Molecular Scanning Analysis of Hepatocyte Nuclear Factor 1α (TCF1) Gene in Typical Familial Type 2 Diabetes in African Americans

Steven C. Elbein, Kui Teng, Kenya Eddings, Denise Hargrove, and Eric Scroggin

Type 2 diabetes mellitus (T2DM) is strongly inherited, but the major genes for this disease have been elusive. In contrast, early-onset, autosomal-dominant diabetes results from at least 5 loci, of which hepatocyte nuclear factor 1α (HNF1α or TCF1) is the most common cause. Mutations in HNF1α also cause later-onset diabetes in some Caucasian populations, but the role of these mutations has not been tested in African American populations. We used a variety of screening methods, including both single-strand conformation polymorphism (SSCP) analysis and dideoxy fingerprint analysis, to search for mutations in 51 African American subjects with onset of diabetes before age 50 years. Potential mutations were confirmed by direct sequencing. We identified 21 different variants, of which 11 were unique to African Americans. Four mutations either altered the amino acid sequence (Gly52Ala and Gly574Ser) or were close to a splice site (intron 1 and intron 10). A 5-nucleotide insertion in intron 1 was present in both diabetic members of a small family, but Gly52Ala, Gly574Ser, and the intron 10 mutation did not segregate with diabetes. Gly574Ser was present in 2 large families and 5% of controls, all of which appeared to share the same common HNF1α haplotype. Surprisingly, radioactive SSCP analysis under 2 room-temperature conditions performed as well as methods using fluorescent labeling that were expected to be more sensitive. We conclude that in African American individuals under age 50, variation in the HNF1α gene is common but unlikely to be a significant cause of T2DM.

TWIN STUDIES and a strong familial aggregation suggest a major inherited susceptibility to typical type 2 diabetes mellitus (T2DM). Nonetheless, probable heterogeneity and epistasis (multiple interacting loci) conspire to make identification of the predisposing genetic loci challenging. In contrast, at least 5 loci have been implicated in early-onset, autosomal-dominant diabetes (maturity-onset diabetes of the young [MODY]).[1-5] Of these loci, *MODY3*, which results from mutations in hepatocyte nuclear factor 1α (HNF1α) and is encoded by the TCF1 gene, is the most common cause of early-onset T2DM (MODY) outside of France.[6,7] Over 41 mutations in HNF1α have been identified.[8] The same mutations have arisen independently in several unrelated families, thus suggesting a number of potential mutational hotspots. Furthermore, individuals carrying high-penetrance HNF1α mutations such as a common frameshift have presented with a variable age of onset and therapy ranging from diet only to insulin.[9] These observations suggested that HNF1α mutations might be a significant cause of relatively early-onset typical T2DM that does not meet criteria for MODY. In support of this hypothesis, Kaisaki et al[6] reported mutations in 9 of 25 German subjects with T2DM onset before age 35, Frayling et al[7] found HNF1α mutations in 2 of 32 English subjects with T2DM onset before age 40, and we reported mutations that altered conserved amino acids in 2 of 36 Caucasian families in which 1 subject had diabetes onset before age 40 years.[9]

Linkage studies have provided additional evidence for a T2DM locus at or near the HNF1α gene on chromosome 12q. Mahtani et al[10] reported a linkage to this region in a subset of Botnian Finnish families with the lowest insulin secretory response, and others also suggested a linkage of T2DM to markers near *MODY3*.[11,12] However, linkage studies would not detect the small but significant subset of families with HNF1α mutations reflected in the studies by Kaisaki et al,[6] Frayling et al,[7] and our group.[9]

African American individuals are a unique population in which Caucasian and African genetic backgrounds are admixed. African Americans are characterized by a higher risk of T2DM, an earlier onset, a higher rate of complications, especially nephropathy, and possibly a relative reduction in insulin secretion.[13-15] The role of HNF1α mutations in populations of African origin has not been characterized. Given the earlier onset and possible insulin deficiency, we hypothesized that mutations of HNF1α were a significant cause of T2DM in African Americans. To test this hypothesis, we used a variety of molecular scanning methods to search for variation in the HNF1α gene among African American subjects ascertained in Arkansas whose T2DM was diagnosed at or before age 50 years. We have identified 21 different HNF1α variants in this population, of which 11 are unique to African Americans. However, in contrast to our studies of Northern European Caucasians, HNF1α mutations do not appear to be an important cause of diabetes in this population.

