

ARTICLE

# Mutation screening of *PTPN22*: association of the 1858T-allele with Addison's disease

Beate Skinningsrud<sup>\*1,4</sup>, Eystein S Husebye<sup>2,3</sup>, Kristina Gervin<sup>4</sup>, Kristian Løvås<sup>2,3</sup>, Anne Blomhoff<sup>1,4</sup>, Anette B Wolff<sup>2</sup>, E Helen Kemp<sup>5</sup>, Thore Egeland<sup>4</sup> and Dag E Undlien<sup>1,4</sup>

<sup>1</sup>Institute of Medical Genetics, University of Oslo, Oslo, Norway; <sup>2</sup>Section of Endocrinology, Institute of Medicine, University of Bergen, Bergen, Norway; <sup>3</sup>Department of Medicine, Haukeland University Hospital, Bergen, Norway; <sup>4</sup>Department of Medical Genetics, Ullevaal University Hospital, Oslo, Norway; <sup>5</sup>School of Medicine and Biomedical Sciences, University of Sheffield, Sheffield, UK

The tyrosine-protein phosphatase non-receptor type 22 (*PTPN22*) gene was recently identified as an important genetic susceptibility factor in several autoimmune diseases. The increased risk has been broadly explained by the 1858T-allele (rs2476601). As two smaller studies on Addison's disease (AD) have shown diverging results, we aimed to elucidate the predisposing effect of the single-nucleotide polymorphism (SNP) 1858CT in a larger population of AD patients, especially focusing on the AD patients with known autoimmune etiology. We also screened for unknown rare or common variants in the *PTPN22* gene that could predispose for AD. The case-control study of Norwegian AD patients ( $n = 332$ ) and controls ( $n = 990$ ) showed a significant association between autoimmune AD ( $n = 302$ ) and the *PTPN22* 1858T risk allele ( $P = 0.016$ ). The association of AD with 1858T was supported by a meta-analysis combining our genotype data with that of others published previously ( $P = 0.003$ ). The mutation screening of *PTPN22* in AD patients ( $n = 332$ ) and controls ( $n = 112$ ) revealed eight missense variants, five of which have not been reported previously. In conclusion, the 1858T-allele is a *PTPN22* genetic susceptibility factor for autoimmune AD. Other rare variants in *PTPN22* do occur, and may also be involved in the pathogenesis. *European Journal of Human Genetics* (2008) 16, 977–982; doi:10.1038/ejhg.2008.33; published online 27 February 2008

**Keywords:** Addison's disease; *PTPN22*; Lyp; autoimmunity; association; resequencing

## Introduction

Autoimmune diseases affect up to 5% of the population and are among the 10 most common causes of death in women.<sup>1</sup> They are characterized by the loss of self-tolerance causing immune-mediated tissue destruction.<sup>2</sup> Autoimmune Addison's disease (AD) occurs due to autoimmune destruction of the adrenal cortex. Approximately half of the patients have additional autoimmune components.<sup>3</sup> AD with concomitant thyroid autoimmunity

and/or type I diabetes is the most common syndrome referred to as autoimmune polyendocrine syndrome type II (APS II).<sup>4</sup> It has long been suspected that several of the genetic determinants may be identical in different autoimmune diseases. This hypothesis is based upon observations of clustering of different autoimmune diseases in families,<sup>5</sup> as well as overlapping linkage peaks in meta-analyses of families with different autoimmune diseases.<sup>6</sup> There is also molecular genetic evidence to support this; for example, human leukocyte antigen (*HLA*) complex genes<sup>7</sup> and cytotoxic T-lymphocyte-associated protein 4 are associated with numerous autoimmune conditions.<sup>8,9</sup>

Tyrosine-protein phosphatase non-receptor type 22 encoded by the *PTPN22* gene has recently been claimed to be the second most important genetic susceptibility factor

\*Correspondence: B Skinningsrud, Institute of Medical Genetics, University of Oslo, Pb1036 Blindern, Oslo N-0315, Norway.

