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Examination of Rare Variants in HNF4 α in European Americans with Type 2 Diabetes

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Abstract

The hepatocyte nuclear factor 4-α *(HNF4*α) gene codes for a transcription factor which is responsible for regulating gene transcription in pancreatic beta cells, in addition to its primary role in hepatic gene regulation. Mutations in this gene can lead to maturity-onset diabetes of the young (MODY), an uncommon, autosomal dominant, non-insulin dependent form of diabetes. Mutations in *HNF4*α have been found in few individuals, and infrequently have they segregated completely with MODY in families. In addition, due to similarity of phenotypes, it is unclear what proportion of type 2 diabetes (T2DM) in the general population is due to MODY or *HNF4*α mutations specifically. In this study, 27 documented rare and common variants were genotyped in a European American population of 1270 T2DM cases and 1017 controls from review of databases and literature implicating *HNF4*α variants in MODY and T2DM. Seventeen variants were found to be monomorphic. Two cases and one control subject had one copy of a 6-bp P2 promoter deletion. The intron 1 variant (rs6103716; MAF = 0.31) was not significantly associated with disease status (p>0.8) and the missense variant Thr130Ile (rs1800961; MAF = 0.027) was also not significantly different between cases and controls (p>0.2), but showed a trend consistent with association with T2DM. Four variants were found to be rare as heterozygotes in small numbers of subjects. Since many variants were infrequent, a pooled chi-squared analysis of rare variants was used to assess the overall burden of variants between cases and controls. This analysis revealed no significant difference (P=0.22). We conclude there is little evidence to suggest that *HNF4*α variants contribute significantly to risk of T2DM in the general population, but a modest contribution cannot be excluded. In addition, the observation of some mutations in controls suggests they are not highly penetrant MODY-causing variants.

Keywords: Type 2 Diabetes; HNF4A; Rare variants

Abbreviations: MODY: Maturity-Onset Diabetes of the Young; T2DM: Type 2 Diabetes; MAF: Minor allele Frequency

Introduction

The hepatocyte nuclear factor 4-α *(HNF4*α) gene codes for a transcription factor which is responsible for regulating gene transcription in pancreatic beta cells [1], in addition to its primary role in regulation of hepatic genes [2]. *HNF4*α has also been implicated in the regulation of glucose transport and metabolism [3]. Disruptions in this gene can lead to maturity-onset diabetes of the young (MODY), an uncommon, autosomal dominant, non-insulin dependent form of diabetes [4]. Several disease-causing mutations have been identified across different populations, usually in the form of pedigree studies [5-10]. These variants in *HNF4*α have been reported to be associated with MODY and/or type 2 diabetes (T2DM) [11-20]; however, the effect of these potential mutations in the general population has rarely been replicated [21]. *HNF4*α mutations have primarily been examined in family studies of early-onset T2DM [22-27], which more closely resembles MODY than does later onset T2DM with its associated age and obesity risk factors. Few studies have attempted to look at the influence of *HNF4*α variants on T2DM risk in the general population, and these studies have often examined a limited number of variants [28].

Many of the rare mutations in *HNF4*α have been found in only a few individuals, and only occasionally have they segregated completely with MODY. Due to a similarity of phenotypes, it remains unclear what proportion of T2DM in the general population is due to MODY. In this study we examined an extensive collection of rare and common *HNF4*α variants to assess their prevalence in a large sample T2DM case-control study and assessed their association with T2DM susceptibility.

Materials and Methods

Samples

A total of 1270 European American T2DM patients were studied. This sample consisted of 637 T2DM patients with end-stage renal disease (T2DM-ESRD), 163 T2DM patients without renal disease (T2DM-Only), and 470 unrelated T2DM patients from the Diabetes Heart Study (DHS) [29]. The control samples consisted of 1017 healthy non-diabetic European American individuals, from the same geographic region (southeastern United States) as the case samples. Ascertainment and diagnostic criteria of participants has been previously described in detail [30-32].

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Variant selection

The variants chosen for genotyping were identified through literature and database (Human Gene Mutation Database [33]) searches documenting either MODY or T2DM association.

