Brief Genetics Report

Lack of Association of the Ala^{45}Thr Polymorphism and Other Common Variants of the NeuroD Gene With Type 1 Diabetes

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Variation in genes necessary for normal functioning and development of β-cells, e.g., NEUROD1, which encodes a transcription factor for the insulin gene and is important for β-cell development, causes maturity-onset diabetes of the young. Some studies have reported an association between a nonsynonymous Ala^{45}Thr (+182G→A) single nucleotide polymorphism (SNP) in NEUROD1 and type 1 diabetes, but this result has not been consistently found. To clarify this, we genotyped Ala^{45}Thr in 2,434 type 1 diabetic families of European descent and Caucasian ethnicity from five different countries. Taking the allele frequency of 36% for Thr^{45} and an odds ratio (OR) of 1.2, this sample provided >99% power to detect an association (P < 0.05). We could not confirm the association (P = 0.77). No evidence of population heterogeneity in the lack of association of Thr^{45} with type 1 diabetes was observed. To evaluate the possibility that another NEUROD1 variant was associated with type 1 diabetes, we resequenced the gene in 32 U.K. affected individuals and identified and genotyped all common SNPs (minor allele frequency >10%; n = 5) in 786 families. We report no evidence of association of these common variants in NEUROD1 and type 1 diabetes in these samples. Diabetes 53: 1158–1161, 2004

The pathogenesis of type 1 diabetes may involve intrinsic functional defects in the insulin-producing β-cells of the pancreas in addition to the extrinsic destruction of these cells by the immune system. Hence, genes that affect β-cell development, function, or apoptosis might conceivably influence susceptibility to type 1 diabetes.

Maturity-onset diabetes of the young (MODY) encompasses a group of single-gene disorders characterized clinically by nonimmune diabetes that develops in the young and is generally inherited in an autosomal-dominant fashion (1). Mutations in genes such as hepatocyte nuclear factor-4α, glucokinase, and hepatocyte nuclear factor-1α, which are necessary for the normal functioning of β-cells, have previously been associated with various subsets of MODY (MODY-1, -2, and -3, respectively). NeuroD is a helix-loop-helix protein that acts as a transcription factor for the insulin gene and plays a pivotal role in the development of pancreatic β-cells (2). Mutations in this gene have been associated with a form of MODY found in Iceland (3). Mice deficient in NeuroD develop severe diabetes and die in the perinatal period with severe hyperglycemia due to the absence of pancreatic β-cells (4). Sequence changes that lead to synthesis of a transcriptionally inactive form of NeuroD have been demonstrated in two families with premature onset of non–immune-mediated diabetes, although not all individuals with these changes developed diabetes (5).

The level of expression of NeuroD influences apoptosis in β-cells; overexpression induces apoptosis in transfected cells (6). β-Cell apoptosis results in the release of numerous autoantigens that may be relevant to the induction of self-tolerance and the prevention of autoimmunity. Genetic variation that increases thymic insulin gene expression correlates with protection against type 1 diabetes, perhaps due to increased tolerance to the insulin autoantigen (7,8). It is therefore possible that polymorphisms in NEUROD1 may affect the development of self-tolerance and hence the genetic predisposition to type 1 diabetes, as well as influence basic β-cell development.

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MODY, maturity-onset diabetes of the young; SNP, single nucleotide polymorphism; TDT, transmission disequilibrium test.

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investigators described a variant in **NEUROD1**.

...in this study, in contrast to the report by Iwata et al. with type 1 diabetes in 138 Danish sibpair families. Of... association between the Ala45Thr variant and type 2 diabetes was observed (online supplementary data, Appendix 1.4 [available at http://diabetes.diabetesjournals.org]).

Interestingly, the gene encoding for this protein has been mapped to chromosome 2q31-q35, where three putative loci have been previously linked to type 1 diabetes (IDDM7, IDDM12, and IDDM13) (9). Mutation scanning in Caucasians followed by case-control and family association studies failed to demonstrate an association between type 1 diabetes and variation in several candidate genes including **HOXD8, NEUROD1**, and IGFFBP5 (10). These investigators described a variant in **NEUROD1** consisting of a G→A transition at the first position of codon 45 in exon 2, resulting in an amino acid substitution (Ala45Thr). The minor (A) allele frequency was 35%. Studies in the Japanese (11) and French (12) populations have failed to show evidence for association of this variant with MODY or type 2 diabetes.

However, Iwata et al. (13) reported an association between this polymorphism and type 1 diabetes in a case-control study in a Japanese population (60 subjects with type 1 diabetes and 174 unaffected control subjects). The relative risk of type 1 diabetes for the variant versus wild homozygote was estimated to be 3.1. A subsequent case-control study performed in a French population with 80% power to detect an effect similar to that reported by Iwata et al. failed to detect an association between the variant and type 1 diabetes (as well as BMI and age at onset of diabetes) (14), although, again, the sample size was small (87 case and 114 control subjects). In another case-control study, Hansen et al. (15) failed to detect an association between the Ala45Thr variant and type 2 diabetes. However, this group reported a positive association with type 1 diabetes in 138 Danish sibpair families. Of note, in this study, in contrast to the report by Iwata et al. (13), the Ala45 NeuroD variant conferred susceptibility to type 1 diabetes, suggesting the possible presence of another (unidentified) functional variant in the gene in linkage disequilibrium with the Ala45Thr polymorphism.