Tel: +47 22 11 98 60; Fax: +47 22 11 98 99;

E-mail: beate.skinningsrud@medisin.uio.no

Received 31 October 2007; revised 24 January 2008; accepted 24 January 2008; published online 27 February 2008

in autoimmune diseases, after the *HLA* gene complex. *PTPN22* encodes a tyrosine phosphatase, also called Lyp, which modulates T-cell activation and signaling events in a way that may trigger development of autoimmune disease.<sup>10</sup> A C to T substitution in nucleotide position 1858, which results in the substitution of arginine (R) with tryptophan (W) (R620W), has been suggested to be a gain-of-function variant.<sup>10,11</sup> The T-allele of this single-nucleotide polymorphism (SNP) has been demonstrated to moderately increase the susceptibility to several autoimmune disorders, for example, type I diabetes,<sup>12–14</sup> rheumatoid arthritis,<sup>15</sup> Graves' disease,<sup>14,16</sup> systemic lupus erythematosus,<sup>17</sup> and juvenile idiopathic arthritis.<sup>18</sup> These associations were confirmed in a meta-analysis by Lee *et al.*<sup>19</sup> Two smaller studies on *PTPN22* 1858CT and AD have, on the other hand, shown diverging results. Velaga *et al.*<sup>16</sup> reported association (odds ratio (OR) for the T-allele equal to 1.69; 95% confidence interval (CI): 1.04–2.73,  $P=0.031$ ) based on genotyping of 104 patients from northeast England, whereas Kahles *et al.*<sup>20</sup> found no association (OR 1.03; 95% CI: 0.63–1.67,  $P=0.913$ ) in 121 German patients. Sample size is crucial in studies of genetic factors with moderate risk, and with the small studies performed on *PTPN22* in AD; it is not surprising that the results are inconsistent.

For this reason, we decided to undertake a larger and statistically more powerful study encompassing a total of 368 AD patients and 1128 healthy controls from Norway and UK to determine whether the *PTPN22* 1858CT variant is associated with AD. Three hundred and two of the Norwegian patients had a verified autoimmune etiology, either by the detection of autoantibodies in their blood or by a secondary autoimmune diagnosis, and were, therefore, of special interest. A meta-analysis of Caucasian cohorts was performed to elucidate the risk of this SNP across populations. Furthermore, in light of the extreme proneness to autoimmunity observed in AD patients, we hypothesized that there may also be other, as yet unidentified, rare variants in this gene, which may predispose to AD. The involvement of infrequent mutations would also be in line with AD, being a rare autoimmune disorder compared to the other diseases previously analyzed for *PTPN22* polymorphisms. Being a rare disorder, the common variant–common disease hypothesis,<sup>21</sup> which postulates that most genetic susceptibility factors in common complex disorders are common variants, is less likely to be applicable. Previous screenings for mutations throughout the *PTPN22* gene are, to our knowledge, limited to 94 type I diabetes patients,<sup>22</sup> 64 parents of type I diabetes patients,<sup>23</sup> 20 psoriasis patients,<sup>24</sup> 48 rheumatoid arthritis patients,<sup>25</sup> and 35 healthy controls.<sup>26</sup> To this end we resequenced the coding part of the gene in 332 Norwegian patients and 112 healthy controls to clarify if there were additional coding variants in *PTPN22* that may predispose for AD. To our knowledge,

this is the most comprehensive sequence analysis of *PTPN22* performed in a study of any autoimmune disease.

