Airway wall splice quantitative trait locus analysis reveals novel downstream mechanisms for known asthma single-nucleotide polymorphisms

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To the Editor:

Genome-wide association studies (GWAS) [1] have identified single-nucleotide polymorphisms (SNPs) associated with the presence of asthma. It is notoriously difficult to link identified SNPs to downstream functional effects leading to increased asthma risk. A possible strategy to do this is to perform expression quantitative trait locus (eQTL) analyses, which pinpoint the direct effects of SNPs on mRNA expression levels. However, eQTL analyses merely focus on the expression level and do not consider alternative splicing. Alternative splicing is a naturally occurring process in which RNA can be spliced into multiple different transcripts, leading to different forms of a protein, which can strongly affect its function [2]. The present study aimed to identify the effect of asthma-related SNPs on alternative splicing of genes expressed specifically in the airway wall, the predominant site where the inflammatory process occurs. We hypothesised that this might reveal novel downstream mechanisms linking previously identified GWAS SNPs to asthma.

To this end, we used genetic and airway wall biopsy transcriptomic data from 146 subjects, including 50 patients with asthma, 25 subjects in clinical or complete asthma remission and 71 healthy controls. Their mean age was 44 years. 52% of the subjects were male and 50% were either current or past smokers. DNA was isolated from blood and RNA was isolated from bronchial biopsies for RNA sequencing as described previously [3, 4]. Splice site usage was quantified by counting split reads (mapping across exon–exon junctions) as described previously [5, 6]. For each intron in the alignment, the chromosome and first and last intron base were recorded. Trimmed mean of the M-values normalisation was used for split-read counts. We used R, RStudio and the package MatrixEQTL [7–9] to identify splice-QTLs and eQTLs from the split-read and transcript count data. Both the splice-QTL and eQTL analyses were performed using the same cohort.

The associated risk for asthma with the genetic variants is well known and described, and was not assessed in the current cohort due to the limited sample size. We decided to pool groups of asthma, controls and remission subjects to increase the power of our analysis, assuming that the downstream effects of the SNPs associated with the expression of genes or splice variants would be the same in asthma and controls.

A total of 1868 SNPs were associated with asthma in the GWAS catalogue. Of those, 149 were splice-QTLs. After combining these results with the eQTL analyses, we found 28 SNPs to be only splice-QTLs, and not eQTLs, of the affected gene. The remaining 121 SNPs were both splice-QTLs and eQTLs, and were not of interest for this analysis as the significance of the finding is probably mostly driven by the eQTL effect. The 28 splice-QTLs involved four unique target genes. These four unique splice-QTL hits are included in the table in figure 1.

The SNP with the largest effect was rs11078928, a splice-QTL for *GSDMB* ($\beta = -2.13$, $p=1.70\times10^{-11}$, false discovery rate (FDR) 3.33×10^{-7}). More precisely, the CC genotype of rs11078928 was associated with the expression of a *GSDMB* transcript lacking exon 6, labelled "c" in figure 1 and known as ENST00000394179.5 in the Ensembl database. The transcript labelled "a" is a transcript including the full

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Studying the effects of asthma SNPs on alternative splicing can lead to new insights into asthma pathophysiology. More specifically, a 17q12 SNP is associated to alternative splicing of *GSDMB*. https://bit.ly/3W49oTs

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Splice QTL results

SNP	Gene	Reference allele	Splice-QTL β-value	Splice-QTL p-value	Splice-QTL FDR	eQTL β-value	eQTL p-value	eQTL FDR	GWAS p-value	Junction
rs13203830	HLA-A	G>A	−98.83	4.94×10^{-23}	1.15×10^{-17}	−0.34	4.59×10^{-4}	0.20	8×10^{-10}	6_29911321_29911898
rs9852988	LPP	A>G	27.28	4.19×10^{-14}	1.52×10^{-9}	−1.26	1.45×10^{-3}	0.37	3×10^{-8}	3_188059508_188123899
rs11078928	GSDMB	T>C	−2.13	1.70×10^{-11}	3.33×10^{-7}	0.94	3.69×10^{-4}	0.18	2×10^{-16}	17_38064468_38065210
rs11042902	MRVI1	C>T	−2.82	2.24×10^{-7}	1.46×10^{-3}	0.44	7.58×10^{-4}	0.27	2×10^{-10}	11_10655619_10711811