SUBJECTS AND METHODS

Subjects

Subjects were selected for (1) African American ancestry, (2) diabetes diagnosis before age 50 years, and (3) family history of diabetes in a first-degree relative. Of 51 subjects meeting these criteria, 44 were selected from our ongoing studies of families ascertained for T2DM in 2 siblings and no more than 1 parent known to have diabetes.[16] An additional 7 individuals were chosen for a family history of diabetes, but no other family members were available for study. In total, 32 families were represented, of which 25 met our strict criteria for familial T2DM (2 affected siblings and no more than 1 affected parent). For all families studied, any available siblings of the proband

From the Division of Endocrinology, Central Arkansas Veterans Health System and University of Arkansas for Medical Sciences, Little Rock, AR.

Submitted May 27, 1999; accepted July 28, 1999.

Supported by Grant No. DK39301 from the National Institutes of Health (National Institute of Diabetes and Digestive and Kidney Diseases), the Department of Veterans Affairs, and a GENNID family acquisition grant from the American Diabetes Association.

Address reprint requests to Steven C. Elbein, MD, Endocrinology 111J/LR, John L. McClellan Veterans Hospital, 4300 W 7th St, Little Rock, AR 72205.

0026-0495/00/4902-0011$10.00/0

sibling pair and any offspring of a diabetic subject were tested by a standard 75-g oral glucose tolerance test. The diagnosis was based on a history of diabetes and current therapy, and a fasting glucose over 7.8 mmol/L, or for subjects not previously on therapy, a 2-hour glucose of 11.1 mmol/L, according to World Health Organization criteria.[17] Patients represented a range of obesity (body mass index, 21.4 to 49.4 Kg/m^2; mean, 31.5), age of onset (range, 20 to 50 years; mean, 39.6), and therapy (27 insulin-treated, 22 treated with oral agents, and 2 newly diagnosed or diet-treated). The population contained 22 men and 29 women. No family met typical criteria for MODY. The control population was 49 healthy African American subjects (mean age, 39.8 years; mean body mass index, 28.3 Kg/m^2; range, 19.8 to 47.0; 32 males and 17 females) who had no known family history of diabetes in a first-degree relative. All subjects provided informed consent according to a protocol approved by the University of Arkansas for Medical Sciences Human Resource Advisory Committee.

Molecular Screening

We used polymerase chain reaction–single-strand conformation polymorphism analysis (PCR-SSCP) to screen all 10 exons and the minimal promoter region as described previously.[9] In previous studies,[9] we used 4 gel conditions. Prior to initiating the current studies, we performed experiments to determine the minimal number of conditions for good sensitivity by testing 19 variants from the promoter, exons 1, 4, 7, 8, and 9, and introns 2, 5, 7, 9, and 10 under SSCP conditions (room temperature and 4°C with 0%, 5%, and 10% glycerol and 0.5X mutation detection enhancement (MDE) gel at room temperature). We were able to detect all known HNF1α variants at room temperature (25°C) with 5% acrylamide (Accugel; National Diagnostics, Atlanta, CA) in 1× Tris-borate-EDTA (TBE) buffer or 0.5× MDE gel (FMC, Rockland, ME) in 0.6× TBE buffer. All products were labeled by incorporation of ^{32}P-dCTP (ICN Pharmaceuticals, Irvine, CA; 3,000 Ci/mmol) and separated on 20-cm × 25-cm gels at 25 W constant wattage for 6 to 8 hours.[9] All amplification products larger than 300 base pairs (bp) underwent restriction enzyme digestion prior to gel electrophoresis so that the largest fragment analyzed was 319 bp.

