Genetic studies in rheumatoid arthritis

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Chapter 5

The *TRAF1-C5* region on chromosome 9q33 is associated with multiple autoimmune diseases

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Abstract

Objectives

The TRAF1-C5 locus has recently been identified as a genetic risk factor for rheumatoid arthritis. Since genetic risk factors tend to overlap with several autoimmune diseases, we aimed to investigate whether this region is associated with Type I Diabetes (TID), Celiac Disease (CD), Systemic Sclerosis (SSc) and Systemic Lupus Erythematosus (SLE).

Methods

We genotyped the most consistently associated SNP, rs10818488, in a total of 735 T1D, 1049 CD, 367 SSc, 746 SLE and 3494 ethnically and geographically matched healthy individuals. The replication sample set consisted of 99 T1D, 272 SLE patients and 482 healthy individuals from Crete.

Results

We detected significant association of the rs10818488 A allele with T1D (OR 1.14, p=0.027) and SLE (OR 1.16, p=0.016) which was replicated in 99 T1D, 272 SLE patients and 482 controls from Crete (OR 1.64, p=0.002; OR 1.43, p=0.002 respectively). Joint analysis of all T1D (N=961) and all SLE (N=1018) patients compared to 3976 healthy individuals yielded an allelic common OR of 1.19 (p=0.002) and 1.22 ($P=2.6x10^{-4}$) respectively. However, combining our dataset with the T1D sample set from the WTCCC results in a non-significant association (OR 1.06, p=0.087). In contrast, previously unpublished results from the SLEGEN study shows significant association of the same allele (OR 1.19, p=0.0038) with an overall effect of 1.22 ($p=1.02x10^{-6}$) in a total of 1577 SLE patients and 4215 healthy individuals.

Conclusion

We report significant association of the *TRAF1-C5* locus in SLE implying that this region lies in a pathway relevant to multiple autoimmune diseases.

Introduction

The region encompassing Tumour Necrosis Factor (TNF) receptor associated factor 1 (*TRAF1*) and complement component 5 (C5) has recently reported it to be a genetic risk factor involved in rheumatoid arthritis (RA)(1). The robustness of this association is demonstrated by its prevalent risk in Dutch, Swedish, Crete(2) and American populations and is further corroborated by both a genome-wide association study(3) and an extensive fine-mapping study we have undertaken(4). Interestingly one consistent RA association signal defined by rs10818488 or its perfect proxy (rs3761847 and rs7021049, Linkage Disequilibrium R^2>0.98) has been identified in this region by three independent studies and has been further confirmed by a large European family-based study(5). Moreover, an association of the opposite allele of rs3761847 in RA has been reported in the Japanese population but no association has been found in a Korean population(6;7). Additionally, we and others have shown that this polymorphism is associated with juvenile (idiopathic) arthritis(JIA)(8;9), indicating its possible role in other autoimmune diseases.

From a functional perspective, TRAF1 is likely to be a negative regulator of TNF Receptor signaling(10) and C5 is a central component of the complement pathway(11). Both molecules are potent immune mediators and so far the question remains whether this region is restricted to arthritis as such or whether it lies in a biological pathway common to other autoimmune diseases. In the present study we aimed to investigate this hypothesis further by considering the role of this locus in four diseases including Type 1 Diabetes (T1D), Celiac Disease (CD), Systemic Sclerosis (SSc) and Systemic Lupus Erythematosus (SLE).

Methods

Sample sets

DNA was obtained from cohorts of T1D, CD, SSc and SLE from The Netherlands and Spain as well as T1D and SLE patients from Crete. Control cohorts were matched to each specific population of origin. The T1D sample set consisted of 556 white Dutch and 306 Spanish patients. The CD sample set comprised 496 Dutch and 553 Spanish CD patients. The SSc sample set consisted of 138 Dutch and 229 Spanish Systemic Sclerosis patients. The SLE sample set included 161 Dutch and 585 Spanish patients. All patients were Caucasian. A common set of controls were used consisting of 1396 Dutch and 2098 Spanish geographically and ethnically-matched healthy individuals. The replication sample set consisted of 272 SLE, 99 T1D patients and 482 geographically and ethnically matched controls originating from Crete. All patients were diagnosed using the appropriate classification criteria and are described extensively in the Supplementary Methods. Informed consent was obtained from all subjects, and the study was approved by the local ethics committee of each center.

