ARTICLE

Replication analysis identifies *TYK2* as a multiple sclerosis susceptibility factor

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In a recent genome-wide association study (GWAS) based on 12 374 non-synonymous single nucleotide polymorphisms we identified a number of candidate multiple sclerosis susceptibility genes. Here, we describe the extended analysis of 17 of these loci undertaken using an additional 4234 patients, 2983 controls and 2053 trio families. In the final analysis combining all available data, we found that evidence for association was substantially increased for one of the 17 loci, rs34536443 from the tyrosine kinase 2 (*TYK2*) gene ($P = 2.7 \times 10^{-6}$, odds ratio = 1.32 (1.17–1.47)). This single nucleotide polymorphism results in an amino acid substitution (proline to alanine) in the kinase domain of *TYK2*, which is predicted to influence the levels of phosphorylation and therefore activity of the protein and so is likely to have a functional role in multiple sclerosis.

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Introduction

Multiple sclerosis is a disease of the central nervous system that is frequently disabling.¹ Although evidence indicates that the condition results from a complex interplay

between environmental and genetic factors,^{2–5} relatively little is known about precisely which genes are involved. Association with the DR15 haplotype from the major histocompatibility complex (MHC) on chromosome 6p21 has been established for more than 30 years⁶ and confirmed in nearly every population studied.⁷ Identification of other susceptibility genes has proven to be decidedly more difficult. Systematic efforts to screen for linkage⁸ together with segregation analysis^{9,10} indicate that susceptibility to multiple sclerosis is likely determined by a series of common variants each exerting only a modest effect on risk. Fortunately, the resources necessary to find such modest genetic effects are now available and the last 2 years have seen remarkable progress in the identification of susceptibility genes of relevance in multiple sclerosis. Through a candidate gene approach prompted by a careful systematic analysis of all available data, association with the interleukin-7 receptor (*IL7R*) gene has been firmly established.^{11,12} In a genome-wide association study (GWAS) involving 931 cases, The International Multiple Sclerosis Genetics Consortium (IMSGC) identified the interleukin-2 receptor (*IL2R*) as a susceptibility gene,¹³ which was confirmed in a very large replication study.¹⁴ Recent studies have also shown a role for CD226 and *CLEC16A* in multiple sclerosis susceptibility.^{15–17}

In parallel with these efforts, we completed a GWAS based on 12 374 non-synonymous single nucleotide polymorphisms (nsSNPs) typed in 975 patients and 1466 controls. This GWAS was performed as part of The Wellcome Trust Case–Control Consortium (WTCCC)¹⁸ (A full list of WTCCC contributors is provided in the Supplementary information online). Although this study failed to identify any unequivocally associated loci, other than those in linkage disequilibrium (LD) with DR15, the most highly ranked markers implicated a number of promising candidate genes. Through extending the analysis of the most promising 17 of these loci, we have now identified another non-MHC susceptibility locus for multiple sclerosis – the tyrosine kinase 2 (*TYK2*) gene.

Materials and methods

Participants

Samples independent of those considered in our WTCCC nsSNP GWAS were collected from Australia, Belgium, Norway, the United Kingdom (UK) and the United States of America (USA). A population-specific breakdown together with patient demographics is shown in Table 1. All patients were diagnosed according to the recognised clinical criteria.^{19,20} DNA was extracted using standard methods and all samples were collected with informed written consent and appropriate ethical approval.

Genotyping

In this analysis, we included the eight SNPs identified in our WTCCC GWAS with a *P*-value < 0.001 and a further 10 SNPs selected from amongst those with a *P*-value < 0.01 in the GWAS that were from genes that we considered to be logical candidates for multiple sclerosis. To maximise efficiency and limit unnecessary expenditure, genotyping was performed in two stages. In the first screening stage, we typed all 18 SNPs in 1481 patients, 1879 controls and 806 trio families (from the Belgian, Norwegian and UK1 populations). SNP genotyping was performed using Applied Biosystems TaqMan methodology according to the manufacturer's recommended conditions. A combined analysis of the screening phase data and the original WTCCC data was then completed. In stage 2 of the study, the top five markers identified from this analysis were then followed up in a larger replication set of 2753 patients, 1104 controls and 1247 families from the US, Australian and UK2 (North West UK and South Wales) populations. Power calculations indicate that in both the screening and replication phases, we have greater than 80% power to find association with a nominal (uncorrected) *P*-value of 0.05 assuming a common risk allele (10% frequency) with an odds ratio of 1.3 (multiplicative model).²¹ By combining the original WTCCC data with the screening and replication phase data, in total 5213 patients, 4453 unrelated controls and 2053 trio families were considered in the analysis of the top five markers.

