Brief Genetics Report

Analysis of the Type 2 Diabetes–Associated Single Nucleotide Polymorphisms in the Genes IRS1, KCNJ11, and PPARG2 in Type 1 Diabetes


It has been proposed that type 1 and 2 diabetes might share common pathophysiological pathways and, to some extent, genetic background. However, to date there has been no convincing data to establish a molecular genetic link between them. We have genotyped three single nucleotide polymorphisms associated with type 2 diabetes in a large type 1 diabetic family collection of European descent: Gly972Arg in the insulin receptor substrate 1 (IRS1) gene, Glu23Lys in the potassium inwardly rectifying channel gene (KCNJ11), and Pro12Ala in the peroxisome proliferative–activated receptor γ2 gene (PPARG2). We were unable to confirm a recently published association of the IRS1 Gly972Arg variant with type 1 diabetes. Moreover, KCNJ11 Glu23Lys showed no association with type 1 diabetes (P > 0.05). However, the PPARG2 Pro12Ala variant showed evidence of association (RR 1.15, 95% CI 1.04–1.28, P = 0.008). Additional studies need to be conducted to confirm this result. Diabetes 53:870–873, 2004

Type 1 and 2 diabetes have been considered genetically and pathophysically distinct diseases, although clinically, it can sometimes be difficult to distinguish between them in individuals with deficient or absent endogenous insulin secretion, a feature common to both categories. Evidence does exist suggesting that there may be shared etiological features (1–3). Occasionally, individuals who develop diabetes late in life initially present in a similar manner to type 2 diabetes, but develop progressive β-cell failure together with evidence of autoimmunity to islet cells, which is characteristic of type 1 diabetes. This so-called latent autoimmune diabetes in adults could suggest an overlap in the pathogenesis of type 1 and 2 diabetes in some cases. Furthermore, a recent study (4) has reported that early in the course of type 1 diabetes, individuals with evidence of islet autoimmunity may have normal fasting glucose but abnormal postprandial glucose tolerance. This pattern of defective postprandial secretion of insulin in response to nutrient intake is often observed in type 2 diabetes as well (4). There has been some evidence of familial clustering of type 1 and 2 diabetes (5,6). However, the HLA region, the major locus in type 1 diabetes, has only infrequently been associated with type 2 diabetes (7). Moreover, both diseases have been associated with variation in the insulin gene but with predisposition determined by opposite alleles of the 5′ variable number tandem repeat locus (8). Recently, genetic studies in type 2 diabetes have begun to reveal disease-associated polymorphisms that have established some level of general acceptance because they have been replicated by independent studies. Two polymorphisms that are well established as associated with type 2 diabetes are the single nucleotide polymorphisms (SNPs) Pro12Ala in the peroxisome proliferator–activated receptor γ gene isoform 2 (PPARG2), resulting from a C>G transversion, and Glu23Lys in the potassium inwardly rectifying channel gene (KCNJ11), resulting from an A>G transition (9). The reported effect sizes are modest (odds ratio [OR] 1.2–1.3) in meta-analyses (9,10). A large collaborative collection of type 1 diabetic families is now avail-
able for genetic analysis (11) with sufficient power to detect effects in the OR range of 1.2–1.3. Hence, in the present report we investigated the possibility that the two type 2 diabetes–associated SNPs were also associated with type 1 diabetes in an effort to begin to establish if overlaps exist in the two disease pathways. We have also tested the insulin receptor substrate 1 (IRS1) Gly972Arg polymorphism because it has been reported to be associated with type 1 diabetes (12), although it is not as well established in type 2 diabetes as the PPARG2 and KCNJ11 SNPs.

The PPARG gene encodes peroxisome proliferator–activated receptor γ (PPARγ), a member of the nuclear receptor superfamily that has regulatory functions in adipocyte differentiation and glucose homeostasis (13). PPARγ is a target for a large family of antidiabetic drugs, the thiazolidinediones, that act as PPARγ agonists and increase hepatic and peripheral insulin action in type 2 diabetes (14). In a meta-analysis of published studies through February 2003 (9), an OR of 1.27 was estimated for the Pro allele of isoform 2 (P value <2 × 10⁻⁸) in type 2 diabetes. This association is supported by functional data, as in vitro studies have shown that the Ala allele decreases the DNA-binding affinity of the PPARγ2, thus reducing its transcriptional activity (15).

