Original Article

Large Genomic Rearrangements in the Hepatocyte Nuclear Factor-1β (*TCF2*) Gene Are the Most Frequent Cause of Maturity-Onset Diabetes of the Young Type 5

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Maturity-onset diabetes of the young (MODY) 5 is caused by mutations in the *TCF2* gene encoding the transcription factor hepatocyte nuclear factor-1β. However, in 60% of the patients with a phenotype suggesting MODY5, no point mutation is detected in *TCF2*. We have hypothesized that large genomic rearrangements of *TCF2* that are missed by conventional screening methods may account for this observation. In 40 unrelated patients presenting with MODY5 phenotype, *TCF2* was screened for mutations by sequencing. Patients without mutations were then screened for *TCF2* rearrangements by the quantitative multiplex PCR of short fluorescent fragments (QMPSF). Among the 40 patients, the overall detection rate was 70%: 18 had point mutations, 9 had whole-gene deletions, and 1 had a deletion of a single exon. Similar phenotypes were observed in patients with mutations and in subjects with large deletions. These results suggest that MODY5 is more prevalent than previously reported, with one-third of the cases resulting from large deletions of *TCF2*. Because QMPSF is more rapid and cost effective than sequencing, we propose that patients whose phenotype is consistent with MODY5 should be screened first with the QMPSF assay. In addition, other MODY genes should be screened for large genomic rearrangements. *Diabetes* 54:3126–3132, 2005

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HNF, hepatocyte nuclear factor; MODY, maturity-onset diabetes of the young; QMPSF, quantitative multiplex PCR of short fluorescent fragments; STS, sequence-tagged site.

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Maturity-onset diabetes of the young (MODY) is characterized by the occurrence of nonketotic diabetes of early onset, typically before the age of 25, caused by primary insulin-secretion defects and inherited as an autosomal dominant trait. Currently, heterozygous mutations in six different genes have been identified as a cause of MODY. These genes encode the enzyme glucokinase (MODY2 subtype) and the following transcription factors: hepatocyte nuclear factor-4α (*HNF*-4α; MODY1), HNF-1α (*TCF1*; MODY3), insulin promoter factor 1 (MODY4), HNF-1β (*TCF2*; MODY5), and neurogenic differentiation factor 1 (MODY6) (1).

In 20–40% of the patients presenting with clinical and family history consistent with MODY, no mutation in the known MODY genes are found (2,3). Part of these so-called MODY-X cases may be caused by mutations in still unidentified genes. Alternatively, some MODY-X cases could result from complex molecular alterations in the known MODY genes that are missed by conventional screening methods.

This hypothesis is supported by the observation that large genomic rearrangements account for up to 20% of the molecular defects responsible for other monogenic diseases (4–7). PCR amplification of individual exons followed by sequencing is currently the standard screening method for MODY mutation analysis. However, in the case of large genomic deletions involving one or several exons, this method would yield false-negative results due to the amplification of the single wild-type allele.

MODY5 encompasses a wide clinical spectrum comprising diabetes, pancreas atrophy with subclinical exocrine deficiency, progressive nondiabetic nephropathy, kidney and genital malformations, and liver test abnormalities (8). Sequence variations in the *TCF2* gene cause MODY5, and all cases described so far have been associated with either heterozygous point mutations or deletion/insertion of a

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TABLE 1
Main clinical characteristics of the patients with MODY5 and of those with no molecular alteration of *TCF2*