In addition to PCR-SSCP analysis, we examined each exon by BESS-T scan mutation detection (Epicentre Technologies, Madison, WI) using the manufacturer's protocol. This method incorporates UTP in place of TTP followed by specific enzymatic cleavage at each UTP site, thus providing the equivalent of a thymidine sequencing ladder under denaturing conditions. Forward and reverse M13 primers were appended to these primer sequences. The exon was first amplified without label, and this amplification product was subsequently reamplified using M13 forward and reverse sequencing primers differentially labeled with LI-COR 700- and 800-nmol/L fluorescent dyes (LI-COR, Lincoln, NE). Each product was examined by polyacrylamide gel electrophoresis under both denaturing and nondenaturing conditions (0.5X MDE) with detection on a LI-COR GR-4200 Sequencer. Each exon thus generated 4 images: a dideoxy fingerprint gel for each strand,[18] and a T-ladder for each strand. Resultant gels were examined visually for deviant banding patterns. Nondenaturing conditions could not be interpreted for 1 or both strands for exons 3 and 4.

Sequence Analysis

Standard amplification primers were modified as already described to include forward and reverse M13 primer sequences appended to the 5′ and 3′ primers, respectively. The initial amplification product was column-purified (Qiaquick PCR purification kit; Qiagen, Chatsworth, CA). Simultaneous sequencing was performed using cycle sequencing with thermostable polymerase and differentially labeled forward and reverse M13 primers as described before. Additionally, the intron 1 insertion (GAGCC) and exon 1 codon 52 variant (Gly52Ala) were blunt-cloned into vector pT7 Blue (Novagen, Madison, WI), and the sequence variation was confirmed from cloned DNA for both alleles.

Allele Typing

Variant alleles were typed by restriction digest or by SSCP (Table 1). All available family members were typed for newly described variants that alter the amino acid sequence, for the intron 1 insertion, and for the intron 10 +6 variant. The frequency of these and other unique variants was determined in 49 control individuals with no family history of diabetes (Table 1).

Because the exon 9 (Gly574Ser) variant was unique to African Americans yet present in several families, we sought to determine whether this variant arose on a single haplotype by typing 7 common polymorphisms that have been described previously: exon 1 codons 17 and 27 (detected by *Xho*I and *Sau*3 AI digests, respectively), exon 7 codons 459 and 487 (detected by enzymes *Pst*I and *Xmn*I, respectively), exon 8 codon 515 (detected by enzyme *Stu*I), and intron 7 (+7) and intron 9 (−24) variants (detected by enzymes *Hea*II and *Nhe*I, respectively). Haplotype phase was established by segregation among family members.

RESULTS

We found 21 variant sequences among the 51 African American members of 32 different families using our 3 screening methods (Table 1). Variant sequences were identified in all PCR fragments except those amplifying exons 5 and 8. Screening detected 11 variants that appear to be unique to African Americans. Among the base changes that alter the amino acid sequence, Ile27Leu, Ala98Val, and Asn487Ser were reported previously[2,6] and were not associated with diabetes or impaired insulin secretion in previous studies.[19] Additional base changes in the promoter region, exon 1 (codons 17 and 92), exon 4 (codon 288), exon 7 (codons 441, 459, and 482), and introns 1, 2, 6, 7, 9, and 10 would not be predicted to alter the protein structure or the known splice consensus sequences, and thus were not expected to alter gene function. The single promoter mutation was outside the conserved minimal promoter region, and not likely to alter gene expression. One common polymorphism in Caucasians (exon 8 codon 515, ACG → ACA) was not present in African Americans. Among the newly identified base changes were 2 missense mutations (Gly52Ala in exon 1 and Gly574Ser in exon 9) and 2 variants that were near the splice-site consensus sequence (intron 1 +5 insertion and intron 10 +6 A/G).

