Diabetologia (2007) 50:214–216
DOI 10.1007/s00125-006-0505-z

RESEARCH LETTER

No major contribution of *TCF7L2* sequence variants to maturity onset of diabetes of the young (MODY) or neonatal diabetes mellitus in French white subjects

S. Cauchi · M. Vaxillaire · H. Choquet · E. Durand · A. Duval · M. Polak · P. Froguel

Received: 1 September 2006 / Accepted: 2 October 2006 / Published online: 9 November 2006

To the Editor: Maturity onset diabetes of the young (MODY), and permanent and transient neonatal diabetes mellitus (NDM), are the most prevalent monogenic forms of diabetes. Dominant activating mutations in the *KCNJ11* gene [1, 2], which encodes the inwardly rectifying Kir6.2 subunit of the ATP-sensitive potassium (K_{ATP}) channel expressed at the surface of the pancreatic beta cell, or in the *ABCC8* gene [3], which encodes the SUR1 regulatory subunit of the channel, account for more than 30% of cases of NDM in the French population. So far, genes involved in the aetiology of MODY include glucokinase (*GCK*) [4] (also occasionally responsible for permanent NDM) and at least five beta cell-expressed transcription factors [5]. Common DNA polymorphisms in most of these genes have also been repeatedly associated with beta cell dysfunction and with type 2 diabetes in a polygenic context [6]. Recently, intronic single nucleotide polymorphisms (SNPs) of the transcription factor 7-like 2 (*TCF7L2*) gene have been associated with type 2 diabetes in different ethnic groups, including French white subjects [7, 8]. The physiological consequences of carrying the rs7903146 T allele, at risk for type 2 diabetes, remain unclear but there are some arguments supporting a primary effect on insulin secretion [8–10].

These data prompted us to evaluate the putative contribution of *TCF7L2* in monogenic forms of diabetes. In this study, we sought variants by direct sequencing of all potential exons and intron–exon junctions of *TCF7L2* in unrelated white individuals with still unexplained NDM (n=28) and MODY (n=17), as described in Table 1. Two other groups were also screened for mutations: 205 non-MODY early onset type 2 diabetes (EOD; age at diagnosis <40 years), and 93 normoglycaemic (NG) subjects (Table 1). The *TCF7L2* gene (formerly known as *TCF4*) is subject to extensive alternative splicing [11], and its exon–intron structure was obtained using the Genbank sequences AJ270770 to AJ270778. Seventeen different exons have been described, eight of them being totally (exons 4, 13, 14, 15 and 16) or partially (exons 7, 9 and 17) alternatively expressed (Fig. 1). Alternative splicing events of exons 14 and 15 change the reading frame in exon 17, leading to three different COOH-terminal ends. When exons 14 and

Electronic supplementary material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s00125-006-0505-z and is accessible to authorised users.

S. Cauchi · M. Vaxillaire · H. Choquet · E. Durand · P. Froguel
CNRS, 8090, Institute of Biology, Pasteur Institute,
Lille 59000, France

S. Cauchi · M. Vaxillaire · H. Choquet · E. Durand · P. Froguel
University of Lille2,
Lille 59000, France

A. Duval
Inserm, U762, Jean Dausset-CEPH Fundation,
Paris 75010, France

A. Duval
University of Pierre and Marie Curie-Paris6, U762,
Paris 75010, France

M. Polak
University of René Descartes Paris5,
Paris 75006, France

M. Polak
Inserm, U0363, Necker Hospital,
Paris 75015, France

P. Froguel (✉)
Genomic Medicine, MRC Clinical Science Centre,
Faculty of Medicine, Imperial College London,
Hammersmith Hospital Campus,
Du Cane Road,
London W12 0NN, UK
e-mail: P.Froguel@imperial.ac.uk

Table 1 Clinical characteristics of patients with unexplained NDM, MODY or EOD, and NG subjects