GWAS data

Genotype counts were obtained for rs10118357 (R^2=1 with rs10818488) from the publicly available WTCCC study(12) consisting of 1960 T1D patients and 2930 ethnically matched healthy individuals from the UK population. For the SLE sample set, genotyope counts were obtained from the SLEGEN study(13) for rs3761847 (R^2=1 with rs10818488) from 720 SLE patients and 2337 healthy individuals. These sample sets are described in further detail elsewhere(12;13).

Genotyping

All samples were genotyped using the Taqman assay (Applied Biosystems) according to the manufacturer's instructions. Each plate consisted of at least 8 positive and 8 negative controls. At least 10% of the samples were genotyped in duplo with no discrepancies observed. The Dutch control sets consists of 511 healthy individuals which were previously genotyped(1). An additional 535 Dutch healthy unrelated individuals were genotyped using allele specific kinetic PCR(14) as well as 715 Dutch controls from Utrecht using the Taqman assay. To compare genotyping methods at least 50 samples were genotyped on each platform (Taqman, allele specific kinetic PCR and RFLP) and revealed a concordance rate of >99%.

Statistical analysis

In the controls, the frequencies were in Hardy-Weinberg equilibrium as determined by the observed versus expected genotype counts. Genotype counts were analysed using SPSS version 12.0. Odds ratios and confidence intervals were calculated using Statcalc. Combining odds ratios across sample sets was performed using the Cochran-Mantel-Haenszel test as implemented in EasyMA and the meta package in R (15). No evidence of heterogeneity of risk effect was observed using the Breslow and Day method (P>0.05) when combining OR. All power calculations were performed using Quanto version 1.2 (http://hydra.usc.edu/gxe). P values below 0.05 were considered significant.

Results

T1D, CD, SSc and SLE samples were genotyped from both the Spanish and Dutch populations for the strongest and most consistent association signal in the *TRAF1-C5* region characterized by SNP rs10818488. Since the Dutch study was largely underpowered to detect modest effect sizes (OR~1.2) we opted for a combined analysis which consisted of a total of 3494 controls and 735 T1D, 1049 CD, 367 SSc, 746 SLE patients. This combined dataset enhanced power to ≥85% to detect effect sizes of 1.2 at P<0.05 with the exception of SSc which only achieved 64% power to detect an odds ratio of 1.2.

The frequency of the rs10818488 A allele was significantly increased in T1D patients (OR 1.14; 95% CI 1.02- 1.28; p=0.027, Table 1). Similarly we found a significant difference in the prevalence of the A allele in SLE patients resulting in an OR of 1.16 (95% CI 1.03-1.31, p=0.016). Patients harboring two copies of the A allele had a 1.3 fold (p=0.01) and a 1.4 fold (p=0.04) increased risk for T1D and SLE respectively as compared to those who carried none. We observed no association with CD (Allelic OR 1.07, 95% CI 0.97-1.18, p=0.18) and SSc (Allelic OR 1.02, 95% CI 0.87-1.19, p=0.84). While the possibility remains that the absence of association in SSc may be due to lack of power to detect OR of 1.2 or lower, the direction of association in the individual studies was opposite (Allelic OR, 95% CI; Spanish 1.16, 0.94-1.43; Dutch 0.85, 0.65-1.09), suggesting that the absence of association may be more likely.

