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The codon 620 Tryptophan Allele of the Lymphoid Tyrosine Phosphatase (LYP) Gene is a major determinant of Graves' Disease

MR VELAGA, V WILSON, CE JENNINGS, CJ OWEN, S HERINGTON, PT DONALDSON, SG BALL, RA JAMES, R QUINTON, P PERROS, SHS PEARCE

Institute of Human Genetics (MRV, VW, CEJ, CJO, SHSP) and School of Clinical Medical Sciences (CJO, SH, PTD, RAJ, RQ, SGB, PP, SHSP), University of Newcastle, Newcastle upon Tyne, UK.

ABSTRACT The lymphoid tyrosine phosphatase (LYP), encoded by the protein tyrosine phosphatase-22 (*PTPN22*) gene, is a powerful inhibitor of T cell activation. Recently, a single nucleotide polymorphism (SNP), encoding a functional arginine to tryptophan residue change at LYP codon 620 has been shown to be associated with type 1 diabetes and other autoimmune disorders. We have used a PCR-restriction fragment (*XcmI*) assay to examine genotypes at the codon 620 polymorphism in 549 unrelated probands with Graves' disease, 104 unrelated subjects with autoimmune Addison's disease and 429 controls. The T nucleotide at the SNP, encoding the tryptophan 620 residue, was present in 151 of 1098 (13.8%) Graves' disease alleles compared to 67 of 858 (7.8%) control alleles (χ^2=17.2, p=3.4x10^{-5}; odds ratio = 1.88, 5- 95% confidence intervals [CI] 1.39 to 2.55). Similarly, the T nucleotide at the codon 620 SNP was present in 26 of 208 (12.5%) Addison's disease alleles vs 7.8% of controls (χ^2=4.63, p=0.031; odds ratio= 1.69, 5- 95% CI 1.04 to 2.73). These data suggest that this LYP polymorphism is a susceptibility allele for Graves' disease with a major effect, and which is likely to have a role in many other autoimmune conditions.

Graves' disease (GD) and autoimmune Addison's disease (AAD), in common with most other autoimmune disorders, have a substantial genetic component to their pathogenesis (1). Data from twin studies suggest that about 80% of the susceptibility to GD is determined by genetic factors (2), with the remainder being influenced by environmental or other factors. Only two loci, namely the cytotoxic T lymphocyte antigen-4 (*CTLA4*) gene and the major histocompatibility complex (*MHC*) have shown unequivocal evidence for a role in GD pathogenesis, between them accounting for about 50% of the inherited component (3,4). Two other loci that are emerging with some evidence to support an association with GD in certain populations are thyroglobulin and CD40 (5-7). However, the strength of the genetic effect at these loci appears to be less than that at *CTLA4* or *MHC*, and these are currently regarded as tentative susceptibility loci until findings are replicated in larger patient cohorts. Other than *MHC* and *CTLA4*, the genetic basis of AAD also remains poorly defined (8). Thus, a substantial part of the inherited component to GD and AAD remains unexplained (1,4,8).

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One important theme to come from the intensive study of the genetics of autoimmune disease susceptibility over the last decade is that many autoimmune disorders have susceptibility loci in common (9). Thus, CTLA4 was originally identified as having a role in GD, but is also a susceptibility locus for type 1 diabetes mellitus (T1D), autoimmune Addison's disease and several other disorders (3,4,8,10,11). Recently, a tryptophan for arginine substitution at codon 620 (R620W) of the lymphoid-specific tyrosine phosphatase (LYP) protein was found to be associated with T1D in North American and Sardinian patient cohorts (12). A similar effect has been confirmed in U.S. cohorts of subjects with rheumatoid arthritis and systemic lupus erythematosus (13,14). This polymorphism, which is present in about 10% of healthy U.S. control subjects, was found to encode an important functional change, such that the tryptophan-bearing LYP allele cannot associate with C-terminal src kinase (Csk), a partner molecule in an inhibitory

complex that regulates key T cell receptor signaling kinases (Lck, Fyn, ZAP-70) (12).

We have examined a substantial cohort of unrelated GD subjects, some AAD subjects and healthy controls for association with the LYP R620W SNP polymorphism, to determine whether this might account for some of the currently unexplained inherited susceptibility to GD.

Patients and Methods

Patients

Blood samples were obtained from five hundred and forty nine unrelated white patients with GD who were attending endocrine and thyroid eye disease clinics at the Newcastle upon Tyne Hospitals Trust. All subjects had biochemical evidence of hyperthyroidism with either positive thyroid autoantibodies (TPO or TBII), thyroid eye disease or a diffuse uptake on ^{99}-Tc radionuclide scanning. Of the 549 GD patients there were 430 women and 40% of the cohort had significant thyroid eye disease (NOSPECS class 3 or worse) (15). Blood samples from one hundred and four subjects with AAD were obtained from the endocrine clinics at the Newcastle upon Tyne Hospitals Trusts, and surrounding district endocrine services. Details of diagnostic criteria for this cohort have been reported previously (8).

Four hundred and twenty nine healthy white subjects were recruited from local offices and factories in Newcastle for use as DNA controls. These subjects all had parents born in the north-east of England. Controls were screened as healthy on the basis of personal history. Thyroid autoantibodies and thyroid function were not tested. The study was approved by the Newcastle and North Tyneside district ethics committee.

