Sequence Analysis of Candidate Genes for Common Susceptibility to Type 1 and Type 2 Diabetes in Mice

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Abstract. Although type 1 and type 2 diabetes are regarded as clinically distinct diseases, several lines of evidence have suggested common genetic factors between the two types of diabetes. The non-obese diabetic (NOD) mouse, an animal model of type 1 diabetes, and the Nagoya-Shibata-Yasuda (NSY) mouse, a model of type 2 diabetes, are derived from the same outbred colony, Jcl:ICR, suggesting a shared susceptibility between the two types of diabetes in mice. Genetic as well as functional studies have supported the possibility that Tcf2, which encodes the transcription factor, hepatocyte nuclear factor 1β (HNF-1β), is a candidate gene for the common susceptibility between NSY and NOD mice. Tsn, encoding thioredoxin which is a redox (reduction/oxidation)-active protein, is also a positional and functional candidate for a common susceptibility gene. To investigate whether either of these two genes is a common susceptibility gene, the coding nucleotide sequences of these two genes were compared among the NSY, NOD and control C3H strains. The coding sequence of Tcf2 of the NOD mouse was identical to that of the C3H mouse, but was different from that of the NSY mouse. The coding sequence of Tsn was identical in the three strains. These data suggest that neither of the two genes is a common susceptibility gene between type 1 and type 2 diabetes in mice.

Key words: Thioredoxin, Hepatocyte nuclear factor 1β, Genetic susceptibility, Diabetes mellitus

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which spontaneously develops type 1 diabetes due to autoimmune-mediated destruction of pancreatic β cells [11], is also derived from the same outbred Jcl:ICR colony [5]. Genetic analysis demonstrated that diabetes in the NSY mouse is polygenic [6, 12], as in the NOD mouse [11, 13]. Although type 1 and type 2 diabetes may well have different underlying mechanisms leading to hyperglycemia, the common ancestor (Jcl: ICR) between the NSY and NOD strains makes it possible that a shared genetic factor(s) contributes to diabetes development in the two models.

By quantitative trait locus (QTL) mapping, we have previously mapped a major QTL for glucose intolerance in the NSY mouse, Niddln, on mouse chromosome 11 [6]. A support interval for Niddln [6] overlaps the interval where a susceptibility gene for type 1 diabetes, Idd4, was mapped in the NOD mouse [14]. Niddln was shown to be associated with impaired insulin secretion, suggesting that the effect of Niddln is via insulin-producing β cells [6]. The function of Idd4 was reported to be related to metabolic β cell failure under immune attack, but not to autoimmunity itself [14]. Hepatocyte nuclear factor (HNF-1β) is a transcription factor and was suggested to play a role in pancreatic β cells [15]. Tcf2 encoding HNF-1β is located in the interval where Niddln and Idd4 have been mapped [6, 14, 16, 17]. Mutations of the human HNF-1β gene have been shown to cause a monogenic form of type 2 diabetes (maturity-onset diabetes of the young: MODY), which is characterized by impaired β cell function [18]. In addition, recent studies demonstrated that subsets of patients diagnosed as having type 1 diabetes had mutations in genes encoding the HNF family [19]. These results suggest that Tcf2 is a positional as well as functional candidate gene for common susceptibility between type 1 and type 2 diabetes.

Oxidative stress has been shown to be involved in the pathogenesis of type 1 diabetes [20]. Thioredoxin (TRX), a redox (reduction/oxidation)-active protein, has been shown to have strong antioxidative and antiapoptotic activity [21]. We have reported that pancreatic β cell-specific overexpression of TRX prevents the development of type 1 diabetes without affecting insulitis in NOD mice, indicating a protective effect of TRX against the autoimmune destruction of β cells under autoimmune attack [22]. Therefore, it is possible that mutation of the TRX gene (Txn) may contribute to the susceptibility to type 1 diabetes. In addition to the contribution of oxidative stress to type 1 diabetes, recent studies have implicated oxidative stress in the dysfunction of β cells in type 2 diabetes. Ihara et al. reported that pancreatic β cells from type 2 diabetic rats were subjected to oxidative stress [23], and Kaneto et al. that antioxidant treatment was shown to provide beneficial effects in preventing β cell dysfunction in type 2 diabetes in mice [24]. Therefore, dysfunction of antioxidative molecules, such as TRX, may be involved in the pathogenesis of not only type 1, but also type 2 diabetes. Recently, Txn was shown to be located on mouse chromosome 4 [25], where a QTL for glucose intolerance in NSY mice (Ueda H, et al., unpublished data) has been mapped. Since type 1 diabetes susceptibility loci, Idd9 and Idd11, have also been mapped to chromosome 4 in NOD mice [26, 27], Txn can be regarded as a functional and positional candidate gene for common susceptibility between type 1 and type 2 diabetes in mice.

In this study, to test whether Tcf2 or Txn is a common susceptibility gene for type 1 and type 2 diabetes in mice, we determined the nucleotide sequences of these two candidate genes in animal models of type 1 (NOD) and type 2 (NSY) diabetes.

