	7.7	0	NA	12.5
No mutations found (*n*)				
ABCC8	32	12	16	24
KCNJ11	32	12	–	24
INS	32	12	16	–
GCK[d]	–	–	15	2[e]
HNF1A	–	–	15	–
No abnormalities in chromosome 6q24 (*n*)	NA	12	NA	NA
No autoantibodies associated with type 1 diabetes (*n*)	32	12	16	NA

Data presented as mean±SD

Data presented as median age at diagnosis in [a]months, [b]days or [c]years (interquartile range)

[d]EID patients are now screened for a *GCK* mutation when both parents are known to have impaired fasting glycaemia or diabetes

[e]Persistent HI patients with a family history of diabetes or hypoglycaemia (in first or second degree relatives) were screened for *HNF4A* (n=5) or *GCK* (n=2) mutation, respectively

NA, not applicable

human chromosome 2q24, and encodes a 355 amino acid protein (MIM# 608058). The genomic sequences were analysed in 11 fragments spanning the promoter and 5′-untranslated regions (UTR) (from nucleotide c.−1100), all five exons, the exon–intron boundaries and the 3′-UTR (up to c.*1921) (primer sequences and PCR conditions are given in Table 1 of the Electronic supplementary material [ESM]). Amplicons were sequenced on a 3730xl DNA Analyser (Applied Biosystems, Foster City, CA, USA) using a standard protocol. Electrophoregram reads were assembled and analysed with the Variant Reporter software (Applied Biosystems).

Analysis of the genomic sequence of *G6PC2* revealed 19 rare (minor allele frequency ≤0.05) and nine frequent (minor allele frequency >0.05) variants (Table 2). All variants were present in the heterozygous state. They included four exonic and five intronic variants; the other extragenic variants were located in the promoter region and in the 3′-UTR. All four coding variants were located in exon 5 of *G6PC2* (Table 2). Three of them were non-synonymous and had been described previously, with an assigned rs number from the dbSNP build 129 database (p.Y207S-rs2232323, p.V219L-rs492594 and p.S342C-rs2232328). The synonymous variant

Table 2 *G6PC2* gene variants identified in the patients with unexplained EID, MODY or persistent HI, and control individuals

rs number	SNP	Position on chromosome 2[a]	Part of gene	Minor allele frequency					
				Permanent EID (n=32)	Transient EID (n=12)	MODY (n=16)	Persistent HI (n=28)	Controls[b] (n=147)	HapMap CEU population
rs540524	c.−912A>G	169.465.176	Promoter	0.33	0.25	0.47	0.32	0.28	0.30
Novel	c.−752C>T	169.465.336	Promoter	0	0.04	0	0	0	NA
rs2232314	c.−713C>T	169.465.375	Promoter	0	0	0	0.02	0	0
rs1402837	c.−488C>T	169.465.600	Promoter	0.14	0.29	0.22	0.20	0.21	0.30
rs2232315	c.−410G>A	169.465.678	Promoter	0.02	0	0	0	0.01	0.03
rs573225	c.−301A>G	169.465.787	Promoter	0.33	0.46	0.28	0.30	0.42	0.33
rs34746523	c.−294T>C	169.465.794	Promoter	0	0	0.03	0	0.003	NA
rs2232316	c.−280G>A	169.465.808	Promoter	0.22	0.08	0.09	0.16	0.11	0.10
rs2232318	c.220–42G>A	169.467.159	Intron 1	0	0.04	0.03	0.02	0.04	NA
Novel	c.327+91G>C	169.467.402	Intron 2	0.02	0	0	0	0	NA
rs2232320	c.328–147C>T	169.469.114	Intron 2	0.02	0	0	0.04	0.003	0
Novel	c.328–19G>A	169.469.242	Intron 2	0.03	0	0	0	0	NA
rs560887	c.439–26C>T	169.471.394	Intron 3	0.23	0.33	0.22	0.27	0.37	0.30
rs2232323	c.620A>C. p.Y207S	169.472.387	Exon 5	0.03	0.04	0	0	0.01	0.025
rs492594	c.655G>C. p.V219L	169.472.422	Exon 5	0.55	0.38	0.56	0.48	0.37	0.37
Novel	c.699G>A. p.L233L	169.472.466	Exon 5	0	0	0.03	0	0	NA
rs2232328	c.1025C>G. p.S342C	169.472.792	Exon 5	0.26	0.08	0.09	0.20	0.12	0.09
Novel	c.*24T>C	169.472.859	3′UTR	0.02	0	0	0	0	NA
rs2232329	c.*27G>A	169.472.862	3′UTR	0	0	0	0.02	0.01	NA
Novel	c.*64C>T	169.472.899	3′UTR	0.02	0	0	0	0	NA
Novel	c.*688A>C	169.473.523	3′UTR	0	0	0	0.01	0.02	NA
rs570876	c.*788T>A	169.473.623	3′UTR	0.45	0.63	0.44	0.52	0.62	0.37
Novel	c.*877G>A	169.473.712	3′UTR	0	0	0	0.04	0	NA
rs567243	c.*1148A>G	169.473.983	3′UTR	0.58	0.38	0.56	0.50	0.38	0.36
Novel	c.*1290T>C	169.474.125	3′UTR	0.02	0	0.06	0.02	0.003	NA
Novel	c.*1770A>G	169.474.605	3′UTR	0	0	0.03	0	0	NA
rs10176348	c.*1774C>T	169.474.609	3′UTR	0	0	0	0.02	0	0
Novel	c.*1851T>C	169.474.686	3′UTR	0	0	0	0.02	0	NA