	Molecular alteration of *TCF2*		
	Yes	No	*P*
n	28	12	
Age at latest follow-up (years)	42.8 ± 13.2 (13–73)	55.3 ± 15.4 (21–71)	0.01
Age at first manifestation (years)	24.2 ± 14.8 (1–58)	42.1 ± 18.2 (1–60)	0.004
Diabetes			
Age at diagnosis (years)	28.1 ± 13.1 (10–58)	46.3 ± 14.3 (17–61)	0.002
Symptoms at diagnosis (yes/no)	11/17	2/10	NS
BMI at diagnosis (kg/m^2)	23.8 ± 3.8 (15–32)	27.4 ± 3.1 (21.6–32.5)	0.004
HbA_{1c} at last examination (%)	7.2 ± 1.2 (5.5–9.5)	7.2 ± 1.5 (5.3–10.8)	NS
Insulin therapy (yes/no)	20/8	2/10	0.002
Kidney disease			
Age at diagnosis (years)	28.9 ± 16.9 (1–63)	47.8 ± 17.1 (1–66)	0.002
Presence of kidney cysts (yes/no)	21/7	10/2	NS
Other kidney morphological abnormalities (yes/no)*	14/14	1/11	0.01
Creatinine clearance at latest examination (ml/min per 1.73 m^2)	47.0 ± 26 (5.5–108)	52.7 ± 27.2 (23.9–96)	NS
Systolic blood pressure (mmHg)	127 ± 17 (104–170)	139 ± 12 (117–160)	0.01
Diastolic blood pressure (mmHg)	75 ± 7 (60–90)	77 ± 9 (64–95)	NS
Liver disease			
Liver tests abnormalities (yes/no)	23/4	4/6	0.01
First-degree family history			
Diabetes (yes/no)	12/16	10/2	0.03
Renal disease (yes/no)	11/17	5/7	NS

Data are means ± SD (range). Comparisons between groups were performed using Mann-Whitney test or Fisher's exact test where appropriate. HbA_{1c} normal values, 4–6%. *Renal agenesis, renal atrophy, and pelvicaliceal abnormalities.

few base pairs, with the exception of a 75-bp deletion in one case (8–22). However, no mutation in *TCF2* is detected in ~60% of the patients presenting with MODY5 phenotype (8).

Here, using the technique of quantitative multiplex PCR amplification of short fluorescent fragments (QMPSF) that allows detection of gross genomic rearrangements, we found large deletions in the *TCF2* gene in almost one-half the adult patients with a MODY5 phenotype but no point mutation. No obvious phenotypic differences were observed between these patients and those with *TCF2* mutations. Altogether, point mutations, small deletions/insertions, and large genomic rearrangements of *TCF2* accounted for 70% of the cases of patients presenting with a clinical phenotype consistent with MODY5.

RESEARCH DESIGN AND METHODS

Forty unrelated Caucasian patients (Table 1) were recruited from clinical departments that participate in the French Group for the Study of MODY on the basis of the following clinical characteristics: *1*) Fasting plasma glucose ≥6.1 mmol/l (110 mg/dl) suggesting a MODY phenotype that is diagnosed before the age of 40 or later in the absence of obesity (BMI <30 kg/m^2) or in the context of a family history suggesting autosomal dominant inheritance. All patients were negative for islet-cell antibodies and GADAs. 2) Kidney morphological abnormalities (presence of cysts and/or reduced kidney size and/or pelvivcaliceal abnormalities) and/or impaired renal function (creatinine clearance <80 ml/min per 1.73 m^2) in the absence of persistent proteinuria (albumin excretion ≥0.5 g/24 h) and of diabetic retinopathy.

Clinical history of diabetes and of renal involvement was recorded using a standardized protocol. Creatinine clearance was calculated according to Cockcroft formula. Imaging studies of the kidneys consisted of ultrasonography and intravenous urography or computed tomography scan. Genital tract abnormalities were assessed by ultrasonography. All participants gave written informed consent.

Molecular analysis. The minimal promoter, the coding regions, and exon-intron boundaries of *TCF2* gene were screened for mutations by direct sequencing as described previously (9). Subjects who were negative for mutations were then screened for *TCF2* rearrangements by QMPSF analysis.