Methods

Genomic DNA was extracted from peripheral blood samples and used as a template in a subsequent PCR reaction. A 220bp amplicon of the *PTPN22* gene (encoding the LYP molecule) was PCR amplified in a 25μl reaction using the following oligonucleotides: F tcaccagcttcctcaacca-ca; R gataatgttgcttcaacggaattt. Thirty five cycles of PCR were performed using a final concentration of 2.5mM $MgCl_2$ and an annealing temperature of 60^{o}C. The amplicon was digested with the restriction enzyme *XcmI* overnight at 37 ^{o}C and the digestion products resolved on a 3% agarose gel, stained with ethidium bromide. The *XcmI* cleaved the PCR product in the presence of the T nucleotide (encoding the tryptophan allele). Positive controls whose genotype had been determined by direct DNA sequencing were used on each plate. Ten percent of genotypes were repeated by a second laboratory worker (VW) blind to original genotype assignations to verify the accuracy of the assay.

A power calculation showed that given the odds ratio (1.7) found in T1D subjects (12) and a 20% minor (tryptophan) allele frequency (from US subjects), our GD study had 95% power to detect an effect (α=0.05). Statistical analysis was performed using a χ^2 test on 2x2 and 2x3 contingency tables. Uncorrected P values are shown. Odds ratios were calculated according to Woolf's method.

Results

The T nucleotide at the LYP codon 620 SNP, which encodes the tryptophan carrying allele, was found to be significantly over-represented in 549 subjects with Graves' disease compared to 429 controls. One hundred and fifty one of 1098 (13.8%) GD alleles were found to encode this tryptophan allele, compared to 67 of 858 (7.8%) control alleles (χ^2=17.2, p=3.4x10^{-5}; odds ratio = 1.88, 5- 95% CI 1.39 to 2.55). Full genotype and allele data are shown in table 1.

Similar findings were made in the subjects with AAD, where the tryptophan encoding LYP variant was found in 26 of 208 (12.5%) AAD alleles, compared to the 7.8% prevalence of this allele in controls (χ^2=4.6, p=0.031; odds ratio = 1.69, 5-95% CI 1.04 to 2.73) (table 1). Genotype frequencies of both cases and controls were found to be in Hardy-Weinberg equilibrium.

Discussion

Our study examined the LYP R620W SNP and has demonstrated highly significant association of

TABLE 1. Genotypes and alleles of the LYP codon 620 C→ T (R620W) SNP

	Graves' disease (n=549)	Addison's disease (n=104)	Controls (n=429)
Genotypes			
CC	404 (73.6%)[a]	81 (77.9%)[c]	365 (85.1%)
TC	139 (25.3%)	20 (19.2%)	61 (14.2%)
TT	6 (1.1%)	3 (2.9%)	3 (0.7%)
Alleles			
C	947 (86.2%)[b]	182 (87.5%)[d]	791 (92.2%)
T	151 (13.8%)	26 (12.5%)	67 (7.8%)

[a.] $\chi^2 = 19.0$, $p=7.6x10^{-5}$, compared to controls.
[b.] $\chi^2 = 17.2$, $p=3.4x10^{-5}$; odds ratio for the T allele 1.88, 5- 95% CI 1.39 to 2.55, compared to controls.
[c.] $\chi^2 = 5.5$, p=0.065, compared to controls.
[d.] $\chi^2 = 4.6$, p=0.031; odds ratio for the T allele 1.69, 5- 95% CI 1.04 to 2.73, compared to controls.

Graves' disease with the tryptophan encoding allele as compared to controls. The odds ratio of nearly 1.9 for this LYP allele (table 1) suggests that it has a marked predisposing effect to GD in our cohort, such that the LYP tryptophan allele, although not highly prevalent, may have the strongest predisposing effect to GD of any locus currently identified. This compares to an odds ratio of about 1.5 found at the CTLA4 locus, which is estimated to contribute about 30% of the total inherited susceptibility to GD (3,4,10). A lesser effect of the LYP 620W allele was found in AAD subjects (odds ratio 1.68). However, the confidence intervals are broad and the effects at LYP in both of our cohorts are similar in magnitude to those recently found in TID and other autoimmune conditions (12-14).

The molecular events associated with T cell receptor (TCR) ligation, and the downstream signaling pathways that regulate T lymphocyte activation have proven fruitful in yielding candidate genes that predispose to autoimmunity. In GD, AAD and T1D, the MHC and CTLA4 loci have substantial effects on disease susceptibility. Similarly, ZAP70 kinase, which also interacts with the TCR complex, has recently been implicated in a murine model of rheumatoid arthritis (16). The R620W change was shown to produce an LYP molecule that is unable to bind Csk (12). The LYP-Csk interaction appears necessary for optimal inhibitory activity (17), a lack of this interaction predicts that key TCR-associated kinases (Lck, Fyn and ZAP70) may be able to induce cellular activation unchecked. Nevertheless, it is possible that the R620W polymorphism is just one contributing polymorphism linked to a broader autoimmune susceptibility haplotype at this locus. In addition, it would be predicted that polymorphism of the genes involved in molecular events surrounding TCR engagement/ signaling would cause a general predisposition to several forms of autoimmune disorder. Our novel data concerning LYP in GD and AAD subjects is consistent with this. Studies of the LYP codon 620 polymorphism in other autoimmune endocrine disorders are warranted, based on these findings.

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Address for correspondence: Simon Pearce, Institute of Human Genetics, Central Parkway, Newcastle upon Tyne, NE1 3BZ, U.K. Email: s.h.s.pearce@ncl.ac.uk.