

SHORT COMMUNICATION

Variation within the *CLEC16A* gene shows consistent disease association with both multiple sclerosis and type 1 diabetes in Sardinia

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Variation within intron 19 of the *CLEC16A* (*KIAA0350*) gene region was recently found to be unequivocally associated with type 1 diabetes (T1D) in genome-wide association (GWA) studies in Northern European populations. A variant in intron 22 that is nearly independent of the intron 19 variant showed suggestive evidence of association with multiple sclerosis (MS). Here, we genotyped the rs725613 polymorphism, representative of the earlier reported associations with T1D within *CLEC16A*, in 1037 T1D cases, 1498 MS cases and 1706 matched controls, all from the founder, autoimmunity-prone Sardinian population. In these Sardinian samples, allele A of rs725613 is positively associated not only with T1D (odds ratio = 1.15, *P* one-tail = 5.1×10^{-3}) but also, and with a comparable effect size, with MS (odds ratio = 1.21, *P* one-tail = 6.7×10^{-5}). Taken together these data provide evidence of joint disease association in T1D and MS within *CLEC16A* and underline a shared disease pathway.

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Multiple sclerosis and type 1 diabetes are serious chronic disorders with a putative, only partially understood, autoimmune pathogenesis. Both diseases are most common in European and derived populations and within Europe are more prevalent in northern latitudes and relatively rarer proceeding southward, with the notable exception of the Mediterranean island of Sardinia. In Sardinia, multiple sclerosis (MS) and type 1 diabetes (T1D) not only have a much higher frequency compared with surrounding populations, but they also show an increased probability of co-occurrence, in the same individuals and in the same families.¹ A similar trend has been observed in other populations.²

The human leukocyte antigen region on chromosome 6p21 contains the major component of inherited risk in both diseases, albeit with a much stronger effect size in T1D than in MS. However, the inheritance of MS and

T1D as well as their co-occurrence, is only partially explained by shared genotype variation within this region.³

The recent advent of first generation genome-wide association (GWA) studies is beginning to allow a more systematic clarification of the genetic bases of these diseases, although only a few populations have been assessed so far, and a significant portion of the genetic factors involved in T1D—and even more dramatically in MS—remains to be determined.

Of the associated variants recently noted in these first generation GWA surveys, the *CLEC16A* gene on 16p13 is of particular interest. Two variants (rs725613 and rs12708716) located in intron 19 of this gene and in perfect LD ($r^2 = 1$) with each other were found to be significantly associated with T1D in two independent studies of Northern Europeans and Americans.^{4,5} Furthermore, another variant (rs6498169), located in intron 22 of the same gene and independent ($r^2 = 0.2$ in public databases) from the block of polymorphisms associated with T1D, showed some suggestive evidence of association with MS in a subsequent GWA scan.⁶ Even if one of the polymorphisms, rs12708716, reported to be associated with T1D by Todd *et al.*⁵ was incorporated in the assay employed in the MS GWA study,⁶ no information was reported regarding its association with

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MS, and thus it could not be concluded whether the associations observed in the two diseases were related.

To assess whether variation in the *CLEC16A* gene is consistently associated in T1D and MS, we selected a marker, rs725613, within *CLEC16A*, representative of the previously reported associations with T1D, and genotyped it in Sardinian T1D and MS sample sets. In selecting this SNP, we considered the previously reported unequivocal associations with T1D and their r^2 values obtained from the Genome Variation Server (<http://gvs.gs.washington.edu/GVS/>). The first step of our study was to establish whether rs725613 was also associated with T1D in Sardinia. After we had confirmed the association of this variant with T1D, it was interesting to assess whether the same variant was also associated with MS in sample sets from the same population.

Marker rs725613 was thus typed in 1037 independent T1D cases (453 sporadic patients and 584 affected children derived from the families), 1498 independent MS cases (1088 sporadic patients and 410 affected children derived from the families) and 1706 matched controls (712 Sardinian blood donors and 994 affected family-based controls⁷ deriving from T1D and MS family trios). Affected family-based controls frequencies are based on the alleles that are never transmitted from the parents to affected children and provide allelic frequencies comparable to those detected at the general population level in the absence of population stratification.^{7,8} The detailed description of the samples assessed in the study is presented in Table 1.

Tests of association for each disease were assessed by comparing allelic and genotypic frequencies detected in all patients and controls for rs725613 using both family and case-control data. The results of analysis of association are presented in Table 2. Allele A of rs725613, with a frequency of 47% in T1D patients, was significantly over-represented when compared with the control frequency of 43% (P one-tail 5.1×10^{-3} , OR = 1.15). Similarly, with a frequency of 48%, the same allele was also significantly more common in MS patients when compared with the control frequency (P one-tail 6.7×10^{-5} , OR = 1.21).

To rule out the possibility of spurious results because of population stratification, we reassessed disease association using the transmission disequilibrium test¹¹ on trio families that were already included in the overall association tests. Allele A was significantly more transmitted from parents to affected children with T1D (Transmission = 55.6%, P one-tail 3.5×10^{-3}) and with MS (Transmission = 57.0%, P one-tail 4.3×10^{-3}).

Genotypic analysis indicates that the A/A genotype conferred an increased risk for both diseases compared with the C/C baseline genotype, with a more striking effect in MS than in T1D (P one-tail 4.5×10^{-3} , OR = 1.35 for T1D and P one-tail 3.1×10^{-5} , OR = 1.5 for MS), whereas no significant association was detected for the A/C heterozygous individuals relative to the same C/C reference genotype (see Table 2). These results seem to suggest a recessive-like model of the inherited risk at *CLEC16A*, although this needs to be reassessed in much larger sample sets.