## Materials and methods

### Patients and controls

Three hundred and thirty-two Caucasian patients with AD (206 women, 126 men) were recruited from the Norwegian registry of organ-specific autoimmune diseases (Supplementary Table 1). The mean age of the patients was 53 years (range 15–92 years), the mean age at diagnosis was 36 years (range 1–86 years) and the mean duration of AD was 18 years (range 0–54 years). Either low basal serum cortisol together with high adrenocorticotropic hormone (ACTH) or a pathological ACTH stimulation test was used to diagnose adrenal insufficiency. In the total cohort of men and women, 128 patients (39%) had isolated AD and 177 (53%) had APS II (all having AD concomitant with thyroid disease and/or type I diabetes). Autoantibodies against 21-hydroxylase (21OH) were present in 266/328 (80%) of the patients (four had unknown 21OH autoantibody status), 89 of them having isolated AD. Of the remaining 66 patients, 9 had antibodies against side-chain cleavage enzyme (SCC) or glutamic acid decarboxylase (GAD), which indicate an autoimmune etiology. Another 27 (8%) had other autoimmune manifestations (eg vitiligo, pernicious anemia, alopecia and premature gonadal failure) also indicating autoimmune etiology of AD. Only 30 patients, all with isolated AD, lacked autoantibodies, had unknown autoantibody status, no associated autoimmune features or other known causes of adrenal failure. Thus, the vast majority of patients (302 of 332) were interpreted to have autoimmune AD. Patients with AD as a component of autoimmune polyendocrine syndrome type I were excluded. A total of 990 anonymous healthy Norwegian blood donors, none of whom had AD were used as controls. The 36 British AD cases and 138 healthy controls were recruited by University of Sheffield as described elsewhere.<sup>27</sup> Informed consent has been obtained by all participants.

### Measurement of autoantibodies

Antibodies against 21OH, GAD and SCC were assayed by a radioimmunoassay against 35S-labeled recombinant human autoantigens expressed by *in vitro* transcription and translation, as described by Ekwall *et al.*<sup>28</sup> Positivity for the assays was governed by whether the test absorbance was greater than 3 SD above the negative control value.

### Single-nucleotide polymorphism genotyping

The 1858CT (rs2476601) SNP was genotyped by standard conditions using a Custom TaqMan<sup>®</sup> SNP Genotyping Assay, and analyzed on ABI PRISM<sup>®</sup> 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Secondary to the mutation screening, the same

technique was used to analyze five of the missense variants in an extended number of controls to define their frequency in the general population. Direct sequencing, as described below, was performed for the same purpose for the other two missense variants.

### Mutation screening

The BigDye<sup>®</sup> terminator v3.1 chemistry (Applied Biosystems) was used for bidirectional sequencing of all 21 exons of the *PTPN22* gene, in addition to the 5'UTR, 3'UTR and intron–exon boundaries in all 332 Norwegian AD patients and 112 anonymous Norwegian blood donors (randomly chosen from the 990 blood donors analyzed for the SNP 1858CT). Primers were designed using Primer3 ([http://frodo.wi.mit.edu/cgi-bin/primer3/primer3\\_www.cgi](http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi)),<sup>29</sup> and optimized with 60°C annealing temperature to enable the polymerase chain reactions (PCRs) to be run simultaneously in 5 µl reactions in 384-well plates. PCR clean-up was performed with Exo-SAP (USB Corporation, Cleveland, OH, USA), while the sequence reactions were purified using Montage<sup>™</sup> SEQ384 filter plates on a vacuum manifold (Millipore, Bedford, MA, USA) according to recommendations from the manufacturers. Sequences were read by an ABI3730 DNA analyzer and analyzed using the SeqScape v2.1.1 software (Applied Biosystems). Unsuccessful sequences were redone obtaining 100% coverage. All identified novel coding variants were assessed for functional importance using PolyPhen (<http://genetics.bwh.harvard.edu/pph/>),<sup>30</sup> a bioinformatic tool for prediction of possible impact of an amino acid substitution of a protein, based on both phylogenetic and structural information. Any changes in splicing-site motifs due to nucleotide substitutions close to exon–intron junctions were predicted by the use of ESEfinder v3.0 (<http://rulai.cshl.edu/cgi-bin/tools/ESE3/ese finder.cgi>).<sup>31,32</sup>