Genotyping and quality control

Genotyping of the single nucleotide changes and small deletions was performed using the Sequenom Mass Array platform (Sequenom; San Diego, CA). Genotypes of the 6bp deletion [11] (were determined by fragment analysis on an ABI 3700xl (Applied Biosystems; Foster City, CA). ABI Prism GeneMapper v.3.0 software was used for analysis. Discordance between blind duplicate samples included in the genotyping was 0.04%. SNPs and samples which did not meet a 90% call rate were removed from the analysis.

Statistical analysis

The program SNPGWA (http://www.phs.wfubmc.edu/public_bios/sec_gene/downloads.cfm) [34] was used to obtain Hardy-Weinberg Equilibrium p-values, as well as minor allele frequencies, and to test for

Variant	Position[a]	AA[b]	NT[c]	Associated	Study Design	Population	# of ind.[d]	Total Study Size	Reference
Tyr16Term	42417906	16a	C>G	MODY	Unpublished results	UNKNOWN	1 family	UNKNOWN	Ellard, et al 2006[8]
Ser34Term	42468124	43	C>A	MODY	Screening study	European Caucasians	3 (1)	48 HNF1 neg	Pearson, et al 2005[37]
t-del 75	42468247		TtC	MODY	Screening/family study	Danish	6 (6)	20 MODY/10 non-MODY	Moller, et al 1999[38]
aa-del 99	42469466		CaaG	MODY	Single family case study	Swedish		1 family	Lehto, et al 1999[39]
Arg154Term	42475850	163	C>T	MODY	Multi-family study	German	6 (6)	12 families	Lindler, et al 1997[40]
Asp206Tyr	42476711	215	G>T	MODY	Screening study	European Caucasians	5 (5)	48 HNF1 neg	Pearson, et al 2005[37]
IVS-5	42476717		G>A	MODY	Screening study	European Caucasians	1 (1)	48 HNF1 neg	Pearson, et al 2005[37]
Arg244Gln	42481796	253	G>A	MODY	Case study	Japanese	2 (2)	3 (single family)	Hara, et al 2002[36]
Gln268Term	42481867	277	C>T	MODY	Single family case study	US Caucasians	51 (42)	93 (single pedigree)	Yamagata, et al 1996[4]
Glu276Gln	42481893	285	G>C	MODY	Single family case study	UK	7 (5)	12 (single pedigree)	Bulman, et al 1997[41]
Arg301Gln	42486108	310	G>A	MODY	MODY recruitment	Danish	1 family	78 + 351 family members	Johansen, et al 2005[7]
Ile314Phe	42486146	323	A>T	MODY	Screening study	European Caucasians	2 (2)	28 HNF1 neg	Pearson, et al 2005[37]
Val393Ile	42490463	402	G>A	MODY	NIDDM pedigrees	French	1 (1)	19 families	Hani, et al 1998[42]
Pro436Ser	42491627	445	C>T	MODY	Atypical T1D	Mediterraneans	1 (1)	8 atypical T1D	Aguilera, et al 2004[43]
IVS-1	42433044		A>C	T2D	Case/Control	Finnish	MAF 0.34	786 case/619 ctrl	Bonnycastle, et al 2006[15]
P2Del	42463359			T2D	182 Diabetic with Nephropathy	US Caucasian	6 (5)	188 (182 + fam)	Price et al 2000[11]
Gly115Ser	42469514	124	G>A	T2D	Multi-family study	US/Canadian Caucasians	6 (6)	53 families	Malecki, et al 1999[22]
Asp126His	42475765	135	G>C	T2D	EOT2D vs T1D/T2D ctrl	Mexican	1*(1)	40 EOT2D / 20 T1D / 20 T2D	Aguilar-Salinas, et al 2001[23]
Asp126Tyr	42475765	135	G>T	T2D	EOT2D vs T1D/T2D ctrl	Mexican	1*(1)	40 EOT2D / 20 T1D / 20 T2D	Aguilar-Salinas, et al 2001[23]
Thr130Ile	42475778	139	C>T	T2D	Case/control	Japanese	2 (2)	100 case/ 100 ctrl	Sakurai, et al 2000[44]
Arg154Gln	42475849	163	G>A	T2D	EOT2D vs T1D/T2D ctrl	Mexican	1 (1)	40 EOT2D / 20 T1D / 20 T2D	Aguilar-Salinas, et al 2001[23]
IVS-4	42476557		G>A	T2D	Single family case study	Filipino	3 (3)	UNKNOWN	Gragnoli, et al 2004[24]
Val160Ile	42476573	169	G>A	T2D	Single family case study	Filipino	3 (3)	3 (single family)	Gragnoli, et al 2004[24]
Arg324His	42486177	333	G>A	T2D	182 Diabetic with Nephropathy	US Caucasian	1 (1)	182 unrelated	Price, et al 2000[11]
Met364Arg	42486267	373	T>G	T2D	Multi-family study	European Caucasians	5 (5)	15 families (108 individuals)	Pearson, et al 2007[45]
Met 398Thr	42490452	398	T>C	T2D	Screening study	Japanese	1 (1)	74 T2D cases	Fukushima-Uesaka, et al 2006[14]
Ile454Val	42491681	463	A>G	T2D	Multi-family study	US/Canadian Caucasians	4 (3)	53 families	Malecki, et al 1999[22]
Pro430Leu	42491583	430	C>T	N/A					rs6031602

