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A functional variant of IRS1 is associated with type 1 diabetes in families from the US and UK

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Abstract

A collection of 767 multiplex type 1 diabetes families from the US and UK were tested for linkage to the IRS1 gene and for allelic association with a specific variant of IRS1, G972R. Pedigree disequilibrium testing revealed preferential transmission of the 972R allele to affected offspring in these families ($P = 0.02$). Linkage analyses conditioning on status at IRS1 position 972 suggest the possibility of interaction with an unidentified locus on chromosome 8.
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Introduction

Type 1 diabetes (T1D) arises from autoimmune destruction of the insulin-secreting β cells of the pancreas resulting in a complete dependence on exogenously administered insulin. Both family and twin studies suggest that a portion of the risk for T1D is genetically determined [1,2]. Given its autoimmune etiology, candidate T1D susceptibility genes have typically been studied based on their roles in the immune system. Genes with primarily metabolic roles, such as in insulin signaling or secretion, are more commonly considered as candidates for insulin resistant syndromes such as type 2 diabetes. One such gene is IRS1, whose product plays a critical role in signal transduction by the insulin receptor and is associated with an increased risk of type 2 diabetes [3].

In a recent study of Italian T1D patients, Federici et al. [4] observed an increased frequency of carriers of a specific variant of IRS1, G972R, relative to controls. G972R carriers have reduced fasting insulin and C-peptide levels, suggesting an effect of this variant on glucose stimulated insulin secretion [5–7]. In the current study, a large cohort of T1D multiplex families ascertained in the US and UK and previously studied by genome-wide scanning for linkage to T1D [8], were genotyped for the IRS1 G972R variant in order to test the reported association with T1D in an independent population by transmission analysis as well as to explore possible statistical interactions with other reported T1D susceptibility loci.

Materials and methods

The cohort of T1D affected sib pair families used in the current study has been previously described [8]. These families were obtained from the Human Biological Data Interchange (HBDI) ($N = 411$) and diabetes UK Warren I ($N = 356$) repositories. All families studied were of white European ancestry and were ascertained in the US or UK. The median age at onset of T1D in this population was 12 years with a range of 2 months to 36 years. Genome scan data from 767 families were used in the current analysis. Genotyping for the IRS1 G972R variant was performed on 510 of these families for which DNA was available.

The IRS1 G972R variant was genotyped by the single-strand conformation polymorphism (SSCP) method [9]. Genomic DNA (20 ng) was amplified with the following primers 5′-GGA AGA GAC TGG CAC TGA

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GG and 5′-CTG ACG GGG ACA ACT CAT CT. Products were radiolabeled during amplification by incorporation of [^{33}P]dATP and separated on 1X mutation detection enhancement (MDE) gels (Cambrex Bio Science, Rockland, Maine) supplemented with 5% glycerol. Genotypes were confirmed for representative samples of each genotype by nucleotide sequencing of the amplification products.

Multipoint linkage analyses were performed using the S (pairs) option of Genehunter Plus and maximized LOD scores were calculated using an exponential model with δ constrained between 0 and 2 [10]. Subset analyses were performed by assigning individual families weights 1 or 0, and recalculating LOD scores with these weights after the initial linkage analysis was complete. Transmission of the 972R variant to affected offspring in T1D families was assessed using the pedigree disequilibrium test (PDT), a variant of the transmission/disequilibrium test (TDT) that correctly handles data derived from pedigrees containing multiple affected individuals [11,12].

Results

An examination of LOD scores on the long arm of chromosome 2 for linkage to T1D in 767 multiplex families from the US and UK revealed little evidence of linkage in the region of the IRS1 gene (LOD < 0.1). Linkage was further assessed in subsets of families characterized by high or low human leukocyte antigen (HLA) encoded risk. Neither a high-risk subset, consisting of 159 families in which all affected individuals were heterozygous for the combination of the HLA DRB1*0401—DQB1*0302 and DRB1*0301—DQB1*0201 haplotypes, nor a low-risk subset, including 35 families where affected siblings had neither the DR4 or DR3 risk haplotypes ($N = 19$), or were discordant for allele sharing at HLA ($N = 16$), displayed any increased evidence of linkage to T1D in the region containing IRS1.

A total of 2118 individuals from 510 multiplex type 1 diabetes families were genotyped for the G972R variant. The 972R allele was preferentially transmitted to affected offspring from heterozygous parents (56% of 309 transmissions, $\chi^2 = 5.3$, $P = 0.02$). Among families with parents informative for transmission of this variant, there were no significant differences between affected offspring to whom the 972R or the 972G alleles were transmitted in either age at disease onset or HLA genotype distribution. The mean age at onset among individuals inheriting the 972R allele was 9 years as compared to 10 years among those inheriting the 972G allele. HLA-DR3/4 heterozygous individuals were the most common in both groups, constituting 47% of the 972R population and 46% of the 972G population.