QMPSF consists of the simultaneous amplification of multiple short exonic fragments. A limited number of cycles limits the PCR to the exponential phase of the amplification process and enables thus obtainment of a semiquantitative estimation of each PCR product. The analysis is based on the comparison of the peak heights generated from the tested DNA sample and control DNA (4). A heterozygous exon deletion will lead to a twofold reduction of the height of the corresponding peak. Primers were designed for the promoter and the exons 1 to 8 of *TCF2* gene. A 5′ extension, consisting of a rare combination of 10 nucleotides preceding the exon-specific sequence, was added to primers as described previously (6). The forward primer of each pair was 5′ end-labeled with 6-FAM fluorochrome. Amplified DNA fragments were separated using the POP6 polymer on an ABI PRISM Genetic Analyzer 3100 sequencer (Applied Biosystems) according to the manufacturer's instructions. Data were analyzed using the Genescan 3.7 software (Applied Biosystems). In all experiments, we included two control DNA, one with a complete *TCF2* heterozygous deletion and the other with a known heterozygous *TCF2* mutation (8,23).

To confirm complete *TCF2* deletions, we used real-time quantitative PCR based on SYBR-Green I fluorescence. Real-time PCR was performed using an ABI 7900 Sequence Detection System (Applied Biosystems). Primers were designed for the nine exons of *TCF2* gene, and the PCR was carried out in a 20-μl reaction using the SYBR-green I PCR master mix (Applied Biosystems) using 300 nmol/l of each primer and 20 ng DNA. We used as reference the β-globin gene (*HBB*). The number of copies was determined using the $2^{-\Delta\Delta CT}$ method where $\Delta\Delta CT = CT_{TCF2\ amplicon} - CT_{reference\ gene}$ (24).

To define the boundaries of *TCF2* deletions, we used a panel of seven markers: D17S1818, D17S1787, D17S934, and D17S1868 located upstream of *TCF2* gene and D17S927, D17S1872, and D17S798 located downstream of *TCF2* gene. We also designed five sequence-tagged sites (STSs) in the neighboring genes *TRIP3*, *AATF*, *MRPL45*, and *MEL18* and in the genomic contig AC113211. To confirm single-exon deletion, a second set of exon-specific primers was defined to exclude a false-positive result due to unreported polymorphisms affecting primer amplification. The deletion size was determined by long-range PCR using the Expand long Template PCR kit (Roche Diagnostics, Meylan, France) according to the manufacturer's instructions. All primer sequences and amplification conditions can be found in the online appendix (available at http://diabetes.diabetesjournals.org).

RESULTS

***TCF2* alterations.** We have screened 40 unrelated subjects presenting with a clinical phenotype consistent with

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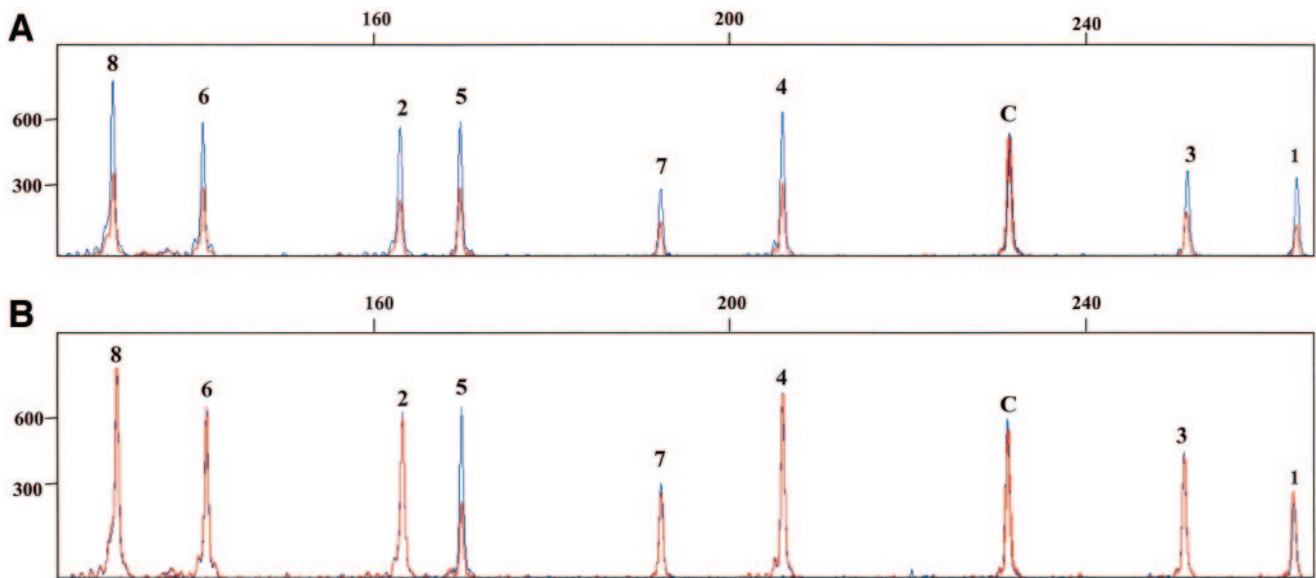