[a]Position refers to the genomic location on Chromosome 20, as determined in HG18/mar2006. [b] Amino Acid change, NP_787110.2 (except for Tyr16Term, which comes from an alternative isoform of the gene: NP_000448.3). [c] Nucleotide change. [d] Number of individuals with the mutation (number of mutation carriers with the phenotype of interest). *These are the same individual, who had two different mutations at the same codon. EOT2D: Early-onset Type 2 diabetes. N/A: not applicable

Table 1: Characteristics of reported *HNF4α* variants.

associations between individual variants and T2DM susceptibility. Due to the small numbers, empirical p-values were estimated by carrying out 10000 permutations of the dataset and assessing the departure of the dataset from expectation. Additionally, a chi-square test of the rare variant burden in cases and controls was performed in GraphPad InStat Version 3.10 (GraphPad Software; La Jolla, CA).

Results

A total of 21 non-synonymous coding variants were selected for analysis, along with 2 small (1-2 base pair) deletions, 3 splice-site modifiers, and a 6 base pair deletion in the P2 promoter of this gene (Table 1). Only two of the polymorphisms were listed in dbSNP and have rs numbers: a splice site alteration in intron 1 (IVS1 = rs6103716) and the threonine to isoleucine at codon 139 (listed as Thr130Ile in the literature) referred to as rs1800961. Table 1 lists the variants, a primary reference and characteristics of the prior variant description. Frequency of the variants ranged from single observations (e.g. Arg154Gln) to low frequency (Thr130Ile). The great majority of variants have been observed only in European-derived samples.

Variants were genotyped in a collection of DNAs composed of 1270 T2DM cases and 1017 controls. Characteristics of the cases and controls (Table 2). Individuals with T2DM had a high BMI and were, on average, diagnosed at an older age than those with T2DM complicated with renal failure. The controls were from the same region, and had lower BMI, were younger, and consisted of more females than the case sample cohorts. The mean age at recruitment for controls (53.9 years) was approximately 5 years later than the mean age of T2DM diagnosis in cases, and well beyond the age at which MODY would have presented. In prior studies we have reported that our sample is of a relatively homogenous European background [35].

A total of 17 variants were found to be monomorphic in this sample (Table 3). Of the six variants that were found to be rare and polymorphic, two (IVS4 and Val393Ile) were found only as heterozygotes in cases only, one (Arg244Gln) was found as a heterozygote in controls only, and two (Thr130Ile and Val454Ile) were found as heterozygotes in both cases and controls. There were two cases that had one copy of a 6bp P2 deletion previously described in our laboratory [13], and one control that had a copy. No deletion-homozygotes were found in either cases or controls. The intron 1 variant (IVS1/rs6103716) was present at a minor allele frequency of 31%. Thr130Ile/rs1800961 was the next most

Cohort	N	BMI (kg/m²)	Age at Recruitment (yrs)	Age at T2DM onset (yrs)	Gender (% Female)
T2DM-ESRD	637	29.57 (7.08)	65.29 (10.38)	45.20 (13.84)	49.5%
T2DM-Only	163	32.03 (7.96)	63.93 (10.24)	49.43 (13.42)	61.5%
DHS	470	32.97 (6.65)	62.96 (8.52)	51.59 (9.32)	51.1%
Total Cases	1270	31.15 (7.22)	64.23 (9.75)	48.14 (12.63)	51.7%
Controls	1017	28.37 (5.67)	53.90 (15.09)	NA	65.7%

Values are presented as: mean (standard deviation) unless otherwise stated

Table 2: Demographic characteristics of the study population.

