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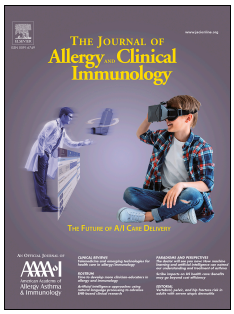

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TSLP disease-associated genetic variants combined with airway TSLP expression to influence asthma risk

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ABSTRACT

Background: Thymic stromal lymphopoietin (TSLP) is an epithelial-derived cytokine important in initiation of allergic inflammation. Single nucleotide polymorphisms (SNPs) in *TSLP* are associated with asthma, yet studies have shown inconsistent associations between circulating TSLP and asthma. Studies that integrate the combined effects of *TSLP* genotype, TSLP mRNA, circulating TSLP levels, and asthma outcome are lacking.

Objective: To recruit a novel cohort based on asthma-relevant *TSLP* SNPs and determine their impact on TSLP mRNA expression and TSLP circulating protein levels, and their individual and combined effects on asthma.

Methods: We developed an algorithm to prioritize *TSLP* SNPs and recruited 51 carriers and non-carriers based on *TSLP* genotypes. We quantified TSLP mRNA in nasal epithelial cells and circulating TSLP levels in plasma. We determined associations of defined *TSLP* risk genotypes and/or TSLP mRNA and protein levels with asthma.

Results: TSLP mRNA expression, but not circulating TSLP, was significantly increased in asthmatics compared to non-asthmatics (P=0.007; OR=1.44). Notably, 90% of children with the defined *TSLP* risk genotypes and high nasal TSLP mRNA expression (top tertile) had asthma compared to 40% of subjects without risk genotypes and with low TSLP expression (bottom tertile) (P=0.024). No association between circulating TSLP and asthma was observed.

Conclusion: Collectively, these data suggest childhood asthma is modified by the combined effects of *TSLP* genotype and TSLP expression in the nasal epithelium. The increased asthma risk likely manifests when genetic variation enables eQTL in the *TSLP* locus to elevate TSLP. It is important to consider both biomarkers when factoring asthma risk.

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Clinical Implications:

TSLP genotype and nasal mRNA expression may be useful biomarkers to aid asthma prediction and/or identify individuals who would benefit from therapy targeting this pathway.

Capsule Summary:

Asthma risk is modified by the combined effects of *TSLP* genotype and TSLP expression in the nasal epithelium rather than circulating TSLP. These results suggest asthma risk is increased only when genetic variation enables eQTL in the *TSLP* locus to elevate TSLP mRNA expression.

Key Words:

TSLP, asthma, genotype, gene, biomarker, gene expression, mRNA

Abbreviations:

TSLP: Thymic stromal lymphopoietin

SNP: Single nucleotide polymorphism

NEC: Nasal epithelial cell

Th: T helper

AD: atopic dermatitis

eQTL: expression quantitative trait locus

MAF: minor allele frequency

GTEx: Genotype-Tissue Expression

FAIRE: formaldehyde-assisted isolation of regulatory elements

TF: Transcription factor

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GCPCR: Greater Cincinnati Pediatric Clinic Repository

CCAAPS: Cincinnati Childhood Allergy and Air Pollution Study

LAR: legally authorized representatives

ELISA: enzyme-linked immunosorbent assay

LD: linkage disequilibrium

sfTSLP: short form TSLP

lfTSLP: long form TSLP

CEU: Utah Residents (CEPH) with Northern and Western European Ancestry

ASW: Americans of African Ancestry in South West USA

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INTRODUCTION

Thymic stromal lymphopoietin (TSLP) is an epithelial cell-derived immune cytokine that regulates T helper (Th) inflammatory 2 responses(1, 2). TSLP contributes to the initiation and maintenance of the allergic immune response, is a marker for defects in epidermal barrier differentiation, and is strongly implicated in the pathogenesis of atopic dermatitis (AD) and asthma(1, 3-5). Accordingly, a drug targeting TSLP as a therapeutic target in clinical trials demonstrated decreased airway inflammation and allergen sensitivity(6-9).

Genetic variation in *TSLP* modifies risk for allergic disease among patients carrying risk alleles in skin barrier genes(3, 10). Numerous SNPs in *TSLP* have been associated with AD and asthma(3, 10-24). The impact of *TSLP* genetic variation may be context dependent. Our group demonstrated that asthma risk partly results from independent associations of variants and epistasis between skin-related genes, *TSLP* and *SPINK5*, and that this effect differs by race(10). A recent study found that *TSLP* SNPs increased the risk of asthma among children with AD, especially among those who also had allergic sensitization(20). TSLP mRNA expression in skin and serum protein levels are increased in AD(25-27). TSLP mRNA expression is increased in nasal epithelial and bronchial airway tissues in asthma(28, 29). Disease-associated expression quantitative trait locus (eQTL) in the *TSLP* locus correlate with TSLP expression(14). These studies have been helpful in highlighting the allergic role of TSLP and in identifying potentially relevant genetic variants; however, which SNPs are the most informative in contributing to allergic disease pathogenesis and prevalence remains unclear.