FIG. 1. Detection of *TCF2* exonic deletions by QMPSF in patients with MODY5. QMPSF consists of the simultaneous amplification of multiple short exonic fragments labeled with a fluorochrome. The analysis is based on the comparison of the peak heights generated from the tested DNA and control DNA samples. The horizontal line indicates the size of PCR products expressed in bp; the vertical line indicates the intensity of fluorescence expressed in arbitrary units. Peak numbers correspond to *TCF2* exons, and the "C" peak corresponds to the exon 3 of *TCF1* used as a standard sample for adjusting peak heights. Fluorescence profiles of the patient (red) and those of a control subject (blue) are superimposed. Their relative heights are determined by adjusting to the same height the peaks of the standard samples. *A*: A complete *TCF2* deletion characterized by a twofold reduction of the height of all peaks. *B*: Deletion of exon 5 detected by a twofold reduction of the corresponding peak.

MODY5 (Table 1). A molecular alteration of *TCF2* was found in 70% (n = 28) of them. The molecular defect was either a point mutation (n = 18) or a gross genomic rearrangement (n = 10). In all cases of genomic rearrangement but one, a complete heterozygous deletion of *TCF2* was observed (Fig. 1*A*). All deletions were confirmed using the real-time quantitative PCR method for the nine exons of *TCF2* (data not shown). Genotyping of neighboring microsatellite markers and STSs revealed that the deletion encompasses a genomic region of at least 1.2 megabases. The 5′ breakpoint was located between *TCF2* and the STS in the AC113211 contig, which is 131 kilobases away from *TCF2*. The 3′ breakpoint was located between D17S1872 and the STS in exon 5 of *TRIP3* gene (Fig. 2).

A single-exon deletion of exon 5 was observed in the remaining case of genomic rearrangement (Fig. 1*B*). We designed specific primers in the introns 4 and 5 and amplified by long-range PCR the rearranged fragment. Direct sequencing of the PCR product showed that the deletion extended from nucleotide −294 in intron 4 to nucleotide +704 in intron 5, with loss of 1,159 nucleotides plus an insertion of 55 nucleotides at the deletion junction. A sequence homology search (www.ncbi.nih.gov/BLAST/) revealed that the inserted sequence was identical to the complementary sequence of a fragment located in intron 4, between IVS4+19314 and IVS4+19369, and inserted in inverse orientation (Fig. 3). Repetitive sequence elements and short repeats are known to be involved in gross genomic deletions through the formation of secondary structures between single-stranded DNA ends at breakpoint junc-