		Cases			Controls			Chi-Square	HWE
Marker	**Position**	**1/1**	**1/2**	**2/2**	**1/1**	**1/2**	**2/2**	**p-value**	**p-value**
P2Del	42465339	626	2	0	906	1	0	**0.748**	0.967
Tyr16Term	42417906	1174	0	0	972	0	0	NA	1
IVS1	42433044	549	501	112	458	417	90	**0.729**	0.842
Ser34Term	42468124	1174	0	0	972	0	0	NA	1
TDEL	42468247	1175	0	0	971	0	0	NA	1
AADEL	42469466	1175	0	0	972	0	0	NA	1
Gly115Ser	42469514	1175	0	0	972	0	0	NA	1
Asp126	42475765	1174	0	0	970	0	0	NA	1
Thr130Ile	42475778	1091	**71**	**4**	913	**52**	0	**0.149**	0.153
Arg154Gln	42475849	1173	0	0	967	0	0	NA	1
IVS4	42476557	1172	**1**	0	972	0	0	**0.991**	1
Val160Ile	42476573	1173	0	0	972	0	0	NA	1
Asp206Tyr	42476711	1133	0	0	900	0	0	NA	1
IVS5	42476717	1167	0	0	963	0	0	NA	1
Arg244Gln	42481796	1175	0	0	970	**1**	0	**0.991**	1
Gln268Term	42481867	1169	0	0	964	0	0	NA	1
Glu276Gln	42481893	1174	0	0	971	0	0	NA	1
Arg301Gln	42486108	1172	0	0	970	0	0	NA	1
Ile314Phe	42486146	1172	0	0	971	0	0	NA	1
Arg324His	42486177	1168	0	0	972	0	0	NA	1
Met364Arg	42486267	1172	0	0	970	0	0	NA	1
Met398Thr	42490452	1164	0	0	972	0	0	NA	1
Val393Ile	42490463	1133	**1**	0	968	0	0	**0.991**	1
Pro430Leu	42491583	1175	0	0	972	0	0	NA	1
Ile454Val	42491681	1167	**1**	0	959	**1**	0	**0.983**	1

Position refers to the genomic location on Chromosome 20, as determined in HG18/mar2006
1 refers to the major allele, 2 refers to the minor allele.

Table 3: Genotyping results by variant, stratified by cases and controls.

Variant	2 Degree of Freedom test p-value	Empirical 2 Degree of Freedom test p-value	Dominant model p-value	Empirical Dominant model p-value	Additive model p-value	Empirical Additive model p-value	Recessive model p-value	Empirical Recessive model p-value
IVS1	0.897	0.993	0.919	0.996	0.774	0.852	0.643	0.4402
Thr130Ile	0.237	0.0197	0.355	0.0299	0.241	0.0105	0.117	0.0004
IVS4	0.844	0.5	0.680	0.113	0.801	0.5	0.923	0.5
Arg244Gln	0.966	0.5	0.793	0.908	0.803	0.5	0.915	0.5
Val393Ile	0.833	0.5	0.656	0.819	0.777	0.5	0.938	0.5
Ile454Val	0.955	0.5	0.837	0.790	0.901	0.5	0.913	0.5

The empirical p-value is the number of instances that the permuted chi square value exceeds the unpermuted chi square value divided by the total number of permutations. This analysis was performed using 10,000 permutations.

Table 4: Empirical P-values.

	Rare alleles	Non-rare alleles	P-value
Cases	82 (3.6%)	2268	0.22
Controls	54 (2.9%)	1890	

Table 5: Chi-squared analysis of combined rare alleles in cases vs. controls.

common variant (2.6% controls, 3.4% cases). Four homozygotes for the minor allele were present in cases, while none were found in the control sample. The Ile454Val variant was present in both cases and controls, but in small numbers (one in each sample), and as a heterozygote only. The intron 4 variant and the Val393Ile were present in only the cases as heterozygotes (N = 1). Arg244Gln was the only variant typed which was present in only controls as a heterozygote.