The KCNJ11 Gly972Lys variant has also been consistently reported (10,16,17) to be associated with type 2 diabetes. KCNJ11 encodes for Kir6.2, which comprises one of the two subunits of the β-cell ATP-sensitive potassium channel that regulates insulin secretion. A meta-analysis combining published case-control studies up to September 2002 (10) gave an OR of 1.23 (95% CI 1.12–1.36, \( P = 1.5 \times 10^{-5} \)) for the Lys allele, suggesting that this allele is associated with type 2 diabetes. In addition, functional data shows that the Lys variant may alter type 2 diabetes susceptibility by increasing the threshold ATP concentration necessary for insulin release, thus inducing spontaneous overactivity of pancreatic β-cells (18). Increasing the metabolic activity of β-cells might confer susceptibility to type 1 diabetes as well (19).

The insulin receptor substrate proteins (IRS1 and IRS2) are expressed in a variety of insulin responsive cells and tissues, mediating metabolic and growth-promoting actions of insulin and IGF-1. Observations from knockout mice suggest a role for these proteins in regulation of β-cell function (20), and the human IRS1 Gly972Arg variant has been reported to be associated with type 2 diabetes (9). Meta-analysis, based on 3,408 case subjects and 5,419 control subjects from studies before January 2002 (21), resulted in an OR of 1.25 (95% CI 1.05–1.48). Of direct relevance to our study is a recent study (12) reporting an association of this variant with type 1 diabetes, with an OR of 2.5 (\( P = 0.0008 \)) in a case-control study and a transmission/disequilibrium test (TDT) in simplex families giving a P value <0.02.

Therefore, we genotyped these three polymorphisms in 2,434 type 1 diabetic families of European descent. Parental genotype frequencies were found to be consistent with Hardy-Weinberg equilibrium. TDT and conditional logistic regression analyses were performed, and the results are summarized in Table 1. In this large dataset of type 1 diabetic families, we were unable to confirm the reported association of IRS1 (12), although we had ≥95% power to detect the reported effect (OR 2.5, \( P = 0.0008 \), minor allele frequency = 8%). Similarly, the KCNJ11 Gly972Lys variant was not associated with type 1 diabetes, as a nonsignificant deviation from 50:50 transmission from heterozygous parents was observed.

The PPARG2 Pro12Ala SNP, however, showed some evidence of association with the more common Pro allele conferring risk to type 1 diabetes (relative risk [RR] 1.15, 95% CI 1.04–1.28, \( P = 0.008 \)), as is the case for type 2 diabetes. TDT results indicated a transmission to affected individuals of 54% (769 of 1,437 informative transmissions, \( P = 0.008 \)). To confirm that this result was not due to genotyping error, we regenotyped the PPARG2 Pro12Ala SNP on the reverse strand and obtained 99.77% concordance. In agreement with the reports in type 2 diabetes, the minor allele (G) is protective in type 1 diabetes. Genotype RRs were calculated by conditional logistic regression analysis for the Pro12Ala SNP, giving values of 0.85 (0.76–0.95, \( P = 0.005 \)) and 0.87 (0.61–1.24, \( P = 0.44 \)) for the C/G and G/G genotypes, respectively, relative to the C/C genotype. Conversely, relative to the G/G genotype, the C/G and C/C genotypes have RRs of 0.98 (0.69–1.39) and 1.15 (0.81–1.64), respectively. The nonsignificant result for the G/G genotype (and the risks relative to the G/G genotype) is likely a result of its low frequency.

Our failure to replicate the Italian study (12), which reported an association between IRS1 Gly972Arg and type 1 diabetes, is perhaps not surprising given the modest sample size previously studied. Nevertheless, we genotyped this SNP with two technologies, as family studies of rare variants may be compromised because apparent undertransmission of alleles can be observed due to genotyping errors. A concordance rate of 99.74% was observed between genotypes generated by the two meth-