GSDMB variants

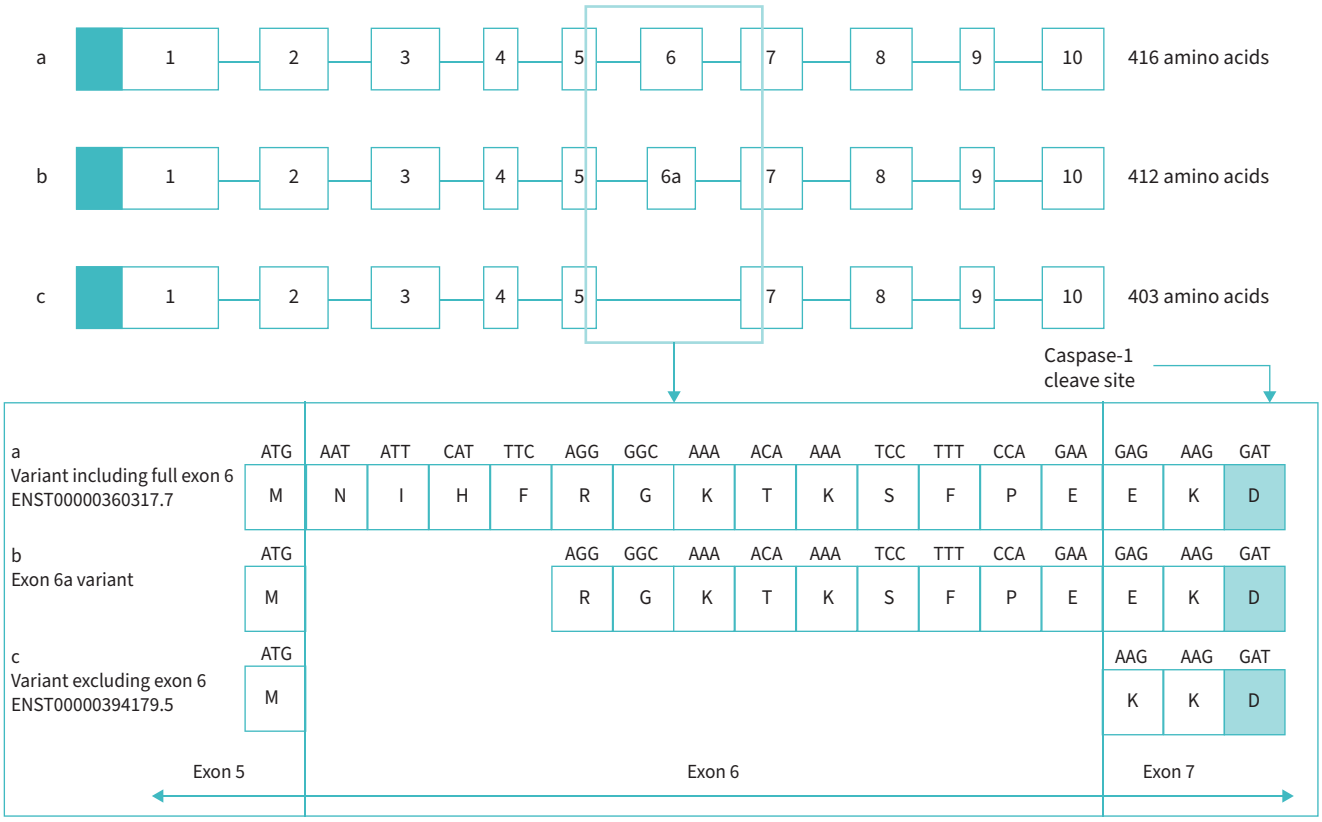

FIGURE 1 Results of the splice quantitative trait locus (QTL) analyses including the splice-QTL hits with splice-QTL false discovery rate (FDR) <0.05 and expression quantitative trait locus (eQTL) FDR >0.05, sorted by splice-QTL FDR (top). Schematic representation of the different gasdermin B (*GSDMB*) splice variants on an exon level including the length in amino acids on the right (middle). Variants a, b and c match in the middle panel and bottom panels. The differences between *GSDMB* splice variants on the nucleotide and amino acid levels (bottom). SNP: single-nucleotide polymorphism; GWAS: genome-wide association study; HLA: human leukocyte antigen; LPP: lipoma-preferred partner; MRVI1: murine retrovirus integration site 1 homologue.