More recently, Cinek et al. (16) in a small case-control study of 285 Czech children with type 1 diabetes and 289 control children reported that the Thr45 NeuroD variant increased susceptibility to type 1 diabetes.

Despite the putative association of this polymorphism with type 1 diabetes, the functional consequences of this polymorphism are unclear, as activation of the human insulin promoter by Thr45 NeuroD does not seem to be different from Ala45 NeuroD (15). This is in marked contrast to the effects of the rare Arg111Leu and His206InsC variants of **NEUROD1** that have been reported to be associated with type 2 diabetes. These variants reduce the activity of the rat insulin-2 promoter in vivo, suggesting interference with the transcription of insulin and other β-cell-specific genes in subjects carrying these variations (5).

**TABLE 1**

Transmission analysis of the Ala45 Thr polymorphism of **NEUROD1** in 2,434 families (sets 1 and 2)

<table>
<thead>
<tr>
<th>DIL reference number</th>
<th>Parental minor allele frequency (%)</th>
<th>TDT</th>
<th>GTRR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>U</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>2402 (Ala45Thr)</td>
<td>36.6</td>
<td>1510</td>
<td>1494</td>
</tr>
</tbody>
</table>

Values for the Thr45 allele from heterozygous parents to affected children with correction for multiple affected siblings. The minor allele frequency is an average from all parents. DIL, Diabetes and Inflammation Laboratory; GTRR, genotype relative risk; T, transmitted; U, untransmitted.

**TABLE 2**

Common polymorphisms identified by resequencing of **NEUROD1** in 32 randomly selected probands

<table>
<thead>
<tr>
<th>DIL reference number</th>
<th>Position relative to initiation codon</th>
<th>IUB code</th>
<th>Minor allele frequency (%)</th>
<th>Within coding sequence?</th>
<th>Sequence context</th>
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</thead>
<tbody>
<tr>
<td>4565</td>
<td>36</td>
<td>r</td>
<td>33</td>
<td>5’ UTR</td>
<td>GCAGGAGGC[A/G]CGGGGTCC</td>
</tr>
<tr>
<td>2402</td>
<td>1799</td>
<td>r</td>
<td>34</td>
<td>Ala to Thr</td>
<td>ACCTCGA[A/G]CCATGAAC</td>
</tr>
<tr>
<td>4566</td>
<td>4571</td>
<td>r</td>
<td>8</td>
<td>No</td>
<td>TGGTCCAG[A/G]AAAAATTTA</td>
</tr>
<tr>
<td>4567</td>
<td>5033</td>
<td>w</td>
<td>8</td>
<td>No</td>
<td>ACATTTGA[A/T]GACTACCC</td>
</tr>
<tr>
<td>4568</td>
<td>7124</td>
<td>s</td>
<td>21</td>
<td>No</td>
<td>CGTGCCCAG[C/G]ATTATGT</td>
</tr>
</tbody>
</table>
type 1 diabetic families. By using a SNP discovery-sequencing panel of 32 affected individuals, we had 88% probability of detecting SNPs with minor allele frequencies of 3.3%, 96% probability for 5% frequency, and 99.8% for 10% frequency.

One variant (DIL4564) in the region 5' to the gene was extremely rare (minor allele frequency <0.001%); association testing was not performed for this SNP. In addition to the Ala456Thr (DIL2402), of the SNPs identified by resequencing, one SNP (DIL4565) was located in the 5' untranslated region, while the remainder were in the region 3' to the gene (Table 2). D' and r^2 values for the common polymorphisms were calculated (online supplementary data, Appendices 1.1 and 1.2).

To evaluate the possibility of other variation in NEUROD1 contributing to the pathogenesis of type 1 diabetes, all other common variants were genotyped using a two-stage approach. This strategy utilizes two subsets of families with type 1 diabetes. One subset is initially a two-stage approach. This strategy utilizes two subsets of diabetes, all other common variants were genotyped using NEUROD1 polymorphisms were calculated (online supplementary data).

***Transmission analysis of DIL4566 in sets 1 and 2 (T 728, U 690: P_{TDT} = 0.31; P_{GTRR} = 0.32). DIL, Diabetes and Inflammation Laboratory, GTRR, genotype relative risk; T, transmitted; U, untransmitted.

<table>
<thead>
<tr>
<th>DIL reference number</th>
<th>Parental minor allele frequency (%)</th>
<th>TDT</th>
<th>GTRR</th>
</tr>
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<tbody>
<tr>
<td>DIL4565</td>
<td>36.4</td>
<td>525</td>
<td>548</td>
</tr>
<tr>
<td>DIL4566</td>
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<td>312</td>
<td>268</td>
</tr>
<tr>
<td>DIL4567</td>
<td>13.0</td>
<td>257</td>
<td>287</td>
</tr>
<tr>
<td>DIL4568</td>
<td>37.6</td>
<td>473</td>
<td>450</td>
</tr>
</tbody>
</table>

**References**

13. Iwata I, Nagauchi S, Nakashima H, Kondo S, Koga T, Yokogawa Y, Akashi T, Shibuya T, Umeno Y, Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraogo Ouedraog...