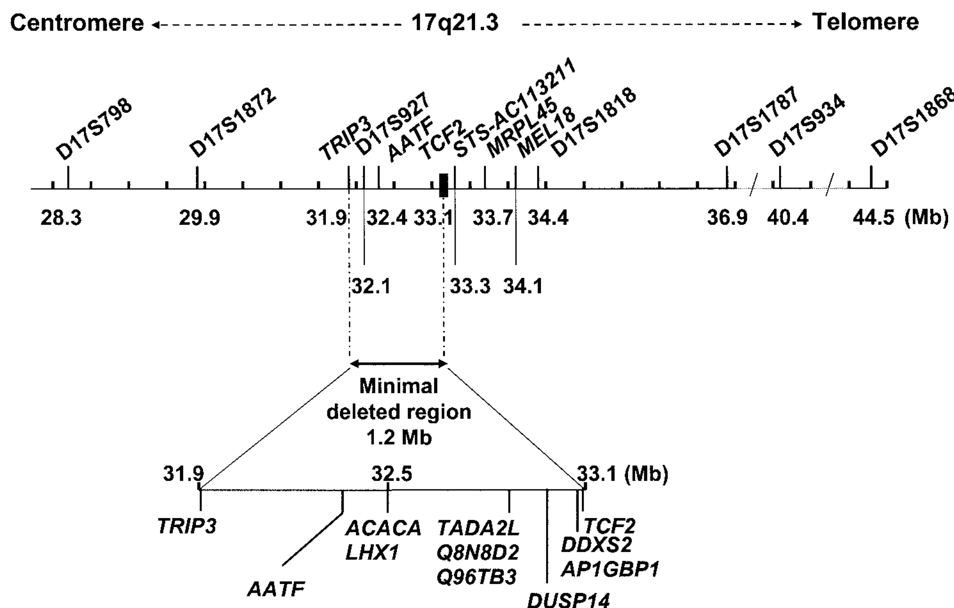

FIG. 2. Schematic representation of the *TCF2*-deleted region. Boundaries of the deletion were defined by genotyping microsatellite markers and STSs located within neighboring genes *TRIP3*, *AATF*, *MRPL45*, and *MEL18* or in the genomic contig AC113211. The common minimal deletion encompasses a genomic region of 1.2 Mb delimited by *TRIP3* and *TCF2*. Seven genes, *AATF*, *ACACA*, *LHX1*, *TADA2L*, *DUSP14*, *DDXS2*, and *AP1GBP1*, and two predicted proteins, Q8N8D2 and Q96TB3, are located within the deleted region.

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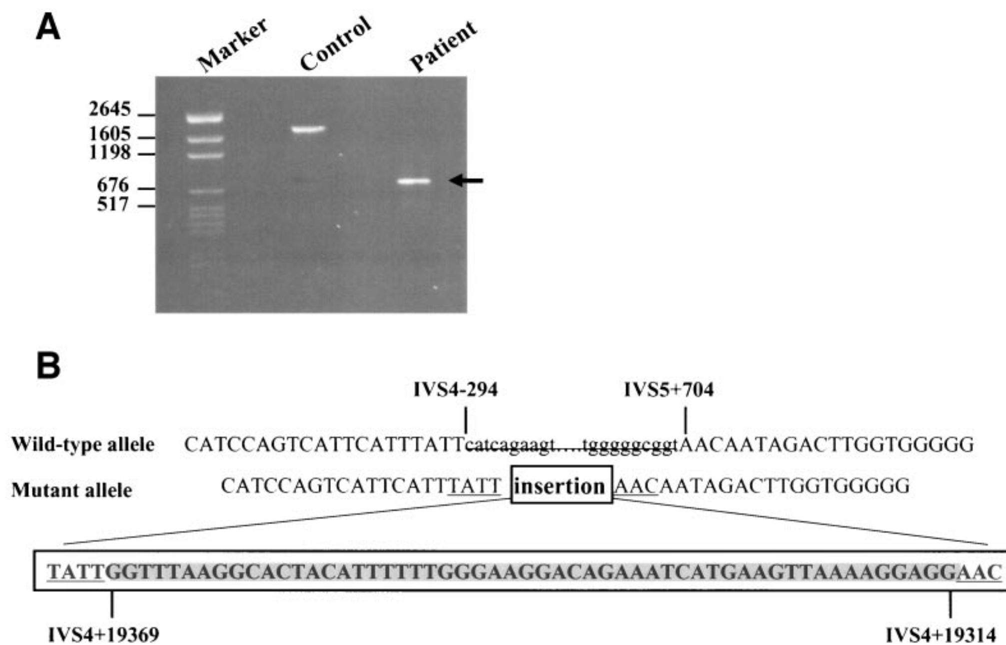