None of the variants in this association analysis, including the relatively common Thr130Ile/rs1800961, showed significant evidence of association with T2DM in European Americans. Due to of the challenge of analyzing very low frequency events, permutation analyses were performed with 10,000 permutations as part of the association analysis (Table 4). As the empirical p-value refers to the fraction of times the permuted p-value is less than the unpermuted p-value, it provides support for the accuracy of the p-values for association generated.

The overall burden of mutations between cases and controls were also analyzed for association (Table 5). The cumulative variants in cases and controls were calculated by adding the number of rare alleles in each group and comparing them to the number of non-rare alleles in a chi-square analysis. 82 (3.6%) rare alleles were observed in the cases and 54 (2.9%) in the controls. The chi square test did not show a significant difference between the two (p=0.22).

Discussion

This study evaluated a collective group of reported T2DM and MODY associated mutations in *HNF4*α in a sample of over 2000 European American T2DM cases and controls. Overall there was little evidence that *HNF4*α mutations contribute to T2DM susceptibility in the general population. In the cumulative burden test there was an excess of variants in T2DM cases compared to controls (3.6% versus 2.9%) but statistical significance was not detected (p=0.22). Due to the rarity of most of the variants that were evaluated, even analysis of a substantially larger sample might not result in significant differences. We do suggest, however, that *HNF4*α variants contribute a modest, but tangible, risk for T2DM susceptibility in the population. If the trend observed in this study were to hold, we estimate a sample of over 20,000 would be required to achieve even nominal (p-value ≤0.05) significance. Thus the influence of *HNF4A* is truly modest.

Consistent with these results, prior reports of *HNF4*α analysis have utilized small samples, Jafar-Mohammadi et al. [28] found the more common Thr130Ile variant (approximately 6.5% of the alleles in our sample) to be associated with T2DM in a meta-analysis of 14,279 cases and 26,835 controls, with an additive p-value of 2.1 x 10^{-5} and an odds ratio of 1.20 (95% CI: 1.10-1.30). Although the p-values for association of Thr130Ile with T2DM in this study did not reach the threshold of significance (p=0.23 2DF; p=0.25 under the additive model), the odds ratios are in the same direction and of a similar magnitude for the additive model (OR 1.22; 95% CI: 0.87-1.71). It is also noteworthy that in Jafar-Mohammadi et al. [28] 8 of the 11 cohorts for which genotype information was available did not reach even nominal significance for association, underscoring the modest risk associated with this variant.

Sookoian et al. [20] also included the Thr130Ile variant in their meta-analysis of common *HNF4*α variants. Studies containing Thr130Ile in this analysis came from 5 publications totalling 15,020 T2DM cases and 15,010 controls. They found no evidence of association of the major allele at this variant under a fixed model (p=0.160), although nominal significance was detected under a random model (p=0.045, OR=0.770 (95% CI: 0.595-0.995)). They did clarify that the effect was driven primarily by association in the Japanese and Pima Indian populations (which made up 2 of the 5 and 1 of the 5 datasets in that meta-analysis, respectively).

None of the other variants which were evaluated reached significance in this study. If variants are "private" mutations, i.e. only seen in a single family, population-based studies have little ability to detect meaningful effects. It is particularly of note that some of the variants examined in this study were reported to be MODY causing (Arg244Gln, Ile454Val), while in this study they were found in healthy individuals without diabetes. This suggests that they are not highly penetrant causal variants of MODY.

Also, several of the *HNF4*α mutations evaluated in this study were first identified in individuals of non-European descent (e.g. Arg244Gln, identified in a single Japanese family) [14,23,24,36]. Since the sample studied here was European Americans from the southeast U.S., rare variants observed in other populations are unlikely to be observed. Our results are consistent with this supposition.

In summary, we found that *HNF4*α variants do not contribute significantly to risk of T2DM in this population of European Americans. However, there is an enrichment of rare mutations in T2DM cases compared to controls, which may reflect a modest contribution to risk in the general population. Additionally, the presence of reported MODY-causing mutations in controls suggests that the variants are not highly penetrant.

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