Interestingly, although the negatively associated allele C of the rs725613 represents the minor allele in the European (average frequency in the controls ranging from 28 to 43% in the dbSNP build 128) and Asian (average frequency ranging from 17 to 30% in the SNPdb) populations, in both Sardinians and Africans (average frequency in the controls of 65% in the SNPdb), it is the common allele. This, coupled with the observed small genetic effects, indicates that variation in the *CLEC16A* region cannot explain either the high prevalence of T1D and MS or the co-inheritance of these diseases in Sardinia.

Overall, the available data highlight not only the existence of shared pathways but also the complexity of the different associations in different autoimmune diseases. For instance, the same *CLEC16A* variant associated with both T1D and MS does not show any indication of association with Graves' disease.⁵ Reciprocally, the SNP +1858C>T within the *PTPN22* gene is positively associated with T1D, rheumatoid arthritis and Graves' disease, but not with MS. Furthermore, although the causal variant within *PTPN22* has been fine-mapped¹² and its functional consequence delineated,^{13,14} no

Table 1 Demographic characteristics of the patients and controls

	MS family trios	MS case subjects	T1D family trios	T1D case subjects
Female	288 (70%)	744 (68%)	265 (45%)	254 (56%)
Male	122 (30%)	344 (32%)	319 (55%)	199 (44%)
Total	410	1088	584	453
Age at disease onset (years)	6–50,	5–60,	4–17,	1–17,
(range and mean (\pm s.d.))	25.1 (\pm 7.4)	31.3 (\pm 9.96)	7.8 (\pm 4.1)	8.7 (\pm 4.4)
<i>MS disease course</i>				
Bout onset	381 (93%)	967 (89%)		
Primary progressive	29 (7%)	121 (11%)		

All T1D and MS patients were of Sardinian origin. T1D patients were diagnosed according to standard clinical criteria whereas MS patients were diagnosed according to the McDonald criteria.⁹ Regarding MS disease course, both relapsing remitting and secondary progressive forms were considered together as Bout onset course. The 410 MS family trios include 310 families from the MS centre of Cagliari and 100 families from the MS centre of Sassari whereas the 1088 MS sporadic patients include 812 cases from the MS centre of Cagliari and 276 from the MS centre of Sassari. The 584 T1D family trios and 453 sporadic cases were mainly collected from the Sardinian Paediatric Regional Centre for Diabetes in the Brotzu Hospital in Cagliari and include patients from the whole island. The control group of 712 Sardinian blood donors were recruited from the transfusional blood centres of Cagliari (N 566) and Sassari (N 146) and were selected to be matched as much as possible for intra-regional origin of their grandparents with the sporadic cases. The study was subject to ethical approval by the Ethical Committees of Cagliari and Sassari and informed consent was obtained from all the participants.

Table 2 Results of association analysis of variant rs725613 within *CLEC16A*

rs725613 ^a	T1D		MS		Controls		P one-tail T1D	OR T1D	P one-tail MS	OR MS
	N	%	N	%	N	%				
<i>Allele</i>										
A	969	0.47	1436	0.48	1473	0.43	5.1×10^{-3}	1.15 (1.03–1.29)	6.7×10^{-5}	1.21 (1.10–1.34)
C	1105	0.53	1560	0.52	1939	0.57				
Total	2074		2996		3412					
<i>Genotype</i>										
A/A	219	0.21	352	0.23	303	0.18	4.5×10^{-3}	1.35 (1.08–1.69)	3.1×10^{-5}	1.50 (1.23–1.84)
A/C	531	0.51	732	0.49	867	0.51	7.1×10^{-2}	1.14 (0.96–1.37)	1.4×10^{-1}	1.09 (0.93–1.28)
C/C	287	0.28	414	0.28	536	0.31	1	1	1	1
Total	1037		1498		1706					

^aMarker rs725613 was typed using TaqMan probes and an ABI PRISM 7900 HT System (Applied Biosystems). Hardy–Weinberg equilibrium was tested using Pedstats (www.sph.umich.edu/csg/abecasis/Pedstats/index.html) and did not show significant departure in either patients or control groups. Frequencies of AFBAC controls were computed as described by Thomson.⁷ Disease association was assessed using the UNPHASED software¹⁰ by arranging the data points from patients and controls into a 2 × 2 contingency table and testing them with the Pearson χ^2 test. Genotype OR and apposite significance were computed as pairwise odds ratios using the C/C as reference genotype.

obvious relevant polymorphism has yet been found in *CLEC16A* that can account for the disease associations, and even the function of the gene itself is not well understood.

CLEC16A belongs to the C-type lectin family and is expressed in immune cells. It can be noted that, the C-type lectins, such as the pancreatitis-associated protein, have an anti-inflammatory function and can inhibit NF- κ B activation, downregulating cytokine production and adhesion molecule expression in inflamed tissue. Furthermore, at least two distinct isoforms of *CLEC16A* have been reported (UCSC Genome Browser, NCBI Build 36.1): the longer, carrying the negatively associated allele C (AB002348) was isolated from the brain, whereas the shorter, carrying the positively associated allele A (BC112897), was found in lymphocytes. Hence, it is possible that alternative splicing mechanisms can account for the effect of *CLEC16A* in autoimmunity.

Despite the modest effect sizes, the as yet unidentified disease-predisposing mutation, or even the lack of a clear role of the protein and its consequence in autoimmunity, and the available data on *CLEC16A* highlight the power of the joint analysis of different autoimmune diseases and provide relevant mechanistic clues that should be investigated further both genetically and biologically.

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