We then proceeded to replicate our significant findings in T1D and SLE in the genetically homogeneous population of Crete. Since this largest island of Greece consists of 650,000 inhabitants who share the same genetic and cultural background as well as a common environment, it represents a “geographically isolated” gene pool which may enhance the detection of risk alleles that may be diluted in larger continental populations(16). We observed an 11% increase and an 8% increase in the A allele in T1D and SLE patients, respectively, when compared to controls (Table 2). This resulted in a 1.6 fold increased risk for T1D (p=0.002) and a 1.4 fold increased risk for SLE (p=0.002) in the Crete population. Overall analysis of T1D and SLE in all three datasets (Spanish, Dutch and Greek) reveals a common OR of ~1.2 (p=0.002 and $p=2.6x10^{-4}$ respectively) for both diseases.

We also analysed our data in combination with rs10118357 (perfect proxy of rs10818488, $R^2=1$) for T1D obtained from the Wellcome Trust Case Control Consortium (WTCCC)(12) study as well as a meta-analysis of the effect of SNP rs3761847 (perfect proxy of rs10818488, $R^2=1$) in the SLE Genetics consortium (SLEGEN) study(13) (Table 2). The WTCCC T1D dataset comprising 1960 patients and 2930 healthy individuals displayed no association with an OR of 0.99 (95% CI 0.91-1.08, p=0.837). Combining all four datasets (2794 patients and 6906 controls) generated an overall OR of 1.06 (95% CI 0.99-1.13, p=0.087) indicating that the effect size is very modest and that our study is largely underpowered to detect a significant effect. Previously unpublished data from the SLEGEN study in contrast, show a significant increase in the rs10818488 A allele frequency in 720 SLE patients (43%) as compared to 2337 healthy individuals (39%) (OR 1.19, 95% CI 1.06-1.35, p=0.0038), independently replicating the association of this locus with SLE. Combining all datasets consisting of 1577 SLE patients and 4215 controls shows a significant association with OR 1.22 (95% CI 1.12-1.31, $p=1.02x10^{-6}$).

Table 1. Association of rs10818488 in the *TRAF1-C5* locus with autoimmune diseases.

		Controls	T1D	CD	SSc	SLE
	Initial Sample Set					
Spanish	AA	169	45	84	38	101
	AG	663	150	251	104	276
	GG	564	111	218	87	208
	A(%)	1001(36%)	240(39%)	419(38%)	180(39%)	478(41%)
Dutch	AA	392	106	100	21	28
	AG	1060	305	249	68	84
	GG	646	145	147	49	49
	A(%)	1844(44%)	517(46%)	449(45%)	110(40%)	140(44%)
	OR(95% CI)*	-	1.14 (1.02-1.28)	1.07 (0.97-1.18)	1.02 (0.87-1.19)	1.16 (1.03-1.31)
	P	-	0.027	0.181	0.842	0.016
	Replication Sample Set					
Crete	AA	32	1			26
	AG	214	77			148
	GG	236	21			98
	A(%)	278(29%)	79(40%)			200(37%)
	OR (95% CI)		1.64 (1.18-2.28)			1.43 (1.14-1.80)
	P		0.002			0.002
	Joint Sample Set					
	Combined OR¥ (95% CI)		1.19 (1.07-1.32)			1.22 (1.09-1.35)
	P		0.002			$2.6x10^{-4}$

T1D – Type 1 Diabetes, CD- Celiac Disease, SSc- Systemic Sclerosis and SLE- Systemic Lupus Erythematosus. * Represents the combined allelic OR between the Dutch and Spanish sample set. ¥ Represents the combined allelic OR between Dutch, Spanish and Crete sample sets. All OR were combined using the Mantel-Haenszel fixed effects as implemented in EasyMA and the Meta package in R.

Table 2. Meta-analysis of rs10818488 in the *TRAF1-C5* locus with Genome Wide Association Studies.

