Published in final edited form as:
Nat Genet. ; 44(1): 3–5. doi:10.1038/ng.1037.

Rare and functional SIAE variants are not associated with autoimmune disease risk in up to 66,924 individuals of European ancestry

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Note: Supplementary information is available on the Nature Genetics website.

AUTHOR CONTRIBUTIONS D.A.vH and J.A.T. designed the study and wrote the manuscript. D.A.vH. performed statistical analysis. K.A.H. and D.J.S. performed a major part of the genotyping and sequencing. All other authors contributed to sample collection and/or genotyping. All authors reviewed and approved the final manuscript.

COMPETING FINANCIAL INTERESTS The authors declare no competing financial interests.

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To the Editor:

Recently, rare loss-of-function genetic variants in the sialic acid acetylesterase (*SIAE*) gene were reported to predispose to multiple human autoimmune diseases[1]. Surolia *et al.*, in a pooled analysis of ten autoimmune diseases, identified twelve distinct non-synonymous *SIAE* risk variant genotypes, present in 24 of 923 (2.60%) cases versus 2 of 648 controls (0.31%, P=0.0002, odds ratio 8.6), that were considered to be "functionally-defective *SIAE* alleles"[1] owing to either esterase activity or secretion defects. These non-synonymous markers comprised one common (SIAE-M89V, rs78778622) and eleven rare allele frequency variants. The secretion-defective homozygous SIAE-89V/89V (rs78778622 GG) genotype was reported in 8 of 923 cases (0.87%) but none of 648 control subjects[1]. To date, compared to common variant genome wide association studies, there are few studies reporting rare variants of large effect predisposing to clinically typical autoimmune disease phenotypes, although much recent enthusiasm for exome sequencing in these genetically complex conditions.

We sought to replicate and extend these *SIAE* findings in a much larger independent study of autoimmune and chronic immune diseases (atopic eczema, coeliac disease, Crohn's disease, Graves' disease, Hashimoto's disease, juvenile idiopathic arthritis, multiple sclerosis, rheumatoid arthritis, sarcoidosis, systemic lupus erythematosus, type 1 diabetes and ulcerative colitis). Individuals were of white European-origin from five geographic regions. Common (i.e. minor allele frequency >5%) genetic risk variants substantially overlap between multiple autoimmune and immune-mediated disorders[2], and we therefore considered that the analysis pooled across autoimmune diseases performed by Surolia *et al.* was rational, and performed the same analysis on our data.

In 66,924 subjects, we found SIAE-89V/89V genotype frequencies to be similar between cases (12 autoimmune diseases, comprising 99.6% of the cases in the original report[1]) and controls (Table 1), observing 60 SIAE-89V/89V homozygous UK control subjects (0.32%). We found SIAE-89V/89V homozygotes in all control collections, and at similar genotype frequencies to cases. To confidently exclude any bias owing to population stratification or admixture, we genotyped SIAE-M89V in 4,805 independent European-origin parent/affected offspring trios (five autoimmune diseases, Table 2). No support for SIAE-M89V risk was observed.

We then studied eight additional rare *SIAE* variants in 43,378 subjects (ten diseases and controls). These eight variants, along with SIAE-89V/89V, were reported as the "functionally-defective *SIAE* alleles" present in 21 of 24 (88%) defective genotype carrying cases by Surolia *et al.*[1] Functionally-defective genotype burden did not differ between cases (ten diseases) and controls (Table 1, Supplementary Table 1). We did not observe an excess of transmissions (Supplementary Table 2) of the eight rarer variant *SIAE* alleles in 2,286 parent/affected offspring (Crohn's disease, coeliac disease, type 1 diabetes) trios, nor any evidence for mis-inheritance to suggest *de novo* mutations.

Our data therefore do not support the genetic association findings of Surolia *et al.*[1] We note that a linkage signal would be predicted for variants of the large effect size reported[1,3], yet none was observed at *SIAE* in type 1 diabetes in a large recent study[4]. We have no reason to doubt the reported effect of the variants on *SIAE* function[1], but importantly, even when non-synonymous variants in a gene appear, and are then experimentally proven, to be functionally relevant this may not alter the prior probability that such variants affect disease susceptibility since the coding exome contains so many thousands of functional rare variants that do not influence phenotypes[5,6]. Fine-scale population sub-structure may confound rare variant association studies, be difficult to detect with current common SNP principal component methods, and only be definitively excluded by family based association analysis.

Investigators performing common variant genome-wide association studies have developed guidelines for reporting novel associations, including both stringent statistical thresholds (necessitating large sample sizes) and independent replication datasets. Similar approaches, ideally including family-based association analysis, should be applied to rare variant studies, as we described previously in the analysis of the *IFIH1* gene in type 1 diabetes[7].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Surolia I, et al. Functionally defective germline variants of sialic acid acetylesterase in autoimmunity. Nature. 2010; 466:243–7. [PubMed: 20555325]
2. Smyth DJ, et al. Shared and distinct genetic variants in type 1 diabetes and celiac disease. N Engl J Med. 2008; 359:2767–77. [PubMed: 19073967]
3. Risch N, Merikangas K. The future of genetic studies of complex human diseases. Science. 1996; 273:1516–7. [PubMed: 8801636]
4. Morahan G, et al. Tests for genetic interactions in type 1 diabetes: linkage and stratification analyses of 4,422 affected sib-pairs. Diabetes. 2011; 60:1030–40. [PubMed: 21266329]
5. Tarpey PS, et al. A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation. Nature genetics. 2009; 41:535–43. [PubMed: 19377476]
6. Durbin RM, et al. A map of human genome variation from population-scale sequencing. Nature. 2010; 467:1061–73. [PubMed: 20981092]