### Statistical analysis

Power calculation (<http://pngu.mgh.harvard.edu/~purcell/gpc/>) for the association study of SNP 1858CT in the Norwegian AD patients was based on the OR of 1.69 reported by Velaga *et al*,<sup>16</sup> a risk allele frequency of 0.116,<sup>18</sup> cases ( $n = 332$ ), controls ( $n = 990$ ) and significance level 0.05. This gave a statistical power of 88.8%. Comparisons of allele, genotype and haplotype frequencies in cases and controls were performed by Pearson  $\chi^2$  analysis using SPSS v14.0, the same program that was used for calculation of OR. Haplotype predictions were done by the software PHASE v2.1,<sup>33</sup> which has been reported to be the most accurate algorithm for haplotype construction with a 5.2–5.9% error rate in phasing the genotypes of unrelated individuals.<sup>34</sup> Hardy–Weinberg equilibrium was checked by the web-based tool at <http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>.

### Meta-analysis

PubMed (<http://www.ncbi.nlm.nih.gov/>) was searched using the search terms 'PTPN22', 'Lyp' and 'Addison's'. All studies found, who gave genotype data for 1858CT, were included in a meta-analysis together with our own data from the present study. The two English cohorts were recruited from different geographical areas. Breslow–Day homogeneity of OR analysis and Mantel–Haenszel common OR estimation were performed using SPSS v.14.0.

### Results

#### Association analyses of the PTPN22 SNP 1858CT

In the Norwegian population, we found a significant association between the *PTPN22* 1858T-allele and AD patients with known autoimmune etiology ( $n = 302$ ) with an OR 1.39 (95% CI: 1.06–1.82,  $P = 0.016$ ). Including all Norwegian AD patients ( $n = 332$ ) lowered the risk to an OR of 1.29 (95% CI: 0.99–1.68,  $P = 0.055$ ). Genotype frequencies of both cases and controls were in Hardy–Weinberg equilibrium. Genotype and allele frequency distributions are listed in Table 1. To confirm this association across populations, we performed meta-analysis with the other available genotyping data. Our meta-analysis included our Norwegian autoimmune AD patients and the Norwegian controls, a British population (recruited from the Sheffield district) and genotype results from two populations published earlier, a German<sup>20</sup> and a British (recruited from the Newcastle upon Tyne district).<sup>16</sup> Breslow–Day analysis did not detect any significant heterogeneity of OR ( $P = 0.554$ ) between the populations. The meta-analysis confirmed the association of the 1858T risk allele with AD with a common OR of 1.36 (95% CI: 1.11–1.66,  $P = 0.003$ ) (Table 2).

#### Mutation screening of PTPN22

The mutation screening of the *PTPN22* gene revealed eight missense variants, of which five have not been reported previously (Table 3). Moreover, ten novel non-coding variants were found (Supplementary Table 2). An additional novel variant was detected in the 3'UTR. Three intronic variants were positioned within four bases away from an exon–intron junction, and might, therefore, affect splicing. We only had access to leukocyte RNA from one patient with a nucleotide change close to an exon–intron junction; the c.197–4 in intron 2 adjacent to exon 3. Analysis to elucidate if this variant caused skipping of the exon 3 did, however, not show such a consequence in this patient's leukocytes (data not shown). All novel base changes were rare variants with a minor allele frequency less than 1% in the normal population. Segregation of the variants in the patients' families could not be investigated since samples from family members were unavailable.

Eight known non-coding or 3'UTR SNPs were located inside the sequenced regions thereby providing genotypes

**Table 1** Genotype and allele frequency distributions of the *PTPN22* SNP 1858CT in the Norwegian and UK datasets

Disease	n	Alleles T vs C					Genotypes TT and CT vs CC					
		T	C	OR <sup>a</sup>	95% CI	P	TT	CT	CC	OR <sup>a</sup>	95%CI	P
<i>Norway</i>												
AD	332	90 (13.6)	574 (86.4)	1.29	0.99–1.68	0.055	7 (2.1)	76 (22.9)	249 (75.0)	1.30	0.97–1.74	0.078
AAD	302	87 (14.4)	517 (85.6)	1.39	1.06–1.82	0.016	7 (2.3)	73 (24.2)	222 (73.5)	1.41	1.04–1.90	0.025
ctr	990	214 (10.8)	1766 (89.2)				12 (1.2)	190 (19.2)	788 (79.6)			
<i>UK</i>												
AD	36	10 (13.9)	62 (86.1)	1.37	0.64–2.97	0.418	2 (5.6)	6 (16.7)	28 (77.8)	1.18	0.48–2.86	0.723
ctr	138	29 (10.5)	247 (89.5)				2 (1.4)	25 (18.1)	111 (80.4)			

AD: total Addison's disease, AAD: verified autoimmune Addison's disease, ctr: controls.