	WTCCC		SLEGEN	
GWAS Data Set	Controls	T1D	Controls	SLE
AA	362	598	339	138
AG	1011	1410	1135	344
GG	587	922	863	238
A (%)	2930 (44%)	1960 (44%)	2337 (39%)	720 (43%)
OR * (95% CI)		0.99 (0.91-1.08)		1.19 (1.06-1.35)
P		0.837		0.0038
Joint Sample Set				
Combined OR¥ (95% CI)		1.06 (0.99-1.13)		1.22 (1.12-1.31)
P		0.087		1.02×10^{-6}

T1D – Type 1 Diabetes and SLE- Systemic Lupus Erythematosus. * Represents the combined allelic OR between the cases and controls in the GWAS sample set. ¥ Represents the combined allelic OR between Dutch, Spanish, Crete and GWAS sample sets. All OR were combined using the Mantel-Haenszel fixed effects as implemented in EasyMA and the Meta package in R.

Discussion

We report here for the first time reproducible association of the *TRAF1-C5* region with SLE complementing the already consistent finding of this variant with RA and juvenile arthritis. Remarkably, the same allele that predisposes to RA and juvenile arthritis also predisposes to SLE lending support to the hypothesis that this region may contribute to a shared pathway involved in RA, JIA and SLE. While the possibility also remains that other additional alleles at this locus may be involved in these diseases, complementary studies undertaking further fine-mapping as well as sequencing will yield further insight into the most likely causal alleles at this locus.

We also observed a difference in allele frequencies in healthy populations of Dutch (44%), Spanish (36%) and Greek (29%) origins. To address this difference in population, each patient-control sample set was geographically and ethnically matched. Data from HapMap (www.hapmap.org) support these observations with the G allele (minor allele in Caucasians) frequency of rs3761847 (perfect proxy of rs10818488) varying from 48% in Caucasians of European descent, 31% in Gujarati Indians in Texas, 42% in Japanese in Tokyo, 66% in Mexicans in Los Angeles, 59% in Yorubans in Nigeria and 74% in Luhya in Kenya. However, underlying population stratification is difficult to account for as panels of markers characterizing each population in our study thoroughly have not as yet been described. In our study, association of the *TRAF1-C5* locus has been observed in more than one sample set considerably reducing the chances of false positive findings due to population stratification.

The association of the *TRAF1-C5* region has not been reported in the recently published genome-wide association studies (GWAS) in SLE(13;17;18) and the GWAS of T1D in WTCCC study(19). However, while an association with RA was initially not detected in the WTCCC , a follow-up report showed a significant association of the same allele in the UK population although with much a much lower effect size(20). In line with this observation, a meta-analysis of GWA studies in RA has recently illustrated that much larger sample sizes are required to detect the commonly observed low penetrance of genes in autoimmune diseases like RA(21). It is also not uncommon that modest effects are not detected in such largescale studies as exemplified by the association of TNFSF4, a gene identified by family-based association studies(22), that did not surface in the recently performed GWAS. Likewise BANK1, a gene identified in the GWA scan by Kozyrev et al was not identified by the other two GWA studies that employed 500K SNPs. However, while our study combined with the GWA of the WTCCC does not provide sufficient evidence of the role of the *TRAF1-C5* locus in T1D, data obtained from the SLEGEN study confirms our findings of this locus in SLE. Interestingly, this association is absent in a well-powered Japanese case-control study and small study in the Columbian population(6;23). As with many genetic loci, it is highly likely that ethnic differences exist in the contribution of this locus to SLE.

TRAF1 has been reported to be a negative regulator of the TNF receptor signalling cascade(10;24) and high levels of TNF has been detected in both human and murine lupus, it is likely that a dysregulation of the function and/or expression of this molecule could be involved in the inflammatory processes in SLE(10;25). Currently, controlled clinical trials involving the blockade of TNF are being conducted(26). Although the haplotype block encompassing rs10818488 does not encompass the C5 coding region(4), this central component of the complement system is also a likely functional candidate in SLE. The prominent role of complement activation in humans and murine models of SLE as well as the beneficial effect of blocking C5 anaphylatoxin in murine lupus models point to the likely role of this molecule in SLE(27;28). In summary, we report here for the first time an association of the *TRAF1-C5* locus with SLE which, in combination with previous findings of an increased risk to RA and juvenile arthritis, indicates that this region is likely to be part of a shared mechanism underlying several autoimmune diseases.

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