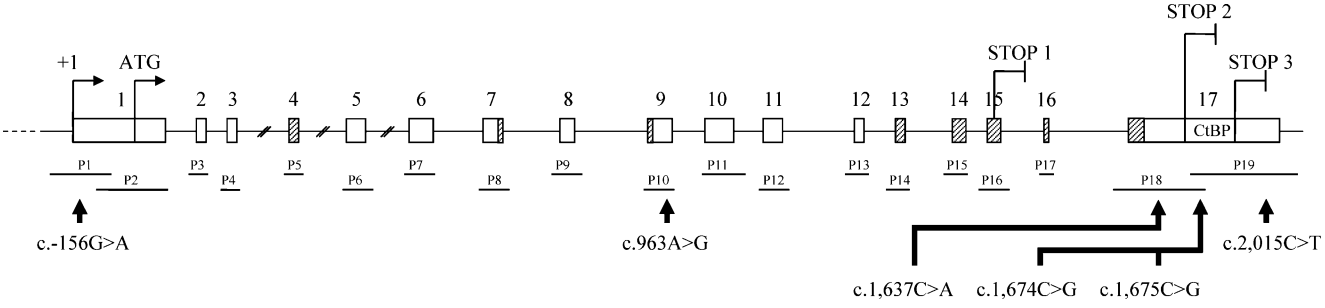
Variable	NDM	MODY	EOD	NG
n	28[a]	17	205	93
Sex ratio (male/female)	13/15	10/7	123/82	35/58
Age at examination (years)	4 [1–21][b]	35±11	52±13	58±15
Age at diagnosis (years)	53 [1–260][c]	18±7	35±8	na
Fasting glycaemia (mmol/l)	18±9	9±4	9±3	5±1
BMI (kg/m^2)	0.06±1.28[d]	24±4	25±3	22±2
Birth weight (g)	2,161 (1,080–3,600; <3–75)[e]	na	na	na

[a] Seventeen patients with permanent and 11 with transient NDM

Data presented as mean±SD except in the following cases: [b] data presented as median age at examination in years (range); [c] data presented as median age at diagnosis in days (range); [d] data presented as mean BMI z-score±SD; [e] data presented as mean birth weight (range of birth weight; range of percentiles adjusted for gestational age)

na Not applicable

15 are transcribed together, it creates a short COOH-terminal end (STOP 1). When only one of these two exons is transcribed, it creates instead a long COOH-terminal end (STOP 3) and when both are lacking, there is a medium COOH-terminal end (STOP 2). Nineteen PCR products were necessary to cover all exons and intron–exon junctions (Electronic supplementary material Table 1). Each fragment was sequenced using an ABI3730*xl* DNA analyser. Sequence variants were confirmed by re-amplifying and re-sequencing a fresh dilution of DNA. No mutations were found in unexplained NDM and MODY subjects. In the other screened subjects, six rare SNPs, all in the heterozygous state, were identified: one in the 5′-untranslated part of the gene (c.−156G>A), four in the coding region (c.963A>G, c.1,637C>A, c.1,674C>G and c.1,675C>G) and one in the 3′-untranslated region (c.2,015C>T) (Fig. 1). No rs numbers have been assigned to these rare SNPs according to the dbSNP build 126 database. No subjects were carriers of more than one SNP. Subjects carrying one of these mutations did not show any specific features, such as a



SNP	c.–156G>A	c.963A>G	c.1,637C>A	c.1,674C>G	c.1,675C>G	c.2,015C>T
Position on chromosome 10	114,700,350	114,900,819	114,915,359	114,915,396	114,915,397	114,915,737
Number of subjects						
EOD (*n* = 205)	3	2	12	2	4	1
NG (*n* = 98)	0	0	7	1	2	0
Possible transcripts						
STOP 1	5'UTR	ACA>ACG Thr→Thr	3'UTR	3'UTR	3'UTR	3'UTR
STOP 2	5'UTR	ACA>ACG Thr→Thr	CCC>CAC Pro→Thr	3'UTR	3'UTR	3'UTR
STOP 3	5'UTR	ACA>ACG Thr→Thr	CAC>CAA His→Gln	CCC>CGC Pro→Arg	CCC>CCG Pro→Pro	3'UTR

Fig. 1 *TCF7L2* gene variants and their potential impact on the different possible transcripts: The *TCF7L2* gene sequence was obtained from the Genbank sequences AJ270770 to AJ270778. Seventeen exons have been described [11], eight of them being totally (exons 4, 13, 14, 15 and 16) or partially (exons 7, 9 and 17) alternatively expressed, as noted by hatched squares. Nineteen PCR amplifications were needed to cover the overall gene. The SNP locations are displayed by base numbers counting from the ATG-translation initiation codon (Human Genome Variation Society recommendation) and from the p-arm telomere of chromosome 10 (http://genome.ucsc.edu/cgi-bin/hgGateway). The codon locations are not displayed due to multiple possible transcripts. For each SNP, the variant nucleotide is *underlined*

more severe diabetic phenotype or differences in glucose-lowering treatment, compared with non-carrier EOD individuals.