FIG. 3. Confirmation and characterization of the exon 5 *TCF2* deletion. *A*: Long-range PCR with the forward primer located in intron 4 and the reverse primer in intron 5. Whereas the expected size of the normal fragment is 1,852 bp, an abnormal fragment (arrow) of ~700 bp was detected in the patient with the exon 5 deletion. Fragment sizes of the marker are indicated in bp. The wild-type fragment is absent in the patient DNA because of the preferential amplification of the short fragment during PCR. *B*: The breakpoints of the deletion were characterized by sequencing. Deleted nucleotides are represented by lowercase letters and bared. The 55-bp insertion corresponding to the complementary of the sequence IVS4+19314_IVS4+19369 inserted in inverse direction is shaded and boxed. The sequences flanking the inserted sequence and homologous to breakpoint sequences are underlined.

tions (25). To understand the mutational mechanism underlying the exon 5 deletion, we searched for repetitive sequence elements at the deletion breakpoints using the Repeat Masker program (http://www.repeatmasker.org). We found that both deletion breakpoints were located in repetitive sequences: the 5′ deletion breakpoint in a human LINE-2 (long interspaced element-1, L2) element, and the 3′ deletion breakpoint in a MIR (medium interspaced repeat) element. We also observed that this rearrangement involved two sequences, TATT and AAC, located at the start and the end of the inserted sequence, respectively, and homologous to the breakpoint junctions (Fig. 3).

Sixteen different point mutations were identified in 18 unrelated subjects (Table 2). These mutations included 11 missense mutations resulting in amino acid substitutions, 1 splice-site mutation, 3 nonsense mutations, and 1 frameshift mutation leading to a truncated protein. We have previously reported eight of these mutations (8). The other eight distinct mutations including six missense mutations are novel and affect residues conserved in human, pig,

TABLE 2
Molecular alterations of *TCF2* in 28 patients with MODY5

No.	Location	Nucleotide change*	Protein effect	Occurrence
1	Exon 1	c.143delT	p.Leu48fs	1
2	Exon 1	c.226G>T	p.Gly76Cys	1
3	Exon 1	c.335G>C	p.Arg112Pro†	1
4	Exon 2	c.406C>G	p.Gln136Glu†	1
5	Exon 2	c.406C>T	p.Gln136X	1
6	Exon 2	c.490A>C	p.Lys164Gln†	1
7	Exon 2	c.494G>A	p.Arg165His†	2
8	Exon 2	c.541C>T	p.Arg181X†	1
9	Exon 2	c.544C>T	p.Gln182X†	1
10	IVS2	c.543+1G>T	IVS2+1G>T†	1
11	Exon 3	c.704G>A	p.Arg235Gln	1
12	Exon 4	c.826G>A	p.Arg276Gly	1
13	Exon 4	c.854G>A	p.Gly285Asp	1
14	Exon 4	c.884G>A	p.Arg295His†	2
15	Exon 4	c.883C>T	p.Arg295Cys	1
16	Exon 5	c.1108G>A	p.Gly370Ser	1
17	Exon 5	c.1046–294_1206+704del	p.Gly349_Met402del	1
18	Exons 1–9	c.1_?_1671+?del	p.Met1_Trp557del	9

*Nucleotide nomenclature is based on GenBank reference sequence NM_000458. Mutation nomenclature is given according to recommendations of the Human Genome Variation Society (http://www.hgvs.org/mutnomen/). †Mutation previously reported (8).

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TABLE 3
Main clinical characteristics of the patients with MODY5 due to mutations or deletions of *TCF2*