Gly52Ala was present in 2 of 4 diabetic siblings and 1 nondiabetic sibling in a single family. Surprisingly, this variant required changes in 2 adjacent nucleotides, neither of which is commonly altered. The presence of both nucleotide changes on the same allele was confirmed by sequencing cloned DNA. Although a lack of segregation with diabetes in the single family argues against a causative role for this mutation in diabetes, the glycine at this position is invariant in human, mouse, rat, and hamster. The Gly574Ser variant in exon 9 has not been reported in Caucasians, but was present in 4 families and in 5% of the controls and thus appears to be common among African Americans. This polymorphism did not segregate with diabetes in 2 large families and, unlike Gly52Ala, is not in a highly conserved region of the gene. Haplotype analysis suggested that the Ser574 allele arose on the most common haplotype of the 7 polymorphisms examined. However, because this haplotype is very common, the analysis does not exclude the possibility that this polymorphism arose on several independent occasions.

Table 1. Nucleotide Changes in HNF1α Gene in African Americans

Location	Position	Nucleotide Change	Detection Method	Typing Method	Minor Allele Frequency
Promoter*	−89	T → C	SSCP, FP	SSCP	0%
Exon 1	Codon 17	CTC → CTG	SSCP	*Xho*I	37%
Exon 1	Codon 27	ATC → CTC Ile27Leu	SSCP	*Sau*3AI	18%
Exon 1*	Codon 52	GGC → GCT Gly52Ala	SSCP, BT, FP	SSCP	0%
Exon 1*	Codon 92	CTC → CTT	SSCP†	—	—
Exon 1	Codon 98	GCC → GTC Ala98Val	SSCP, BT, FP	*Hae*III	4.4%
Intron 1	+5	ins GAGCC	SSCP, BT, FP	SSCP, BT	1%
Intron 1	+20	C → A	BT, FP	—	—
Intron 1	−42	G → A	SSCP, BT, FP	—	—
Intron 2*	+53	G → C	SSCP	—	—
Intron 2	−23	C → T	SSCP, BT‡	—	—
Exon 4	Codon 288	GGG → GGC	SSCP‡	—	—
Intron 6	+26	C → T	SSCP, BT, FP	—	—
Exon 7*	Codon 441	ACG → ACA	SSCP, BT	—	—
Exon 7	Codon 459	CTG → TTG	SSCP, BT, FP	—	—
Exon 7*	Codon 482	AGC → AGT	SSCP, BT, FP	—	—
Exon 7	Codon 487	AAC → AGC Asn487Ser	SSCP, FP§	*Xmn*I	12%
Intron 7	+7	A → G	SSCP, FP§	*Hea*II	—
Exon 9*	Codon 574	GGC → AGC Gly574Ser	SSCP, BT, FP	SSCP	5.7%
Intron 9	−24	T → C	SSCP, FP	*Nhe*I	10%
Exon 10*	3′ UT (nt 130)	G → A	SSCP, BT, FP	SSCP	0%

NOTE. Table summarizes the screening of 51 African American subjects with onset of diabetes before age 50 years. Location is the gene fragment; position is the codon or position relative to the exon included in the amplified fragment. Nucleotide (nt) changes did not alter the amino acid sequence unless noted. Detection method indicates the conditions for screening: SSCP, PCR-SSCP under either or both conditions; FP, fluorescent fingerprint analysis using BESS-T product under nondenaturing conditions; BT, BESS-T product under denaturing conditions. Typing method is the method for determining the status of other family members and controls for the amplified product by restriction digest (enzyme listed) or SSCP. Frequency in controls is based on 49 unrelated nondiabetic individuals. Family segregation and control frequencies were not determined for variants that were not expected to alter gene function.

*Variant unique to this population.

†Variant found by sequencing unique SSCP pattern caused primarily by variation at codons 17 and 27.

‡Fingerprint gels not interpretable for this fragment.