Percentages are given in brackets.

<sup>a</sup>Odds ratios comparing allele frequencies in patients to ethnically matched controls.**Table 2** Meta-analysis of the *PTPN22* SNP 1858CT

Ethnicity	Number of Controls	Number of Cases	OR (95% CI) (T vs C allele)	P-value (2-sided)
Norwegian <sup>a</sup>	990	302	1.39 (1.06–1.82)	0.016
UK <sup>a</sup>	138	36	1.37 (0.64–2.97)	0.418
UK (Velaga <i>et al</i> ) <sup>16</sup>	429	104	1.69 (1.04–2.73)	0.031
German (Kahles <i>et al</i> ) <sup>20</sup>	239	121	1.03 (0.63–1.67)	0.913
<b>Mantel–Haenszel common OR estimate:</b>			<b>1.36 (1.11–1.66)</b>	<b>0.003</b>

<sup>a</sup>Current study. Only the Norwegian AD patients with verified autoimmune etiology are included in the meta-analysis.**Table 3** *PTPN22* missense variants detected in Norwegian AD patients and Norwegian healthy control individuals

Amino acid change	Position <sup>a</sup>	Nucleotide change <sup>b</sup>	Location	Detected in <sup>c</sup>		PolyPhen score
				Cases (n = 332)	Controls (n = 685)	
H189L	114199169	A>T	exon 8	1 (0.0015)	—	3.288, probably damaging
R263Q <sup>d</sup>	114196212	G>A	exon 10	8 (0.012)	25 (0.019)	0.967, predicted benign
H370N	114182437	C>A	exon 13	2 (0.003)	2 (0.0015)	2.574, probably damaging
Q456E	114182179	C>G	exon 13	1 (0.0015)	2 (0.0015)	1.647, possibly damaging
P568S	114181843	C>G	exon 13	—	1 (0.0007)	0.363, predicted benign
S692C <sup>e</sup>	114174153	C>G	exon 17	1 (0.0015)	1 (0.0007)	1.893, possibly damaging
R748G	114173745	A>G	exon 18	2 (0.003)	3 (0.002)	0.965, predicted benign

<sup>a</sup>Positions are according to NCBI Build 36.<sup>b</sup>Major alleles are listed first.<sup>c</sup>The number of heterozygote individuals precedes parentheses with minor allele frequencies.<sup>d</sup>R263Q was reported in North Americans<sup>25</sup> and Germans.<sup>24</sup><sup>e</sup>S692C was reported in Japanese.<sup>26</sup>

in the sequenced individuals. These were rs3789612, rs1217419, rs1217418, rs1599971, rs3761935, rs34209542, rs1217412 and rs3811021. They were all in Hardy–Weinberg Equilibrium. Neither of these SNPs showed significant difference in genotype or allele distributions between the patient group and control group using standard  $\chi^2$  tests. Haplotypes of all SNPs with minor allele frequency (MAF) > 5%, that is, excluding rs3789612 and rs34209542, were constructed for both cases and controls (Supplementary Table 3). As noted earlier, the 1858T allele was observed to occur on a single common haplotype.<sup>22,25</sup> These data show that the 112 controls, randomly selected

for sequencing, have an unusually high frequency of the 1858T variant (14.3%) compared to the wider population (10.8%). Neither of the haplotypes displayed any association with AD in the dataset where we had haplotype data.