### Table 1

<table>
<thead>
<tr>
<th>SNP</th>
<th>Reported risk allele for type 2 diabetes</th>
<th>Frequency (%)</th>
<th>TDT</th>
<th>CLR</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRS1 Gly972Arg</td>
<td>Arg</td>
<td>5.9</td>
<td>312</td>
<td>0.77</td>
</tr>
<tr>
<td>KCNJ11 Glu923Lys</td>
<td>Lys</td>
<td>42</td>
<td>1268</td>
<td>0.72</td>
</tr>
<tr>
<td>PPARG2 Pro12Ala</td>
<td>Pro</td>
<td>86</td>
<td>769</td>
<td>0.018</td>
</tr>
</tbody>
</table>

\( P \) values were calculated based on the null hypothesis of no association of the polymorphism. Conditional logistic regression (CLR) \( P \) values are for \( \chi^2 \) on 2 degrees of freedom; RRs are for the reported risk allele. Frequency given is the frequency in unaffected parents of the reported risk allele for type 2 diabetes. *Successfully typed in 2,161 families (2,887 affected offspring). †Successfully typed in 2,090 families (2,734 affected offspring). ‡Successfully typed in 2,355 families (3,157 affected offspring). T, transmitted; U, untransmitted.
odds. It is unlikely that this common SNP could be genetically associated with type 1 diabetes in central Italy and not in other European countries. In these data we found no evidence of population heterogeneity ($\chi^2 [4 \text{ df}] = 1.93, P = 0.75$, in five populations). Hence, our study highlights the importance of large datasets to investigate the multigenic basis of type 1 diabetes, type 2 diabetes, and other common multifactorial diseases.

If the **PPARG2** Pro12Ala association is confirmed in other studies, then a molecular link would be established between type 1 and 2 diabetes involving a protective effect of the minor allele (Ala). Other studies have reported that thiazolidinediones possess anti-inflammatory properties and decrease diabetes incidence in nonobese diabetic (NOD) mice (22,23). Prevention of autoimmune diabetes in NOD mice by thiazolidinediones has been associated with suppression of intercellular adhesion molecule 1 (ICAM1) expression and alteration of Th1/Th2 cytokine balance (24). Interestingly, we have recently reported (25) an association of the Gly241Arg polymorphism of **ICAM1** with type 1 diabetes. It is possible that **PPARy** agonists may be worth testing in prevention of immune rejection of transplanted islets.

**RESEARCH DESIGN AND METHODS**

All families were Caucasian of European descent and were composed of two parents and at least one affected child. The families comprised up to 458 Diabetes U.K. Warren I multiplex from the U.K. (26), 326 U.S. multiplex from the Human Biological Data Interchange (27), 80 Yorkshire simplex from the U.K., 250 Belfast multiplex/simplex (28) from the U.K., 159 Norwegian simplex, 233 Romanian simplex, and 926 Finnish multiplex/simplex (29). All families were Caucasian of European descent and were composed of two parents and at least one affected child. The families comprised up to 458 Diabetes U.K. Warren I multiplex from the U.K. (26), 326 U.S. multiplex from the Human Biological Data Interchange (27), 80 Yorkshire simplex from the U.K., 250 Belfast multiplex/simplex (28) from the U.K., 159 Norwegian simplex, 233 Romanian simplex, and 926 Finnish multiplex/simplex (29). All DNA samples were collected with appropriate ethical approval and informed consent.

**Genotyping.** SNPs were genotyped by the TaqMan 5’ nuclease assay according to the manufacturer’s instructions (Applied Biosystems, Warrington, U.K.). TaqMan primers and probes were designed by Applied Biosystems. The **PPARG2** Pro12Ala SNP was independently typed by the TaqMan 5’ nuclease assay on both strands. **IBS1** Gly972Arg was also genotyped by restriction enzyme digest with Xmal, incorrectly reported as Mvel in Federici et al. (12). All genotyping data were double scored to minimize error.

**Statistical analysis.** All statistical analyses were performed in Stata (http://www.stat.com) using the Genassoc package (http://www-gene.cimr.cam.ac.uk/clayton/software/stata). A modified test was used to evaluate Hardy-Weinberg equilibrium that allows for allelic frequencies to differ between non-insulin dependent diabetes mellitus and glucose intolerance are located in HLA region. **BMJ** 5:777–779, 1993


21. Jellena A, Zeegers MP, Feskens EJ, Dagnelie PC, Mensink RP: Gly972Arg variant in the insulin receptor substrate-1 gene and associ-