§Intron 7, +7 was found primarily on sequencing gels together with codon 459 and 487 mutations, and may not have been detected alone.

The 5-nucleotide insertion (GCGCC) in intron 1 appeared to represent an exact duplication of an adjacent 5-nucleotide sequence, resulting in a tandem repeat potentially affecting the donor splice-site consensus sequence. The insertion was present in both available diabetic members of a single family, but no nondiabetic siblings or older offspring were available for study. No other family but 1 of 49 control individuals carried this variant. The intron 10 +6 variant, which was also near a splice donor site, was present in only 2 of 4 diabetic family members and 1 nondiabetic member of a single family. No other family members or control individuals carried this base change.

DISCUSSION

Mutations of HNF1α are the most important cause of MODY identified to date in Caucasian and Asian families.[20] The role in other populations, particularly US minority populations, is unclear. Several studies have suggested that early-onset T2DM that does not meet criteria for MODY may nonetheless result from HNF1α mutations in Caucasians,[6,9] but no role has been found in older adults and in other populations.[20-22] We are unaware of published reports in populations of African descent. The tendency to an earlier onset of diabetes in African American individuals might suggest a higher prevalence of HNF1α gene mutations versus the rate in Caucasians, but these individuals also tend to be obese, in contrast to typical subjects with HNF1α gene mutations.

The amount of variation in the HNF1α gene among African Americans was striking. In total, 21 variants were identified in 51 individuals representing only 32 families, and 11 variants were unique to this population. Several variants are intriguing. Three sets of 3 polymorphisms occurred together in the same individual, suggesting linkage disequilibrium between variants and necessitating sequence analysis of these alleles to detect mutations. The mutations occurring together included polymorphisms at codons 17, 27, and 98 in exon 1, codons 17, 27, and 92 in exon 1, and codons 457 and 459 and intron 7 in the exon 7 fragment. The 5-nucleotide insertion in intron 1 represents a duplication of an adjacent sequence that has not been reported elsewhere. Although this variant segregates with diabetes in a sib pair, it was also present in a control individual. We did not determine whether mRNA splicing and gene function are altered. The exon 1 Gly52Ala variant required changes in 2 adjacent nucleotides (GGC to GCT). This region contains 3 adjacent glycines. A mutation in codon 53 (GGT) would require only a single nucleotide change. The repetitive nature of the sequence in this region may represent another mutational hotspot leading to this seemingly unlikely event. This variant is in a conserved region of HNF1α but was present in only 2 of 4

diabetic family members, was not present in an individual with impaired glucose tolerance, and was carried by a 41-year-old woman with normal glucose tolerance. Both diabetes from other causes (sporadic cases) and nonpenetrance have been described in true MODY families.[9,20] Nonetheless, this mutation seems unlikely to cause diabetes.

The Gly574Ser was described previously but not characterized in an individual of Afro-Caribbean descent.[23] We found this polymorphism both in families ascertained on a diabetic sibling pair and in 5% of the control population. Based on the frequency among normal control individuals, the nonconserved nature of the protein sequence in this region, the conservative nature of this substitution, and the lack of segregation with diabetes, we propose that this variant has no effect on HNF1α function. Nonetheless, the origin of this very common, private polymorphism is intriguing. Because this variant is both common among unrelated individuals from Arkansas and present in an individual of African descent residing in France,[23] it may be common among native African populations.