## Discussion

Our data comparing Norwegian AD patients and healthy controls demonstrated a significant association of autoimmune AD to the *PTPN22* 1858T allele, underpinning the coupling of autoimmunity to this SNP. The meta-analysis

comprising European populations provides further support that this is a real association. An earlier performed meta-analysis may have suffered from an inadequate sample size to detect this moderate contribution of risk.<sup>19</sup> Moreover, these results let autoimmune AD fall into the line of autoimmune diseases previously shown to be associated with this SNP.

We screened the *PTPN22* gene to clarify if there are any common unknown variants or any rare mutations in *PTPN22* that may predispose for AD. A total of 28 variations were found, 16 of them were novel, including 5 missense, 1 in the 3'UTR and 10 located in introns. This represents the largest screening for *PTPN22* mutations in any disease, and also included 112 healthy controls. Four of the seven missense variants listed in Table 3 were predicted to be damaging to the protein function by PolyPhen.<sup>30</sup> Interestingly, the frequency of missense variants predicted to be damaging were slightly higher in the patient group compared to the controls. Conversely, there was an opposite weak tendency for variants predicted to be benign to be more frequent in the controls. The main contribution came from R263Q that was detected by the heterozygosity rates of 2.4 and 3.9% in Norwegian AD cases and controls, respectively.

The three non-coding variants located closest to an exon–intron junction, that is, c.197–4, c.828 + 4 and c.2882–2 were subjected to *in silico* analysis for prediction of splice sites and binding of serine/arginine-residue proteins (SR proteins) using the program ESEfinder v3.0.<sup>31</sup> Compared to the respective wild-type sequence, the nucleotide substitutions were predicted to have the following motif changes: the mutated allele c.197–4G had two additional predicted SR protein-binding sites (SC35 and SRp55), and the mutant variant c.2282–2C lacked a 3'U2 splicing site. These predicted differences of binding of splicing factors in the two *PTPN22* variants may suggest that they can affect normal splicing. *In vitro* analysis of c.197–4 did, however, not prove any exon skipping in leukocytes, but we cannot rule out that the variant may influence gene splicing in other tissues. However, any pathogenic effect of these variants is, therefore, at present speculative, and needs to be confirmed functionally.

*PTPN22* is a downregulator of the immune system by turning off the T-cell receptor (TCR) signaling when c-src tyrosine kinase (CSK) is bound to the SH3-domain of *PTPN22*. The *PTPN22* 1858T allele has been suggested to be a gain-of-function variant by increasing the enzyme activity,<sup>11</sup> and also by disrupting the binding of CSK to *PTPN22*.<sup>12,15</sup> Two explanations of the increased risk of autoimmunity in carriers of this allele have been proposed: they may have a higher TCR-signaling threshold and require stronger TCR signals for activation, thereby introducing a relative resistance to thymic deletion of autoreactive T cells,<sup>11</sup> or they may have reduced TCR signaling, leading to less effective T-regulatory cell activity.<sup>35</sup>

The effect of the rare variants detected in AD patients in the present study on the function of *PTPN22* is difficult to predict. Some of the variants may modulate catalytic function, others the binding to CSK or other potential binding partners,<sup>36</sup> with a potential result of affected TCR signaling. We examined family history of individuals with rare *PTPN22* variants; however, no firm conclusions can be drawn, largely owing to the small number of individuals involved (data not shown).

Focusing on the spectrum of variations (Supplementary Figure 1) detected in *PTPN22* in this study 10 of the novel variants (63%) were singletons (ie heterozygotes observed in a single individual) and the rest of the novel variants were all rare (ie MAF <5%), demonstrating the fact that the number of rare SNPs increases with depth of sequencing, whereas the number of common variants will plateau as most of them are already identified.<sup>37</sup> The sequencing of *PTPN22* in a total of 888 chromosomes revealed a two-fold density of variations in non-coding sequence of *PTPN22* compared to the coding sequence of the gene, with a density of one variation per 166 and 333 bp, respectively. Even though our study and the HapMap ENCODE project diverge on the basis of selection of sample population and selection of sequence to be screened, it is noteworthy that our frequency variation spectrum and SNP density (compared to their overall SNP-density of one per 279 bp) are very similar. Anyway, our data comprise a large sample size for mutation screening of a single locus and may, therefore, contribute to our understanding of SNP density in genes.