[a] Variant locations are displayed by base numbers counting from the p-arm telomere of chromosome 2 (according to the Base Position feature in the Human (*Homo sapiens*) Genome Browser Gateway Human Mar. 2006 [hg18] assembly (http://genome.ucsc.edu/cgi-bin/hgGateway, accessed 1 July 2008))

[b] Control subjects are non-diabetic white individuals including 31 adults (fasting plasma glucose 4.16±0.22 mmol/l) and 116 children (fasting plasma glucose 4.25±0.29 mmol/l)

CEU, Centre d'Etude du Polymorphisme (Utah residents with northern and western European ancestry); NA, not available

(c.699G>A-p.L233L) had not yet been described: it was found in one MODY patient, but was not present in 294 control chromosomes. We investigated the proband's family and observed no obvious segregation between this novel coding variant and diabetes. Ten novel rare (minor allele frequency <0.05) but non-coding variants were also identified (Table 2). The c.−752C>T variant located in the promoter region was found in one transient EID patient, and was not present in the control group. This sequence variant maps to a non-conserved genomic region across species, according to the UCSC/Penn State Bioinformatics (www.bx.psu.edu/miller_lab/, accessed 1 July 2008). In silico analysis did not predict any transcription factor binding site in the vicinity of c.−752C>T (Genomatix software: www.genomatix.de/, accessed 1 July 2008). Taken together, these data did not support further investigation of the role of c.−752C>T variant. The two new intronic variants (c.327+91G>C and c.328–147C>T) were identified in one and two permanent EID patients respectively, but not in the control group. These intronic variants are located in non-conserved regions across species. Finally, seven novel variants were found in the 3′ UTR (Table 2). No putative microRNA binding sites were predicted in their vicinity according to the TargetScan software (www.targetscan.org/, accessed 1 July 2008).

Our results do not support the notion that *G6PC2* disruption may be a common cause of monogenic forms of beta cell dysfunction in humans, at least in our patient cohorts. These findings differ markedly from large epidemiology studies that showed strong associations between common DNA variations at the *G6PC2* locus and fasting glucose level [6, 7], or even type 2 diabetes in the Chinese population [8]. Such divergent data between the aetiologies of monogenic beta cell disorders and polygenic type 2 diabetes have already been highlighted in previous studies, which did not show that causal mutations in several genes (such as *TCF7L2*, *HHEX* or *SLC30A8*) led to monogenic beta cell dysfunction [10–12], although these gene loci were confirmed to confer risk strongly for type 2 diabetes. Nonetheless, the approach of screening genes known to be involved in polygenic type 2 diabetes for mutations in rare monogenic diabetes has been remarkably fruitful, as exemplified by the pharmacogenetic impact of *KCNJ11* mutations [13, 14].

Following our study based on three limited patient cohorts, albeit representative of rare monogenic forms of beta cell dysfunction, it would obviously be informative to test the association between all the novel variants and fasting glucose level or multifactorial forms of type 2 diabetes at the population level. Finally, further molecular studies are still required to clarify the function of G6PC2 in the pancreatic beta cell, particularly with regard to a role in the glucose phosphorylation pathway.

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