Because of the large amount of genetic variation in HNF1α, this gene provides an excellent test of methods for mutation screening. We based our SSCP conditions on an analysis of 7 gel conditions, including 4 conditions used in our previous studies.[9,24] The 2 conditions used here detected all known variants. Nonetheless, for the present study, we examined 2 fluorescent-based methods that offer the theoretical advantage of higher sensitivity. The commercial BESS-T method incorporates UTP in place of TTP during PCR, followed by enzymatic cleavage that results in a thymidine sequencing ladder when analyzed on a standard sequencing gel. We also examined a variation in which this product is analyzed on a nondenaturing MDE gel, thus resulting in the equivalent of a dideoxy fingerprint analysis.[18] By differentially labeling forward and reverse primers and using both detection channels of the LI-COR sequencer, we were able to examine both strands simultaneously. In published reports using radioactive methods, the sensitivity of dideoxy fingerprint analysis approached 100%.[18,25] We thus anticipated that combined analysis of the same fluorescently labeled PCR product on denaturing and nondenaturing gel electrophoresis would provide a convenient and sensitive means to detect nearly all sequence variation. Surprisingly, neither fluorescent method surpassed radioactive PCR-SSCP for HNF1α. Indeed, PCR-SSCP under these 2 conditions detected at least 18 of 21 sequence variations (86%). Two additional variants (codon 92 and intron 7) were present with other mutations and did not generate unique patterns. Thus, only the intron 1 +20 variant was not easily detectable by SSCP. In contrast, fluorescent fingerprint analysis detected only 13 variants (62%), while the T-ladder alone detected only 12 of 21 (57%) with analysis of both strands. Both methods together detected 15 of 21 variants (71%). The unexpectedly low sensitivity of the fingerprint methods was due to the difficulty in analyzing the large, complicated fluorescent images, the inability to interpret images for exons 3 and 4, and the difficulty in detecting a second mutation in the presence of 1 mutation. Thus, individual mutations in exons 1 and 7 were difficult to detect on fingerprint analysis. Under denaturing conditions, the appearance of a new adenine or thymidine was easy to detect but the heterozygous loss of a single adenine or thymidine was difficult to discern. Furthermore, under denaturing conditions, BESS-T analysis cannot detect a change from guanine to cytosine or from cytosine to guanine. We also found several variant patterns on fluorescent analysis under both denaturing and nondenaturing conditions for which we could find no sequence variant, thus suggesting a substantial false-positive rate for fluorescent detection methods. These fluorescent methods might be more effective for genes with much less variation and where the expected patterns are easier to interpret.

In conclusion, we have used several methods to search for variants in the HNF1α gene in African Americans. We have identified 11 variants that are novel to African Americans, including 2 potential missense mutations. Segregation and population analyses do not suggest an important role for most of these variants in T2DM. Nonetheless, lean African American individuals with an onset of diabetes before age 25 are underrepresented in this study, and HNF1α mutations might explain a more substantial proportion of the diabetes in that subgroup.

ACKNOWLEDGMENT

We thank Linda Wood for assistance in testing the subjects, Phaedra Yount for initial development of the fluorescent detection methods, and the subjects for their voluntary participation.

REFERENCES

1. Froguel P, Zouali H, Vionnet N, et al: Familial hyperglycemia due to mutations in glucokinase. Definition of a subtype of diabetes mellitus. N Engl J Med 328:697-702, 1993

2. Yamagata K, Oda N, Kaisaki PJ, et al: Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY3). Nature 384:455-458, 1996

3. Yamagata K, Furuta H, Oda N, et al: Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY1). Nature 384:458-460, 1996

4. Stoffers DA, Ferrer J, Clarke WL, et al: Early-onset type-II diabetes mellitus (MODY4) linked to *IPF1*. Nat Genet 17:138-139, 1997

5. Horikawa Y, Iwasaki N, Hara M, et al: Mutation in hepatocyte nuclear factor-1β gene (TCF2) associated with MODY. Nat Genet 17:384-385, 1997

6. Kaisaki PJ, Menzel R, Linder T, et al: Mutations in the hepatocyte nuclear factor-1α gene in MODY and early-onset NIDDM: Evidence for a mutational hotspot in exon 4. Diabetes 46:528-535, 1997

7. Frayling TM, Bulman MP, Ellard S, et al: Mutations in the hepatocyte nuclear factor-1α gene are a common cause of maturity onset diabetes of the young in the U.K. Diabetes 46:720-725, 1997