	Mutation	Deletion	*P*
n	18	10	
Age at latest follow-up (years)	45.7 ± 11.6 (34–73)	37.5 ± 15 (13–57)	NS
Age at first manifestation (years)	27.7 ± 13.9 (1–58)	17.8 ± 14.8 (1–52)	0.04
Diabetes			
Age at diagnosis (years)	29.5 ± 12.2 (10–58)	25.7 ± 15 (10–53)	NS
Symptoms at diagnosis (yes/no)	4/14	7/3	0.02
BMI at diagnosis (kg/m^2)	24.9 ± 3.3 (17.8–32)	21.6 ± 4.0 (15–26.9)	NS
HbA_{1c} at last examination (%)	7.2 ± 1.0 (5.6–9.2)	7.1 ± 1.6 (5.5–9.5)	NS
Insulin therapy (yes/no)	14/4	6/4	NS
Kidney disease			
Age at diagnosis (years)	32.4 ± 16.2 (1–63)	22.7 ± 17.2 (1–57)	NS
Kidney cysts (yes/no)	14/4	7/3	NS
Other kidney morphological abnormalities	8/10	6/4	NS
Creatinine clearance at latest examination (ml/min of 1.73 m^2)	40 ± 20.1 (5.5–74)	61.0 ± 31.6 (15.8–108)	NS
Systolic blood pressure (mmHg)	127 ± 18 (104–170)	127 ± 15 (106–150)	NS
Diastolic blood pressure (mmHg)	76 ± 8 (60–90)	73 ± 6 (60–80)	NS
Genital abnormalities			
n/n examined	8/10	4/5	NS
Pancreas morphology abnormalities			
n/n examined	9/15	3/5	NS
Liver disease			
Liver tests abnormalities (yes/no)	14/3	9/1	NS

Data are means ± SD (range).

mouse, *Xenopus*, and salmon *TCF2* sequences. A panel of 212 control chromosomes and 250 chromosomes of patients with type 2 diabetes was screened to exclude that missense mutations may be rare polymorphisms.

Clinical characteristics of the patients with *TCF2* abnormalities. A wide clinical spectrum was observed in the 28 MODY5 patients (Table 1). The first recorded manifestation of the disease in 70% of patients was diabetes, with or without associated renal disease. In 86% of cases, diabetes was diagnosed before the age of 40, and in 71% of cases, subjects were lean (BMI <25 kg/m^2) at diagnosis. At the latest follow-up, 71% of the patients required insulin therapy and all but two patients had some degree of renal impairment not consistent with diabetic nephropathy. The silent course of the renal disease may explain the wide range of the age at diagnosis (1–63 years). In fact, eight patients had renal congenital malformations (unilateral kidney agenesis and/or ureteric stricture, including pelviureteric junction obstruction) that were diagnosed by imaging studies between age 7 and 63. A family history of diabetes in first-degree relatives was reported by 43% of patients, and of renal disease by 39% of them. However, these figures may have been underestimated, because first-degree relatives were not systematically tested in this study. Other features of the MODY5 phenotype were present in the majority of the patients. Various genital tract abnormalities were found in the 80% of the patients who were tested. Pancreas atrophy was observed in 60% of the patients. Lastly, 85% of the patients presented with fluctuating and increased plasma levels of liver enzymes.

A striking finding of this study was that similar clinical features were observed in carriers of point mutations and carriers of large genomic rearrangements (Table 3). Diabetes and renal disease were not more severe in the patients with *TCF2* deletions than in subjects with a point mutation. Although the disease was diagnosed earlier and diabetes was more often associated with symptoms at onset in the former, a large overlap was observed between the two groups for all other clinical characteristics. By contrast, different clinical profiles were observed in patients with no detected abnormality of *TCF2* despite a suggestive phenotype and in those with *TCF2* alterations (Table 1). Subjects without *TCF2* alterations were older at diagnosis of diabetes and renal disease, had a higher BMI at diagnosis of diabetes, less often required insulin therapy (an indirect index of insulin deficiency), and less often had liver test abnormalities. They also reported more often a family history of diabetes. Lastly, in all of these patients but one, renal morphological abnormalities were restricted to the presence of cysts.