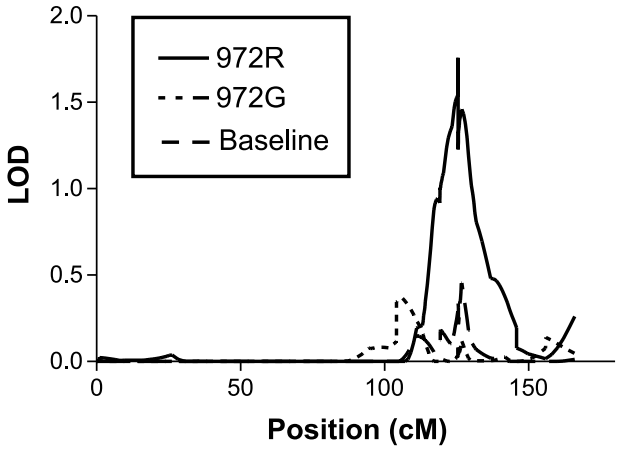

Fig. 1. Multipoint analysis of linkage to T1D on chromosome 8 conditional on IRS1 status. LOD scores are plotted against chromosomal position in centimorgans. Solid line indicates the LOD score in families where any affected member inherited the 972R allele. Fine dashed line indicates LOD scores in families where all affected member inherited the 972G allele. Long dashed line indicates the baseline LOD score in all families genotyped for IRS1.

If the 972R IRS1 variant is contributing to susceptibility to T1D, then families containing 972R positive affected individuals could represent a more homogeneous subset in which to assess the evidence of linkage to T1D. Accordingly, the existing genome-scan data was re-analyzed in three subsets: the group of all 510 families genotyped for IRS1 as a baseline, the 91 families with at least one affected individual carrying the 972R allele, and the 419 families in where all members had the G allele at position 972. For most regions of the genome, the magnitude of the LOD scores obtained was proportional to the sizes of the subsets analyzed, with two exceptions. On chromosome 6q, in the region of the putative *IDDM5* locus, the LOD in the 972R subset was 2.15, while the 972G and baseline LODs were 0.6 and 1.54, respectively. On chromosome 8 (Fig. 1), the LOD in the 972R subset was 1.76 while the LODs in the other subsets were less than 0.3.

Discussion

Given its function in insulin signaling, IRS1 seems an unlikely candidate for a gene involved in susceptibility to T1D. The 972R variant of IRS1 is associated with a moderate impairment of insulin secretion in β cells [5–7], but this characteristic seems more consistent with the phenotype of type 2 diabetes rather than T1D. Federici et al. [4] proposed that the 972R variant might sensitize β cells to apoptosis resulting in the release of potential neo-antigens that might elicit an autoimmune response in the context of a predisposed host immune system. Indeed, 972R islets do contain significantly increased numbers of apoptotic cells and this might contribute to

β cell loss during an ongoing autoimmune process [13]. An alternative, although not mutually exclusive, possibility is that the 972R variant might have effects on lymphocyte differentiation and development that predispose to autoimmunity. IRS1 is expressed in T lymphocytes [14,15] and, while IRS1 knockout mice do not have any gross immunologic abnormalities [16], IRS1 is phosphorylated in response to stimulation with the cytokine IL-4 [17]. IL-4 is a potent inducer of T helper 2 (Th2) lymphocyte differentiation and stimuli that favor Th2 lymphocyte expansion and function are generally protective for T1D [18]. Thus, a defect in IRS1 signaling might act to shift the Th1/Th2 balance of lymphocytes in favor of autoimmunity. In the current study, a large cohort of multiplex T1D families from the US and UK was tested for linkage to IRS1 and/or association with a specific variant, 972R. Although no evidence of linkage to IRS1 was detected, this may reflect the low frequency (8%) of the putative risk allele in this population. PDT analyses in these families revealed preferential transmission of the 972R allele to affected offspring, confirming the previously reported association with T1D and extending it to other populations of European origin. The low frequency of the 972R allele, combined with the modest, but significant, 56% transmission rate means that to attain genome-wide levels of significance for this finding will require much larger numbers of T1D families than are currently available for study worldwide.

A benefit of the family-based approach used here is the availability of existing genome-scan data, allowing testing for interaction with other reported T1D susceptibility loci. Such interactions, if detected, might help to shed light on the mechanism whereby this particular variant could contribute to T1D susceptibility. Although no evidence of interaction with any previously reported locus was detected, increased LOD scores were observed on chromosome 8 in the subset of families in which the 972R allele was segregating. Given the overall magnitude of the LODs observed in this subset analysis, and the need for caution when multiple hypotheses are being tested, this result should be considered preliminary. Larger family collections, such as that being assembled by the T1D genetics consortium (T1DGC) [19] should provide sufficient numbers of transmissions, even for an infrequent variant like the one studied here, to allow robust subset analysis.

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