Despite the strong association of *TSLP* genetic variation and epithelial expression with asthma and AD(14, 28-30), studies have failed to find a consistent association of circulating TSLP (a biomarker of epidermal TSLP production) with asthma and allergic disease(27, 31-34). In fact, one study of circulating TSLP found no positive association with recurrent wheezing,

aeroallergen sensitization, or AD(35). This may be due to several factors. Local TSLP expression in skin cells at mucosal surfaces may be more relevant to disease than circulating levels, which may not accurately reflect local tissue levels(25, 31, 36). Genetic variation may have an impact beyond circulating TSLP protein expression, including on other genes or pathways as we demonstrated with *SPINK5(10)*, which may impact asthma development or persistence. Thus, it is critical to simultaneously examine *TSLP* genotype, local expression, and circulating TSLP and determine their individual and combined impact on asthma.

Herein, we developed an unbiased algorithm to prioritize SNPs affecting *TSLP* gene expression levels. We then recruited a novel cohort of carriers with at least one *TSLP* risk allele from the identified *TSLP* SNPs and non-carriers with none of the identified *TSLP* risk alleles. Next, we systematically evaluated the individual and combined impact of *TSLP* genotypes, nasal mRNA expression, and circulating blood level on asthma to determine the functional and disease impact of these *TSLP* SNPs. We found that short form (sfTSLP) and long form TSLP (lfTSLP) expression in airway nasal epithelial cells, but not circulating TSLP levels, were increased in children with asthma compared with non-asthmatic children, suggesting local regulation of TSLP expression in the airway is important in asthma pathogenesis. Importantly, we found evidence of a combined effect of the identified *TSLP* risk alleles and upper airway TSLP expression such that increased sfTSLP and lfTSLP expression only associate with childhood asthma when the *TSLP* risk variants are present demonstrating a novel genotype-dependent contribution of TSLP expression to asthma risk. Collectively, these results suggest that *TSLP* SNPs and local airway expression of TSLP have synergistic contributions to asthma. The increase in asthma risk is likely manifest only when genetic variation enables eQTL in the *TSLP* locus to elevate lfTSLP.

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METHODS

Study population

Fifty-one participants aged 6 to 18 years from the greater Cincinnati, Ohio metro area who were initially enrolled in the Greater Cincinnati Pediatric Clinic Repository (GCPCR)(37) or the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS)(38) were recruited from May to August 2019 using genotyping information available from these cohorts, as previously described(39-41), for *TSLP* SNPs rs2289277 and rs11466750. These SNPs were selected using the methods described in the Supplemental Materials. The GCPCR includes over 7500 patients and CCAAPS includes 762 participants. Carriers had at least one copy of the risk allele for *TSLP* rs2289277 or rs11466750; non-carriers had zero copies of the risk allele for both SNPs. Carriers and non-carriers were 1-to-1 matched on age (±2 years) to account for potential developmental changes. History of ever asthma and AD diagnosis were defined by medical chart review and patient report of history of doctor diagnosis. Individuals were excluded if they were born at gestational age <36 weeks, current age <6 years, or dependent on oral steroids or immunosuppressive drugs. All participants, or their legally authorized representatives (LAR), provided written informed consent for participation in the study. During the study visit, subjects/LARs completed our validated New Visit Questionnaire(37), which collects AD, allergic rhinitis, food allergy, asthma and allergy symptoms; medical history, family history of allergic disease, medications, diet, home characteristics and environmental exposures. Our Demographic Data Questionnaire(37) captured participants' age, sex, race (American Indian/Alaskan Native, Asian, Black, Native Hawaiian, White, Other, Don't Know), parental ethnicity, and socioeconomic status indicators. Race was categorized as Black and White. This study was approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board.

Statistical analyses were performed in R program. A p-value ≤ 0.05 was considered significant due to the correlation between TSLP measures. The full methods are available in the Supplementary Materials.

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RESULTS

Prioritization of *TSLP* SNPs for analysis

A flow chart of SNP selection is outlined in Figure 1A and described in detail in the Supplemental Materials. Of the 670 SNPs located in the human *TSLP* gene based on the 1000 Genomes Project data, there were 26 SNPs with a MAF ≥0.10. We performed LD expansion (r^2>0.9) to evaluate all genetic association candidates in the defined genomic region and they were all included in the 26 SNPs. Next, we selected 3 SNPs that were associated with allergic disease (including asthma, allergen sensitization, AD, and allergic rhinitis) in a published GWAS(21-24, 42-45) and 11 SNPs that were significant eQTLs in lung, skin, or esophageal mucosal tissues(46, 47) based on data accessed on or prior to March 1, 2019 (Figure 1B, Table 1). We performed genomic functional annotation for these 14 *TSLP* SNPs to prioritize those likely to impact TSLP expression.