DISCUSSION

We have shown in this report that large genomic rearrangements of *TCF2* cause MODY5 and that whole-gene deletion is the most frequent molecular alteration observed in MODY5 patients. In this series, 70% of the patients presenting with a clinical phenotype consistent with MODY5, i.e., presenting with hyperglycemia suggesting a MODY phenotype, associated with either abnormalities of kidney morphology or impaired renal function, were carriers of *TCF2* molecular alterations. Importantly, large deletions of *TCF2* accounted for one-half the patients with a phenotype suggestive of MODY5 but who were so far classified as MODY-X because conventional screening had failed to detect a *TCF2* mutation. These findings demonstrate that MODY5 is actually more frequent than previously estimated. Moreover, point mutations of *TCF2* are sometimes associated with phenotypes restricted to a single organ (8,15). Thus, one cannot exclude *TCF2* gene deletions being associated with more restricted phenotypes, particularly in young subjects with pure renal involvement or in patients with abnormal

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genital tract or pancreas development. In our series, no *TCF2* molecular alteration was detected in 30% of the patients. At least two hypotheses may account for this observation. Molecular alterations of other gene(s) may lead to a phenotype similar to that of MODY5. Alternatively, in adult patients, type 2 diabetes and other abnormalities such as renal failure, renal cysts, and liver test abnormalities may be etiologically unrelated.

Although most of the *TCF2* mutations reported so far were private, mutations affecting the donor splicing site of exon 2 were found in different populations, suggesting that this region is a hot spot for mutations (20). Our results suggest the presence of a second hot spot, because one-fourth of our patients were carriers of a large *TCF2* deletion. The microsatellite and STS analysis revealed in all patients a minimal deleted region of 1.2 megabases. Given that gross genomic rearrangements are not randomly distributed in the human genome, we could hypothesize that the recurrence of this anomaly is due to a common mutational mechanism that could be either a homologous unequal recombination, mediated by similar nonallelic regions such as repetitive sequences, or a nonhomologous recombination occurring between short motifs of homology (26,27). The first mechanism is more frequently involved in gross deletions. By contrast, the presence of short sequences located at the breakpoints and on both sides of the inserted sequence may explain the rearrangement observed in the patient with the deletion of exon 5. Moreover, these sequences are located within AT-rich sequences known to promote secondary structure formation and frequently involved in the occurrence of deletion (25).

In our series, gross rearrangements of *TCF2* were associated with no obvious specific phenotype. We could therefore hypothesize that *TCF2* deletions lead to MODY5 through haplo-insufficient expression of HNF-1β protein, as shown for several *TCF2* mutations (21). Interestingly in MODY1, it has been reported that a balanced translocation that did not disrupt the HNF-4α coding region could affect the gene expression by disrupting regulatory transcriptional elements (28).

The absence of associated clinical defects is remarkable, because the large deletions observed in nine patients span over 1.2 megabases. Similar observations have been reported in other diseases, in particular with cancer-related genes in which genomic deletions of several hundred kilobases have been associated with the classical disease phenotype (29,30). The large deletions we observed involve two sequences encoding putative proteins of unknown function and seven genes for which no link with the pathogenesis of MODY5 is obvious (Fig. 2.).

Our study shows that genomic rearrangements of *TCF2* represent 36% of the molecular defects identified in MODY5 patients. These findings need to be replicated in other populations because they may have important implications for the choice of the diagnosis strategy of MODY5. As already mentioned, such alterations are missed by conventional amplification and screening. Given that clinical presentation provides no firm indication on the molecular mechanism and that QMPSF is more rapid and more cost effective than sequencing, we suggest that the routine molecular screening of *TCF2* should be started with the QMPSF assay. Sequencing should be used in patients for whom the QMPSF assay is negative.

Finally, large genomic rearrangements might be implicated in other MODY subtypes. MODY3, caused by mutations in *TCF1*, is by far the most prevalent MODY subtype in adults. However, point mutations and small insertion/deletions do not account for a large number of patients with a phenotype consistent with MODY3 (31). We suggest that this could be due, at least in part, to *TCF1* deletions. Actually, several studies have shown a linkage between early-onset autosomal-dominant type 2 diabetes and the region including the MODY3 locus. However, so far neither mutations in *TCF1* nor identification of novel locus have been found (32,33). We suggest that patients with the so-called MODY-X should be systematically reassessed for the presence of deletions in the corresponding candidate genes.

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