**Materials and Methods**

Total RNA, extracted from a kidney of an NOD mouse, was subjected to construction of cDNA using cDNA Synthesis System (Gibco BRL, Life Technologies, Inc., Rockville, MD, USA). The cDNA was used as a template for RT-PCR as reported previously [8], and the cDNA sequence of the NOD mouse was determined. Briefly, six segments overlapping the full length of cDNA were amplified by RT-PCR (Fig. 1). Primers were designed according to the published sequence of murine Tcf2 cDNA [28] (Table 1). RT-PCR was performed in 10 μl 10 mM Tris-HCl [pH 8.0], 50 mM KCl, 200 μM of each dNTP, 0.132 μM of each primer, and 0.25 U AmpliTaq DNA polymerase (Perkin-Elmer, Foster City, CA, USA) with 1.5 mM MgCl2. A DNA thermocycler (Perkin-Elmer) was used for the reaction. PCR conditions were as follows: 3 min at 94°C, 40 cycles of 30 sec at 94°C, 1 min at 50°C, 1 min at...
72°C, followed by an elongation step for 7 min at 72°C. PCR products were subcloned into pT7Blue Vector (Novagen, Madison, WI, USA) and subjected to DNA sequencing, and analyzed on an ABI377 sequencer (Perkin-Elmer).

Genomic DNA was prepared by a standard phenol/chloroform method. Our previous analysis of the nucleotide sequence of Tcf2 in the NSY mouse revealed a single nucleotide polymorphism (SNP) (A664G) leading to the amino acid substitution, threonine to alanine, at codon 222 (A1a222Thr) [6]. To investigate the A1a222Thr polymorphism, PCR-RFLP method was adopted in the following strains; NSY, NOD, C3H in addition to C57/BL6 (B6), C57/BL10 (B10). The PCR products were digested with restriction enzyme Hin1I, which cleaves the NSY allele into two bands, but not the wild-type allele [6]. To determine haplotypes around Tcf2, genotypes of three microsatellite markers, D11Mit320, D11Mit195, D11Mit286 (MapPair™; Research Genetics, Huntsville, AL, USA), were determined by PCR based on the protocol for MapPair™. The PCR products were electrophoresed using 9% polyacrylamide gel and then visualized by ethidium bromide staining.

To determine the nucleotide sequence of the entire coding region of Txn, genomic DNA was subjected to PCR amplification. Five sets of primers were designed to cover the five exons of Txn (Table 2), according to the published genomic sequences of the 129/J strain [29]. The PCR products were purified using a QIAquick™ Gel Extraction Kit (Qiagen, Tokyo, Japan) and directly sequenced using an ABI 377 sequencer (Perkin-Elmer).

### Results

The coding sequence of Tcf2 of the NOD mouse was identical to that of the C3H mouse (Table 3), but different from that of the NSY mouse which has an Ala allele [6] instead of a Thr allele at codon 222. The SNP (Thr222Ala) was also tested by PCR-RFLP method (Fig. 2). The variant was observed only in the NSY mouse, but not in the NOD mouse or the
B10 mouse, whose genetic background had been used for mapping Idd4, indicating that the Tcf2 sequence does not vary between the NOD and B10 strains. In contrast, haplotype analysis revealed that microsatellite markers around Tcf2 were polymorphic between the NOD and B10 mice (Table 4).

Regarding the coding region of Txn, the nucleotide sequences were identical in the three strains studied, NOD, NSY and C3H mice. The sequence was also identical to the previously published sequence from a control strain, 129/J [29].

**Discussion**

In the present study, we analyzed the coding sequences of the genes encoding HNF-1β and thioredoxin, both of which are positional and functional candidate genes for common susceptibility between type 1 and type 2 diabetes. The coding sequence of Tcf2 of the NOD mouse was identical to that of the control C3H mouse, but was different from that of the NSY mouse. The Txn coding sequence was identical among the strains analyzed. These data suggest that neither of the two genes is a common susceptibility gene between type 1 and type 2 diabetes in mice.

A region of chromosome 11 encompassing Tcf2 is shown to possess a susceptibility gene for type 2 diabetes (Nidd1n) [6] as well as one for type 1 diabetes (Idd4) [14]. Functional alterations of both Nidd1n [6] and Idd4 [14] were suggested to exist in insulin-producing β cells in the pancreas. Tcf2 is expressed in the pancreas and a β cell line [6], and HNF-1β, encoded by Tcf2, was shown to play a role in pancreatic β cells [15]. Therefore, Tcf2 is a strong candidate for a common susceptibility gene between type 1 and type 2 diabetes. Regarding the Thr222Ala polymorphism of Tcf2, however, the substitution observed in the NSY mouse was not found in the NOD mouse, indicating that the Thr222Ala polymorphism itself is not a common variant responsible for the susceptibility to type 1 and type 2 diabetes in mice. Since codon 222 is located in the DNA binding domain of HNF-1β [30], and alanine and threonine differ in amino acid properties, we cannot rule out the possibility of the direct involvement of this variant in the pathogenesis of type 2 diabetes in the NSY mouse.