8. Gragnoli C, Lindner T, Cockburn BN, et al: Maturity-onset diabetes of the young due to a mutation in the hepatocyte nuclear factor-4α binding site in the promoter of the hepatocyte nuclear factor-1α gene. Diabetes 46:1648-1651, 1997

9. Elbein SC, Teng K, Yount P, et al: Linkage and molecular scanning analyses of MODY3/hepatocyte nuclear factor-1 alpha gene in typical familial type 2 diabetes: Evidence for novel mutations in exons 8 and 10. J Clin Endocrinol Metab 83:2059-2065, 1998

10. Mahtani MM, Widen E, Lehto M, et al: Mapping a gene for type

2 diabetes associated with an insulin secretion defect by a genome scan in Finnish families. Nat Genet 14:90-94, 1996

11. Bowden DW, Sale M, Howard TD, et al: Linkage of genetic markers on human chromosomes 20 and 12 to NIDDM in Caucasian sib pairs with a history of diabetic nephropathy. Diabetes 46:882-886, 1997

12. Shaw JT, Lovelock PK, Kesting JB, et al: Novel susceptibility gene for late-onset NIDDM is localized to human chromosome 12q. Diabetes 47:1793-1796, 1998

13. Harris MI, Flegal KM, Cowie CC, et al: Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. The Third National Health and Nutrition Examination Survey, 1988-1994. Diabetes Care 21:518-524, 1998

14. Freedman BI, Tuttle AB, Spray BJ: Familial predisposition to nephropathy in African-Americans with non–insulin dependent diabetes mellitus. Am J Kidney Dis 25:710-713, 1995

15. Joffe BI, Panz VR, Wing JR, et al: Pathogenesis of non–insulin-dependent diabetes mellitus in the black population of southern Africa. Lancet 340:460-462, 1992

16. Raffel LJ, Robbins DC, Norris JM, et al: The GENNID Study. A resource for mapping the genes that cause NIDDM. Diabetes Care 19:864-872, 1996

17. Harris MI, Eastman RC, Cowie CC, et al: Comparison of diabetes diagnostic categories in the U.S. population according to the 1997 American Diabetes Association and 1980-1985 World Health Organization diagnostic criteria. Diabetes Care 20:1859-1862, 1997

18. Sarkar G, Yoon HS, Sommer SS: Dideoxy fingerprinting (ddE): A rapid and efficient screen for the presence of mutations. Genomics 13:441-443, 1992

19. Urhammer SA, Fridberg M, Hansen T, et al: A prevalent amino acid polymorphism at codon 98 in the hepatocyte nuclear factor-1 alpha gene is associated with reduced serum C-peptide and insulin responses to an oral glucose challenge. Diabetes 46:912-916, 1997

20. Elbein SC: An update on the genetic basis of type 2 diabetes. Curr Opin Endocrinol 5:116-125, 1998

21. Nishigori H, Yamada S, Kohama T, et al: Mutations in the hepatocyte nuclear factor-1 alpha gene (MODY3) are not a major cause of early-onset non–insulin-dependent (type 2) diabetes mellitus in Japanese. J Hum Genet 43:107-110, 1998

22. Yamada S, Nishigori H, Onda H, et al: Mutations in the hepatocyte nuclear factor-1α gene (MODY3) are not a major cause of late-onset NIDDM in Japanese subjects. Diabetes 46:1512-1513, 1997

23. Boutin P, Chevre JC, Hani EH, et al: An automated fluorescent single-strand conformation polymorphism technique for screening mutations in the hepatocyte nuclear factor-1 alpha gene (maturity-onset diabetes of the young). Diabetes 46:2108-2109, 1997

24. Elbein SC, Hoffman M, Qin H, et al: Molecular screening of the glucokinase gene in familial type 2 (non–insulin-dependent) diabetes mellitus. Diabetologia 37:182-187, 1994

25. Liu Q, Sommer SS: Parameters affecting the sensitivities of dideoxy fingerprinting and SSCP. PCR Methods Appl 4:97-108, 1994