To conclude, this study adds autoimmune AD to the list of autoimmune diseases, where *PTPN22* 1858T is a genetic susceptibility factor. The comprehensive resequencing of *PTPN22* did not demonstrate any novel common coding SNP predisposing to AD in Norwegian patients, but our findings show that a number of novel infrequent or private mutations in *PTPN22* do occur, and bioinformatic analyses suggest that they may affect *PTPN22* function and possibly susceptibility to AD. However, their contribution to the AD phenotype needs to be further elucidated by functional studies.

#### Acknowledgements

The study was supported by grants from Eastern Regional Health Authorities and Western Regional Health Authorities, the Ullevaal University Hospital Scientific Advisory Council (VIRUUS) and The Functional Genomics Programme (FUGE) in The Research Council of Norway. Thanks to Morten Mattingsdal for valuable help with bioinformatic analysis. The authors declare that there is no conflict of interest.

#### References

- 1 Walsh SJ, Rau LM: Autoimmune diseases: a leading cause of death among young and middle-aged women in the United States. *Am J Public Health* 2000; 90: 1463–1466.

- 2 Marrack P, Kappler J, Kotzin BL: Autoimmune disease: why and where it occurs. *Nat Med* 2001; **7**: 899–905.
- 3 Gottlieb PA, Fain PR: Genetic basis of autoimmune adrenal deficiency. *Curr Opin Endocrinol Diabet* 2002; **9**: 237–243.
- 4 Eisenbarth GS, Gottlieb PA: Autoimmune polyendocrine syndromes. *N Engl J Med* 2004; **350**: 2068–2079.
- 5 Tait KF, Marshall T, Berman J et al: Clustering of autoimmune disease in parents of siblings from the Type 1 diabetes Warren repository. *Diabet Med* 2004; **21**: 358–362.
- 6 Becker KG: Comparative genetics of type 1 diabetes and autoimmune disease: common loci, common pathways? *Diabetes* 1999; **48**: 1353–1358.
- 7 Ghodke Y, Joshi K, Chopra A, Patwardhan B: HLA and disease. *Eur J Epidemiol* 2005; **20**: 475–488.
- 8 Gough SC, Walker LS, Sansom DM: CTLA4 gene polymorphism and autoimmunity. *Immunol Rev* 2005; **204**: 102–115.
- 9 Ueda H, Howson JM, Esposito L et al: Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 2003; **423**: 506–511.
- 10 Siminovitch KA: PTPN22 and autoimmune disease. *Nat Genet* 2004; **36**: 1248–1249.
- 11 Vang T, Congia M, Macis MD et al: Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. *Nat Genet* 2005; **37**: 1317–1319.
- 12 Bottini N, Musumeci L, Alonso A et al: A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat Genet* 2004; **36**: 337–338.
- 13 Onengut-Gumuscu S, Ewens KG, Spielman RS, Concannon P: A functional polymorphism (1858C/T) in the PTPN22 gene is linked and associated with type I diabetes in multiplex families. *Genes Immun* 2004; **5**: 678–680.
- 14 Smyth D, Cooper JD, Collins JE et al: Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. *Diabetes* 2004; **53**: 3020–3023.
- 15 Begovich AB, Carlton VE, Honigberg LA et al: A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 2004; **75**: 330–337.
- 16 Velaga MR, Wilson V, Jennings CE et al: The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease. *J Clin Endocrinol Metab* 2004; **89**: 5862–5865.
- 17 Kyogoku C, Langefeld CD, Ortmann WA et al: Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. *Am J Hum Genet* 2004; **75**: 504–507.