length exon 6 (ENST00000360317.7 in the Ensembl database). In addition, we identified another splice-QTL involving the same SNP rs11078928 (splice junction 17_38064455_38065210). In this case, the CC genotype of rs11078928 was associated with higher expression of a *GSDMB* splice variant with a truncated exon 6 with 12 base pairs missing from the exon, labelled "b" in figure 1. From here on, we will refer to this variant as the exon 6a variant. The exon 6a variant was found almost exclusively in subjects with a C-allele. Next, a PCR was performed to confirm the existence of this variant. We amplified the RNA from bronchial biopsies and used a validated primer for *GSDMB* and a custom primer for the exon 6a variant. By visual inspection, we found *GSDMB* to be present in the CC and CT genotype, and we confirmed the existence of the exon 6a variant in bronchial tissue of the one subject with remaining RNA material and a CC genotype. This confirmed the existence of the newly identified exon 6a variant in

bronchial tissue. rs11078928 is not an eQTL or splice-QTL of *ORMDL3*, a gene close to *GSDMB* and often linked to the SNP in the literature.

GSDMB is located in the 17q12–21 region, which has been linked to asthma in multiple GWAS [1]. *GSDMB* encodes the gasdermin B protein, which forms pores in the cell membrane upon cleavage by caspase-1 and subsequently leads to the induction of pyroptosis, a programmed form of cell death that coincides with the release of pro-inflammatory damage-associated molecular patterns into the extracellular space [10]. In the intact protein, the carboxy-terminal region inhibits the formation of the pores. After caspase-dependent cleavage, the amino-terminal product will form the pore in the membrane. Previously, both Panganiban *et al.* [11] and Gui *et al.* [12] showed that the CC genotype of rs11078928 results in the exclusion of the full exon 6 in blood. Here, we extend this observation to the airway wall, the primary site of inflammation in asthma [11]. As described by Panganiban *et al.* [11], the alternatively spliced gasdermin B excluding exon 6 prevents gasdermin B-mediated pyroptosis. We show that the C-allele of rs11078928 is not only associated with the exon 6 variant but we now also describe a novel exon 6a variant. This exon 6a variant retains a larger part of the exon 6, possibly maintaining the pyroptosis execution in CC genotype subjects that is lost in the presence of rs11078928. If this is indeed a compensatory mechanism, this would highlight the importance of maintaining, at least homeostatic levels of, pyroptosis that may be needed to preserve sufficient immune response.

Another splice-QTL is rs9852988 and one of the *LPP* transcripts (splice-QTL: β=27.28, p=4.19×10^{-14}, FDR 1.52×10^{-9}) coding for a protein called lipoma-preferred partner. According to Zhu *et al.* [13], the G-allele and minor allele of rs9852988 result in greater asthma risk. We found that the G-allele associated with retention of *LPP* exon 2. The LPP protein has previously been found to be of importance in modulating epithelial (E)-cadherin-modulated cell adhesion [14]. E-cadherin is an essential molecule in the airways and decreased E-cadherin expression has been found in the airway epithelium of asthma patients [15]. In an *in vitro* model, *LPP*-knockdown Madin–Darby canine kidney cells exhibited less adhesion to E-cadherin-coated wells and had lower transepithelial resistance, a measure of tight junction strength [14]. Therefore, the splice-QTL of *LPP* is of interest, because the alternative splice variant could influence epithelial barrier function. Another nominally significant splice-QTL for the same SNP (rs9852988) was associated with a loss of exon 2 in the *LPP* transcript and showed high expression in the major allele (A). The affected exon is in a noncoding region close to the start of the coding region of *LPP* and hypothetically, the presence of exon 2 could decrease the stability of the RNA transcript. The Kozak sequence appears not to be affected by this splice variant, which means the ribosomal binding site remains intact.

In conclusion, we showed that multiple asthma-associated SNPs are splice-QTLs in the airway wall. We have studied two of these splice-QTL hits more extensively. Here, we observed that rs11078928, located in the 17q12 region, affects alternative splicing of *GSDMB* exon 6. This results in either exclusion of the full exon or part of the exon. The variant excluding exon 6 has previously been shown to result in loss of function of the resulting protein, whereas the effect of the newly discovered variant excluding only part of exon 6 remains unknown. We propose that our findings reveal a compensatory mechanism to restore pyroptosis in CC genotype patients. The downstream effects of rs9852988 on splicing of *LPP* have yet to be discovered.

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