Statistical analysis

The genotyping success rate, Hardy–Weinberg equilibrium and heterozygosity for each marker were established using PEDSTATS.²² Mendelian errors in the trio families were identified through PEDCHECK.²³ In total, five Mendelian errors were identified, one for rs1133400 and four for rs7522061. The case–control data sets were tested for heterogeneity using the Breslow–Day test and a Cochran–Mantel–Haenszel test completed (treating each population specific set of samples as a separate stratum) using StatsDirect statistical software. As no significant heterogeneity was identified between the populations, the raw genotypes were combined and an association analysis was completed using UNPHASED v3.08.²⁴

Results

The 18 SNPs were first tested in the screening phase set of samples. A full list of the SNPs and the results in the screening phase set are shown in Supplementary Table S1. Initial testing showed that the marker rs11080149 (from the *OMG* gene) deviated significantly from Hardy–Weinberg equilibrium. As this variant lies in a region of potential copy number variation, the assay was considered to be unreliable and the marker was therefore excluded

Table 1 Demographic feature of affected individuals

Population	Cases	Controls	Trios	Age (years)	Average Disease duration (years)	EDSS	RR/SP%	PP%
<i>Screening phase</i>								
Belgium	368	374	113	47.0	15.0	5.4	91.2	8.8
Norway	638	1026	—	46.3	14.3	4.8	81.4	18.6
UK1	475	479	693	43.8	12.8	4.6	88.2	11.8
WTCCC screen	979	1470	—	43.2	13.5	4.7	87.2	12.8
<i>Replication phase</i>								
Australia	—	—	372	47.2	14.3	3.3 ^a	93.0	7.0
UK2	1531	561	—	44.0	13.0	4.6	91.8	8.2
USA	1222	543	875	44.3	12.4	4.1	94.8	5.2

EDSS, expanded disability status scale; RR, relapse remitting; SP, secondary progressive; PP, primary progressive. The case dataset from UK2 includes 880 from the North West of the UK and 516 from South Wales with the remainder coming from across the whole of the UK (with an emphasis on the South east). The control dataset includes 561 individuals from the 1958 birth cohort.

^aThe EDSS was not determined for these patients; instead, the average 'disease steps' measure is shown.³⁴

Table 2 Results from association testing of the top five SNPs

Gene	Chromosome	SNP	Risk allele	Samples	Screening ^a P-value	Replication ^b P-value	Combined analysis ^c P-value	Odds ratio (95% CI)	RAF (cases)	RAF (controls)
IL7R	5	rs6897932	C	All individuals	3.5×10^{-4}	4.7×10^{-4}	1.3×10^{-7}	1.42 (1.09–1.20)	0.763	0.732
				Case–control	7.3×10^{-6}	8.8×10^{-5}	7.4×10^{-10}	1.20 (1.13–1.27)	0.764	0.730
				Trio families	0.5478	0.1813	0.1834	1.08 (0.96–1.22)	0.761	0.747
TYK2	19	rs34536443	G	All individuals	3.9×10^{-4}	2.6×10^{-3}	2.7×10^{-6}	1.32 (1.17–1.47)	0.966	0.953
				Case–control	2.1×10^{-3}	3.1×10^{-4}	6.8×10^{-6}	1.39 (1.20–1.60)	0.965	0.952
				Trio families	4.2×10^{-5}	0.1910	3.2×10^{-4}	1.58 (1.24–2.02)	0.970	0.954
IFI30	19	rs11554159	G	All individuals	2.0×10^{-3}	8.5×10^{-3}	2.2×10^{-4}	1.10 (1.05–1.16)	0.756	0.737
				Case–control	2.1×10^{-3}	0.1208	0.017	1.08 (1.01–1.16)	0.755	0.740
				Trio families	0.8676	2.5×10^{-4}	3.8×10^{-3}	1.17 (1.05–1.30)	0.757	0.730
FCRL3	1	rs7522061	T	All individuals	2.4×10^{-4}	0.7557	9.1×10^{-3}	1.06 (1.01–1.11)	0.545	0.528
				Case–control	1.0×10^{-3}	0.1432	3.4×10^{-4}	1.11 (1.05–1.18)	0.549	0.523
				Trio families	0.2350	0.1819	0.8508	0.99 (0.90–1.09)	0.536	0.541
SLC4A5 ^d	2	rs17009792	C	All individuals	4.7×10^{-4}	0.7082	0.0191	1.20 (1.03–1.40)	0.980	0.974
				Case–control	9.3×10^{-5}	0.1898	1.4×10^{-3}	1.35 (1.13–1.63)	0.980	0.973
				Trio families	0.2404	0.5411	0.7216	1.07 (0.75–1.52)	0.979	0.978

Association analysis was completed using UNPHASED v3.08. The *P*-values presented are uncorrected.

All alleles are specified with respect to the forward strand of the Ensembl genome browser Release 49 (March 2008).

^aThe screening phase includes 1481 patients, 1879 controls and 806 trio families from the Belgian, Norwegian and UK1 populations. The *P*-value is based on the analysis of this new data in combination with the original WTCCC screen data of 979 patients and 1470 controls.

^bThe replication phase includes 2753 patients, 1104 controls and 1247 families from the US, Australian and UK2 (North West UK and South Wales) populations.

^cThe combined analysis includes the WTCCC screen data, screening phase and replication phase data.

