The role of functionally defective rare germline variants of sialic acid acetylesterase in autoimmune Addison’s disease

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Abstract

Background: Autoimmune Addison’s disease (AAD) is a rare condition with a complex genetic basis. A panel of rare and functionally defective genetic variants in the sialic acid acetylesterase (SIAE) gene has recently been implicated in several common autoimmune conditions. We performed a case–control study to determine whether these rare variants are associated with a rarer condition, AAD.

Method: We analysed nine SIAE gene variants (W48X, M89V, C196F, C226G, R230W, T312M, Y349C, F404S and R479C) in a United Kingdom cohort of 378 AAD subjects and 387 healthy controls. All samples were genotyped using Sequenom iPLEX chemistry to characterise primer extension products.

Results: A heterozygous rare allele at codon 312 (312*M) was found in one AAD patient (0.13%) but was not detected in the healthy controls. The commoner, functionally recessive variant at codon 89 (89*V) was found to be homozygous in two AAD patients but was only found in the heterozygous state in controls. Taking into account all nine alleles examined, 4/378 (1.06%) AAD patients and 1/387 (0.25%) healthy controls carried the defective SIAE alleles, with a calculated odds ratio of 4.13 (95% CI 0.44–97.45, two-tailed P value 0.212, NS).

Conclusion: We demonstrated the presence of 89*V homozygotes and the 312*M rare allele in the AAD cohort, but overall, our analysis does not support a role for rare variants in SIAE in the pathogenesis of AAD. However, the relatively small collection of AAD patients limits the power to exclude a small effect.
contrast to the common disease-common variant hypothesis, there may be a greater role for rare genetic variants in the susceptibility to less prevalent autoimmune diseases (13, 14).

SIAE is a negative regulator of B lymphocyte signalling by acting at inhibitory receptors that attenuate B-cell receptor signalling. Spontaneous autoantibody production has been demonstrated in SIAE-mutant mice on a C57Bl/6 background, suggesting that defects in SIAE function conferred by SIAE variants might contribute to human autoimmunity (15). Genome-wide studies have not demonstrated any association or linkage with SNPs at the SIAE locus in patients with autoimmunity; however, under a multiple rare variants model, this might not be expected. Nevertheless, AAD is rarer than all the previously studied disorders and hence rare variants might have a significant contribution to its pathogenesis. The purpose of our study is to explore whether these rare SIAE variants are associated with AAD, which has not previously been studied (11, 12, 16).

Materials and methods

Three hundred and seventy-eight Caucasian subjects with AAD have been recruited since 1996 through outpatient endocrinology services in the North East of England and the UK Addison’s disease self-help group. The diagnosis of AAD was confirmed by either a subnormal response to the ACTH1–24 stimulation test (using 250 μg of parenteral synthetic ACTH), or a low basal cortisol with a high ACTH level. Patients with APS1, primary adrenal failure owing to infiltrative or infective causes or secondary adrenal failure were excluded. Three hundred and eighty-seven healthy local Caucasian controls were used for comparison (including 113 individuals from a 1958 birth cohort). This study was carried out with approval of the Leeds (East) Research Ethics Committee (Ref 05/Q1206/144).

Table 1 Genotypes for SIAE variants in autoimmune disease cases and controls.

<table>
<thead>
<tr>
<th>Autoimmune Addison’s disease</th>
<th>UK healthy controls</th>
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<tbody>
<tr>
<td>Genotypea</td>
<td>Rare allele/genotype frequency</td>
</tr>
<tr>
<td>W48X</td>
<td>0/0/370</td>
</tr>
<tr>
<td>M89V</td>
<td>2/42/303</td>
</tr>
<tr>
<td>C196F</td>
<td>0/1/374</td>
</tr>
<tr>
<td>C226G</td>
<td>0/0/371</td>
</tr>
<tr>
<td>R230W</td>
<td>0/0/375</td>
</tr>
<tr>
<td>T312M</td>
<td>0/1/372</td>
</tr>
<tr>
<td>Y349C</td>
<td>0/0/360</td>
</tr>
<tr>
<td>F404S</td>
<td>0/0/372</td>
</tr>
<tr>
<td>R479C</td>
<td>0/0/358</td>
</tr>
</tbody>
</table>

aData presented as number of rare homozygote/heterozygote/common homozygote genotypes.

bHomozygous 89*V genotype.
The association between rare SIAE genetic variants and disease susceptibility has recently been explored in some common autoimmune diseases, with conflicting results among various research groups (11, 12, 16, 17). Nevertheless, the loss-of-function rare variants in the TREX1 and CYP27B1 genes have been significantly implicated in SLE and MS patients respectively (13, 14), suggesting the possibility that rare genetic variants may have a role in risk susceptibility for some of the less prevalent autoimmune diseases. We explored the hypothesis that rare SIAE variants would be associated with AAD, one of the least common autoimmune conditions. We demonstrated the presence of two codon 89*V homozygotes and one heterozygous carrier of the 312*M allele in the AAD cohort, which was not significantly different from healthy control genotypes. Thus, our findings extend the negative study of Hunt et al. (12) who showed no differences for nine of the SIAE gene rare variants (including M89V but not T312M) in cohorts of the commoner autoimmune and inflammatory conditions, such as type 1 diabetes, atopic eczema, celiac disease, Graves’ disease and Hashimoto thyroiditis. However, this large cohort of nearly 67 000 subjects did not include patients with the rarer condition of AAD.

While our study had good power to replicate findings of a similar magnitude to those previously seen in other autoimmune conditions (OR 8.6) (11), our analysis is underpowered to detect a more subtle genetic effect, such as is more frequently seen for commoner autoimmune disease susceptibility alleles. We cannot exclude that a future analysis of an enlarged AAD cohort or a family-based genetic association study may cast further light on this question. However, the parameters for declaring significance in studies of rare genetic variants are not yet well defined (18), and the key attribute of reproducibility has not been fulfilled for SIAE variants in autoimmune diseases to date.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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