Therefore, it is unlikely that *TCF7L2* contributes to unexplained MODY or NDM in the study sample of subjects of French white origin. In addition, no significant linkage with MODY was observed at the *TCF7L2* locus on chromosome 10q25 in European families including a set of French families [12]. By re-analysing the markers nearest to *TCF7L2* in French families only, the LOD-score values were consistently below −4 for every genetic model. These observations suggest that *TCF7L2* mutations are not likely to be responsible for MODY in these families. Our study has also led to the identification of six SNPs, three of them only present in EOD subjects (c.−156G>A, c.963A>G and c.2,015C>T), which may be due to the low frequency of these mutations. Recently, four exonic SNPs have been reported in the *TCF7L2* gene of patients with permanent neonatal diabetes [13]. We assumed that Groves et al. used the NM_030756 mRNA sequence as reference sequence. This reference sequence does not take into account exons 4, 15 and 16, as well as the beginning of exons 9 and 17 reported in sequences AJ270770 to AJ270778 submitted by Duval et al. [11]. Two SNPs were found to be common between the two studies, c.879A>G and c.1429C>A (according to the NM_030756 sequence used by Groves et al.) corresponding to c.963A>G and c.1,637C>A, respectively (according to the AJ270770 to AJ270778 sequences used in our study). The two other reported SNPs were not found within our subjects. Further studies, with more participants, are necessary to investigate the potential impact of these SNPs on *TCF7L2* function and/or the WNT signaling pathway. Interestingly, the two non-synonymous polymorphisms (c.1,637C>A, Pro→Thr or His→Gln and c.1,674C>G, Pro→Arg) may change the protein conformation in the transcripts with long or medium COOH-terminal ends. In this regard, transcripts harbouring long COOH-terminal ends have been demonstrated to mediate *TCF7L2* transcriptional repression, as they contain two specific binding domains for C-terminal binding protein (CtBP), an ubiquitous transcriptional repressor [11]. Additional studies are needed to determine which transcript isoforms are expressed in beta cells, given that some of the sequence variants could differentially affect *TCF7L2* isoforms. However, at this stage, there is no functional argument supporting a role of these variants in beta cell function.

Acknowledgements This work was partly supported by the French Governmental Agence Nationale de la Recherche and the charity Association Française des Diabétiques. We thank M. Deweider and F. Allegaert for the DNA bank management, and S. Gaget for his help on phenotype databases. We are indebted to all subjects who participated in this study.

Duality of interest The authors declare that there was no duality of interest associated with this study.

References

1. Gloyn AL, Pearson ER, Antcliff JF et al (2004) Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med 350:1838–1849
2. Vaxillaire M, Populaire C, Busiah K et al (2004) Kir6.2 mutations are a common cause of permanent neonatal diabetes in a large cohort of French patients. Diabetes 53:2719–2722
3. Babenko AP, Polak M, Cave H et al (2006) Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. N Engl J Med 355:456–466
4. Froguel P, Zouali H, Vionnet N et al (1993) Familial hyperglycemia due to mutations in glucokinase. Definition of a subtype of diabetes mellitus. N Engl J Med 328:697–702
5. Vaxillaire M, Froguel P (2006) Genetic basis of maturity-onset diabetes of the young. Endocrinol Metab Clin N Am 35:371–384, x
6. Vaxillaire M, Froguel P (2006) Genetics of type 2 diabetes: contribution of monogenic diabetes to common forms of the disease. Int Diabetes Monit 18:17–22
7. Grant SF, Thorleifsson G, Reynisdottir I et al (2006) Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet 38:320–323
8. Cauchi S, Meyre D, Dina C et al (2006) Transcription factor *TCF7L2* genetic study in the French population: expression in human β-cells and adipose tissue, and strong association with type 2 diabetes. Diabetes 55:2903–2908
9. Cauchi S, Meyre D, Choquet H et al (2006) *TCF7L2* variation predicts risk of hyperglycaemia incidence in a French general population: the D.E.S.I.R. study. Diabetes (in press)
10. Florez JC, Jablonski KA, Bayley N et al (2006) TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. N Engl J Med 355:241–250
11. Duval A, Rolland S, Tubacher E, Bui H, Thomas G, Hamelin R (2000) The human T-cell transcription factor-4 gene: structure, extensive characterization of alternative splicings, and mutational analysis in colorectal cancer cell lines. Cancer Res 60:3872–3879
12. Frayling TM, Lindgren CM, Chevre JC et al (2003) A genome-wide scan in families with maturity-onset diabetes of the young: evidence for further genetic heterogeneity. Diabetes 52:872–881
13. Groves CJ, Zeggini E, Minton J et al (2006) Association analysis of 6,736 U.K. subjects provides replication and confirms TCF7L2 as a type 2 diabetes susceptibility gene with a substantial effect on individual risk. Diabetes 55:2640–2644