^dNot tested in the Australian population.

from further analysis. All other markers were in Hardy–Weinberg equilibrium.

An analysis of the original WTCCC data with the screening phase data was completed and the top five SNPs were chosen for further investigation (see Supplementary Table S1). These markers were followed up in the replication data set and a combined analysis of all the available data (screening and replication set samples) was then completed for these five SNPs (Table 2). The population specific data for the top five markers is available in Supplementary Table S2. In this larger analysis, no

marker showed deviation from Hardy–Weinberg equilibrium and the average genotyping success rate across these five markers was 99.1% (range 98.7–99.4%). There was no statistically significant difference in the genotyping failure rate between cases and controls for any of these markers.

Using the Breslow–Day test, we found no statistically significant evidence for heterogeneity between the different population cohorts or the WTCCC study for any of the top five SNPs. For this reason, it is unsurprising that the results from a Cochran–Mantel–Haenszel analysis showed

no meaningful difference from the results obtained, when treating all the data as if they had been collected from a single population.

The most significant association that we observed was with rs6897932 from the *IL7R* gene ($P = 1.3 \times 10^{-7}$). As this marker is already confirmed as a susceptibility locus^{11,12} in multiple sclerosis it provides a useful positive control for our study and illustrates that screens based on nsSNPs are capable of identifying genuine associations.

The next most strongly associated marker was rs34536443 from exon 21 of the *TYK2* gene ($P = 2.7 \times 10^{-6}$). As with the *IL2R* and *IL7R*, the common allele increases risk at rs34536443. This risk allele has a frequency of 95.3% in the background population and increases risk with an odds ratio of 1.32 (1.17–1.47). In a stratified analysis of the individual replication cohorts, the trend for association was in the same direction for all cohorts, with the only exception being the Belgian population (the smallest cohort analysed).

In the final analysis of all available data, evidence for association was either substantially reduced or largely uninfluenced for each of the other markers studied. One marker retained modest evidence for association (rs11554159 from *IFI30*, $P = 2.2 \times 10^{-4}$) and may represent a genuine association of smaller effect, but very large sample sizes will be needed to confirm such an association.

Discussion

This study reports the first attempt to explore the results that emerged from our WTCCC nsSNP GWAS. Through the typing of selected markers in a large independent cohort of cases, controls and trio families, we have shown evidence of a novel association with rs34536443 in the *TYK2* gene.

Tyrosine kinase 2, part of the Janus family of tyrosine kinases, has a crucial role in signal transduction for a wide range of cytokines, including type 1 interferons, IL10 and IL12.^{25,26} TYK2 deficiency in humans results in severe combined immunodeficiency (SCID),²⁷ with reduced Th1 (and possibly Th17 immune responses) and increased production of Th2 cytokines.²⁵ TYK2 variants have been associated with susceptibility to the autoimmune disease, systemic lupus erythematosus.^{28,29} The detrimental autoimmune response generated by TYK2 is further shown in Tyk2-deficient mice that are shown to be resistant to autoimmune arthritis.³⁰ The SNP rs34536443 is located in exon 21 and codes either a conserved proline (major allele) or alanine at position 1104, in the tyrosine kinase domain. 1104A is predicted to be 'probably damaging' based on the program Polymorphism Phenotyping (PolyPhen) and 'intolerant' based on Sorting Intolerant from Tolerant (SIFT). The shift to a Th1 pro-inflammatory immune response in multiple sclerosis is believed to lead to cell destruction and consequent pathology. The reduced TYK2 function of the 1104A variant could lead to a shift to a protective Th2 response, and/or reduced Th1 or Th17

activation. As the minor allele frequency is higher in the control population, this further supports the possibility that the rs34536443 minor allele may be protective.

IFI30 or IFN γ -inducible lysosomal thiol reductase (*GILT*) is a prime candidate gene in multiple sclerosis as it has a vital role in antigen processing through MHC class II and up-regulated expression on macrophages and microglia in active demyelinating lesions in multiple sclerosis.³¹ *GILT* is expressed constitutively in antigen presenting cells and is induced by inflammatory cytokines, such as IFN γ , TNF α and IL1 β in other cell types.³² Studies in mice have shown that *GILT* deficient T cells show stronger T-cell activation and increased proliferation, suggesting a role for *GILT* in modifying the immune response by controlling T-cell activation.³³ Although the strength of association for rs11554159 was reduced compared with the initial nsSNP screen (despite the trend for association being in the same direction for all cohorts analysed), modest evidence for association remained. As initial findings tend to overinflate the effect size, our replication cohort may be too limited in power to confirm this association. However, given these findings combined with the involvement of *IFI30* in the immune response, this gene warrants further analysis in a much larger cohort.

In summary, we have identified a novel association with the *TYK2* gene. The power to detect the small genetic effect attributed to this SNP has been crucially dependent on the large sample sizes analysed in this study. The consequence of the rs34536443 SNP on TYK2 function and expression in patients with multiple sclerosis needs to be explored, together with the role this gene may have in autoimmunity in general.

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Websites

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