7. Nejentsev S, Walker N, Riches D, Egholm M, Todd JA. Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. Science. 2009; 324:387–9. [PubMed: 19264985]

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Table 1

SIAE variants in autoimmune disease cases and controls

	Samples attempted	SIAE-M89V rs78778622 GG / GA / AA	SIAE-M89V rs78778622 GG frequency	SIAE genotype defective[b]/normal (9 variants)
UK coeliac disease	7728	30 / 846 / 6851	0.388 %	59 / 7669
UK Crohn's disease	2557	7 / 272 / 2277	0.274 %	18 / 2539
UK Graves' disease	2395	7 / 280 / 2106	0.293 %	15 / 2380
UK Hashimoto's disease	416	2 / 47 / 366	0.482 %	4 / 412
UK multiple sclerosis	2970	11 / 345 / 2519	0.383 %	
UK systemic lupus erythematosus	182	1 / 19 / 162	0.549 %	
UK type 1 diabetes	6772	32 / 757 / 5981	0.473 %	60 / 6712
UK ulcerative colitis	2850	7 / 294 / 2549	0.246 %	14 / 2836
British 1958 Birth Cohort controls	7128 (Taqman)[a] 5430 (Infinium)[a]	21 / 843 / 6171	0.299 %	25 / 5405
Cambridge BioResource controls	8352	25 / 921 / 7322	0.302 %	
ECCAC human random controls	480	2 / 57 / 394	0.442 %	
UK Blood Services - Common Controls	2844	12 / 323 / 2507	0.422 %	23 / 2821
UK: autoimmune disease vs. controls			***P*=0.37**	***P*=0.13**
Dutch rheumatoid arthritis	1031 (Taqman)[a] 561 (Infinium)[a]	3 / 112 / 892	0.298 %	1 / 560
Dutch Crohn's disease	1199	4 / 114 / 1080	0.334 %	6 / 1193
Dutch coeliac disease	1123	3 / 128 / 992	0.267 %	4 / 1119
Dutch controls	1147	5 / 140 / 1002	0.440 %	10 / 1137
Dutch: autoimmune disease vs. controls			***P*=0.55**	***P*=0.09**
US juvenile idiopathic arthritis	784	1 / 64 / 718	0.128 %	1 / 783
US controls	634	1 / 64 / 569	0.158 %	4 / 630
US: autoimmune disease vs. controls			***P*=1.00**	***P*=0.18**
German Crohn's disease	1885 (Taqman)[a] 691 (Infinium)[a]	7 / 180 / 1663	0.378 %	3 / 688
German ulcerative colitis	1123 (Taqman)[a] 104 (Infinium)[a]	2 / 109 / 987	0.182 %	2 / 102
German atopic eczema	1678	5 / 185 / 1488	0.298 %	9 / 1669
German sarcoidosis	1781	8 / 183 / 1589	0.449 %	10 / 1771
German controls (collection 1)	1472	5 / 142 / 1291	0.348 %	
German controls (collection 2)	2684	7 / 264 / 2410	0.261 %	12 / 2672
German: autoimmune disease vs. controls			***P*=0.65**	***P*=0.61**

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	Samples attempted	SIAE-M89V rs78778622 GG / GA / AA	SIAE-M89V rs78778622 GG frequency	SIAE genotype defective[b]/normal (9 variants)
Sardinian type 1 diabetes	726	0 / 26 / 700	0.000 %	
Sardinian multiple sclerosis	2294	1 / 84 / 2209	0.044 %	
Sardinian blood donors	2689	1 / 90 / 2598	0.037 %	
Sardinian: autoimmune disease vs. controls			***P*=1.00**	
Meta-analysis	**66924 43378**		***P*=0.45**	***P*=0.44**

[a] substantial overlap between the samples genotyped by Taqman (M89V data only) and Infinium (9 variants) assay

[b] sum of individuals with SIAE-89V/89V homozygous risk genotype, and SIAE-W48X, C196F, G212R, Q309P, R314H, Y349C, F404S, R479C heterozygous risk genotypes (as[1]). A detailed breakdown by each variant is in Supplementary Table 1, and description of genotyping and statistical analysis in Supplementary Methods.

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Table 2

Family based association studies of *SIAE* variants.

Disease	**Sample size**	**Transmission disequilibrium test SIAE-M89V (Transmissions / Non-Transmissions)**
European-origin type 1 diabetes (T1DGC)	906 multiplex families (1703 trios)	170 T / 177 NT
UK systemic lupus erythematosus	124 single affected child-parent trios	10 T / 18 NT
UK multiple sclerosis	1153 single affected child-parent trios	125 T / 122 NT
European-origin coeliac disease	483 single affected child-parent trios	44 T / 55 NT
Dutch Crohn's disease	100 single affected child-parent trios	5 T / 11 NT
Sardinian type 1 diabetes	679 single affected child-parent trios	11 T / 21 NT
Sardinian multiple sclerosis	563 single affected child-parent trios	23 T / 16 NT
Meta-analysis: all autoimmune disease		***P*=0.124**