- 18 Viken MK, Amundsen SS, Kvien TK et al: Association analysis of the 1858C>T polymorphism in the PTPN22 gene in juvenile idiopathic arthritis and other autoimmune diseases. *Genes Immun* 2005; **6**: 271–273.
- 19 Lee YH, Rho YH, Choi SJ et al: The PTPN22 C1858T functional polymorphism and autoimmune diseases – a meta-analysis. *Rheumatology (Oxf)* 2007; **46**: 49–56.
- 20 Kahles H, Ramos-Lopez E, Lange B, Zwermann O, Reincke M, Badenhop K: Sex-specific association of PTPN22 1858T with type 1 diabetes but not with Hashimoto's thyroiditis or Addison's disease in the German population. *Eur J Endocrinol* 2005; **153**: 895–899.
- 21 Lander ES: The new genomics: global views of biology. *Science* 1996; **274**: 536–539.
- 22 Onengut-Gumuscu S, Buckner JH, Concannon P: A haplotype-based analysis of the PTPN22 locus in type 1 diabetes. *Diabetes* 2006; **55**: 2883–2889.
- 23 Zoledziewska M, Perra C, Orru V et al: Further evidence of a primary, causal association of the PTPN22 620W variant with type 1 diabetes. *Diabetes* 2008; **57**: 229–234.
- 24 Huffmeier U, Steffens M, Burkhardt H et al: Evidence for susceptibility determinant(s) to psoriasis vulgaris in or near PTPN22 in German patients. *J Med Genet* 2006; **43**: 517–522.
- 25 Carlton VE, Hu X, Chokkalingam AP et al: PTPN22 genetic variation evidence for multiple variants associated with rheumatoid arthritis. *Am J Hum Genet* 2005; **77**: 567–581.
- 26 Kawasaki E, Awata T, Ikegami H et al: Systematic search for single nucleotide polymorphisms in a lymphoid tyrosine phosphatase gene (PTPN22): association between a promoter polymorphism and type 1 diabetes in Asian populations. *Am J Med Genet A* 2006; **140**: 586–593.
- 27 Kemp EH, Ajjan RA, Husebye ES et al: A cytotoxic T lymphocyte antigen-4 (CTLA-4) gene polymorphism is associated with autoimmune Addison's disease in English patients. *Clin Endocrinol (Oxf)* 1998; **49**: 609–613.
- 28 Ekwall O, Hedstrand H, Grimelius L et al: Identification of tryptophan hydroxylase as an intestinal autoantigen. *Lancet* 1998; **352**: 279–283.
- 29 Rozen S, Skaletsky H: Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol* 2000; **132**: 365–386.
- 30 Ramensky V, Bork P, Sunyaev S: Human non-synonymous SNPs: server and survey. *Nucleic Acids Res* 2002; **30**: 3894–3900.
- 31 Cartegni L, Wang J, Zhu Z, Zhang MQ, Krainer AR: ESEfinder: A web resource to identify exonic splicing enhancers. *Nucleic Acids Res* 2003; **31**: 3568–3571.
- 32 Smith PJ, Zhang C, Wang J, Chew SL, Zhang MQ, Krainer AR: An increased specificity score matrix for the prediction of SF2/ASF-specific exonic splicing enhancers. *Hum Mol Genet* 2006; **15**: 2490–2508.
- 33 Stephens M, Smith NJ, Donnelly P: A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001; **68**: 978–989.
- 34 Marchini J, Cutler D, Patterson N et al: A comparison of phasing algorithms for trios and unrelated individuals. *Am J Hum Genet* 2006; **78**: 437–450.
- 35 Gregersen PK, Behrens TW: Genetics of autoimmune diseases – disorders of immune homeostasis. *Nat Rev Genet* 2006; **7**: 917–928.
- 36 Vang T, Miletic AV, Bottini N, Mustelin T: Protein tyrosine phosphatase PTPN22 in human autoimmunity. *Autoimmunity* 2007; **40**: 453–461.
- 37 International HapMap Consortium: A haplotype map of the human genome. *Nature* 2005; **437**: 1299–1320.

Supplementary Information accompanies the paper on European Journal of Human Genetics website (<http://www.nature.com/ejhg>)