The present study demonstrated that the coding sequence of Tcf2 of the NOD mouse is identical to that of the control C3H mouse, as well as the published sequence of the B6 strain [Mouse Genome Database]. In the initial mapping study for Idd4 [14], the NOD mouse was mated with a congenic strain B10.H-2^nod, and thus the gene responsible for Idd4 must be allelically variant between the NOD and B10 strains. B6 and B10 mice are genealogically close to each other [31], and the genetic markers surrounding Tcf2 as well as the Thr222Ala variant were identical in B6 and B10 mice (Table 4 and Fig. 2), suggesting that the coding sequence of Tcf2 is identical in the B6 and B10 strains. Taken together, the Tcf2 sequence of the NOD mouse is likely to be identical to that of the B10 mouse, whose genetic background was used for mapping Idd4 [14], suggesting that the Tcf2 coding sequence is not responsible for Idd4.

Our present data demonstrated that the coding sequence of Txn does not have any allelic variant among the mouse strains investigated. In addition, comparison of the coding sequence of mouse Txn with that in other species demonstrated high homology across mammalian species (99.0% with rat, and 95.6% with human). The less polymorphic nature in mice as well as the conserved nature of the sequences across the species may reflect the importance of the thioredoxin structure in mammals. Since thioredoxin is shown to be an inducible protein in β cells [32], its expression level may well differ according to stimuli. Therefore, the promoter region of Txn is a theoretical candidate for possessing polymorphism(s) related to an alteration of transcriptional regulation.

**Table 2.** Primer sequences used for sequencing the coding region of Txn

<table>
<thead>
<tr>
<th>Exon</th>
<th>GenBank Accession No.</th>
<th>Primer sequences (5’-3’*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D21855</td>
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<tr>
<td>2</td>
<td>D21856</td>
<td>Forward: TGTTCTTTTCCCTCTTGA &lt;br&gt; Reverse: CTCCTCGTTCATCTGACT</td>
</tr>
<tr>
<td>3</td>
<td>D21857</td>
<td>Forward: ACCTTTTCTTGTGTTTTGA &lt;br&gt; Reverse: CATCCGATTTCCGTACTACG</td>
</tr>
<tr>
<td>4</td>
<td>D21858</td>
<td>Forward: GTTTGTTTTCCTCTCTC &lt;br&gt; Reverse: TCCATCTGATCTGGCTTGG</td>
</tr>
<tr>
<td>5</td>
<td>D21859</td>
<td>Forward: ATAGCCCTTTTATCTCCTCA &lt;br&gt; Reverse: ACACATCAGGCGATTGT</td>
</tr>
</tbody>
</table>

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Table 3. cDNA sequence of mouse Tcf2, nucleotide 355 through 775 (Seg. 2 in Fig. 1)

<table>
<thead>
<tr>
<th></th>
<th>B6 (consensus)</th>
<th>C3H</th>
<th>NOD</th>
<th>NSY*</th>
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<td>C3H</td>
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<tr>
<td>NSY*</td>
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<tr>
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<td>NOD</td>
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<tr>
<td>NSY*</td>
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<tr>
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<td></td>
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<tr>
<td>C3H</td>
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<tr>
<td>NOD</td>
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<td></td>
</tr>
<tr>
<td>NSY*</td>
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</table>

Fig. 2. Thr222 Ala variant of Tcf2 in inbred strains of mice (PCR-RFLP). M, molecular size marker (cX174-HaeIII digest); lane 1, NSY; lane 2, C3H; lane 3, C57BL/6; lane 4, C57BL/10; lane 5, NOD.

Table 4. Microsatellite polymorphism around Tcf2

<table>
<thead>
<tr>
<th>Position</th>
<th>Marker</th>
<th>NSY</th>
<th>C3H</th>
<th>C57/BL6</th>
<th>C57/BL10</th>
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<tbody>
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<td>39.3</td>
<td>D11Mit320</td>
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<td>*124</td>
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<td>43.7</td>
<td>D11Mit193</td>
<td>120</td>
<td>*132</td>
<td>136</td>
<td>136</td>
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<tr>
<td>44.0</td>
<td>Tcf2</td>
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<tr>
<td>49.2</td>
<td>D11Mit286</td>
<td>124</td>
<td>*108</td>
<td>*124</td>
<td>124</td>
<td>*124</td>
</tr>
</tbody>
</table>

*Ref. 6. The nucleotide sequence data in NOD mouse reported in this paper will appear in the DDBJ, EMBL and Genbank nucleotide sequence databases with the following accession number: AB 052659.

Boldface region indicates the codon 222. Dash indicates identity with B6 sequence.
This is also compatible with observations indicating that the 5' untranslated regions are a target for genetic analysis besides coding regions [33]. Further investigations are needed to clarify the possible contribution of the regulatory region of Txn to susceptibility to diabetes. Since a sequence variant was observed in the 5' untranslated region of Tcf2 in the present study, further studies are also needed to clarify the contribution of sequence variants in the 5' untranslated region and other regulatory regions of Tcf2 to susceptibility to diabetes as well.

Acknowledgements

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References

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