TSLP transcription regulatory regions

TSLP is induced during epidermal barrier dysfunction and is involved in pathogenesis of asthma and AD(1, 48). FAIRE analyses performed by our colleagues, Lander et al., identified regulatory regions likely to regulate human TSLP expression in response to epidermal mechanical disruption or genetic dysfunction(49, 50). We performed an analysis of dynamic barrier-specific FAIRE peaks by identifying regions of open chromatin that correlated with lfTSLP expression. To select functional *TSLP* SNPs associated with increased asthma prevalence that are likely associated with TSLP expression, we prioritized SNPs that were located within these FAIRE peaks. *TSLP* rs2289277 is located in intron 2 of lf*TSLP* and in the sf*TSLP* promoter (Figure 2A).

Haploreg in silico analyses

Histone modifications are important markers of function and chromatin state. Variants that affect chromatin at distal regulatory sites frequently also direct changes in gene expression at

associated promoters(51). HaploReg analysis predicted overlap of 8 *TSLP* SNPs (including rs10061842, rs10455025, rs3806932, rs3806933, rs1898671, rs2289277, rs11466749, rs11466750) that might affect the histone mark of enhancers and promoters in asthma relevant tissues (see Table E1 in the Online Repository)(52).

SNPs that alter transcription factor binding motifs have been associated with multiple human diseases and may influence transcription factor binding affinity and alter gene expression and regulation. HaploReg analysis predicted altered motifs for 6 *TSLP* variants (rs1837253, rs3806932, rs2289277, rs10073816, rs11466754, rs56390548)(52). SNP2TFBS analysis predicted altered FOXF2 and Sox motifs associated with *TSLP* (rs1837253), and altered NR2C2 binding associated with rs2289277(53).

DNase I sensitivity is an indicator of open chromatin. DNase I hypersensitive sites typically mark regions that are associated with active histone marks and transcription factor binding, and often overlap with FAIRE peaks(51, 54). HaploReg identified DNase marks associated with *TSLP* rs2289277 and rs3806932 in asthma relevant tissues (52).

SNP algorithm scores

Using a simple algorithm that takes the above information into account, we calculated a cumulative score for 14 *TSLP* SNPs (Figure 1). SNPs were assigned one point for each of the functional characteristics examined (Table 1). The scores ranged from 1 to 5 [median=2 points; IQR: 2-3] out of 6 points possible. Figure 2A shows the LD among 5 *TSLP* SNPs with the highest scores in our SNP matrix analysis and 3 SNPs identified in GWAS associations. There is strong LD in the *TSLP* locus and among the 8 SNPs in Figure 2A in European descent (CEU) populations (median D' = 1.0) suggesting shared information among these variants. Figure E1 shows the LD structure derived from African descent (ASW) populations, which exhibited

weaker LD. Two SNPs (rs2289277 and rs3806932) scored 5 points and were strongly linked (r^2 = 0.97). Three SNPs (rs11466749, rs11466750, rs3806933) scored 3 points. *TSLP* rs3806933 is in strong LD with rs2289277 and rs3806932 (r^2 = 0.97 and 1.0, respectively), and rs11466749 and rs11466750 are strongly linked (r^2 = 0.88). Within these two LD blocks, rs2289277 and rs11466750 each have the highest minor allele frequency. On this basis, we prioritized rs2289277 and rs11466750 in our recruitment strategy. Both of these SNPs are eQTLs and have been associated with asthma and AD(10, 13, 14, 49). *TSLP* rs2289277 is located in intron 2 of lf*TSLP* and in the promoter of sf*TSLP,* while rs11466750 is located in the 3' untranslated region of lf*TSLP* and sf*TSLP.*

Demographics and clinical attributes of the *TSLP* cohort

To investigate the impact of genetic variation on TSLP expression and asthma diagnosis, we recruited individuals with 1 or 2 alternate alleles for *TSLP* disease-associated SNPs rs2289277 and/or rs11466750 as carriers using defined risk genotypes and age-matched non-carriers who had 2 copies of the reference allele at both SNPs (n=51, Figure 2B). The cohort included individuals of median age 16.1 years [range: 10.5-17.9 years], majority male sex (54.9%) and white race (58.8%) (Table 2). Carriers with the *TSLP* risk genotypes had a nominally higher prevalence of asthma diagnosis (p=0.064) and significantly higher prevalence of having both asthma and AD diagnosis (p=0.022). Carriers and non-carriers did not differ significantly by age, sex, or race.

***TSLP* risk genotype carriers have increased TSLP mRNA expression in nasal epithelial cells**

In order to investigate the impact of genetic variation on TSLP expression at the local and systemic level, we examined TSLP expression in nasal epithelial cells (NEC) and circulating TSLP levels in plasma comparing carriers with the risk genotypes to non-carriers. Both sfTSLP

289 and lfTSLP transcripts have been reported(30, 32, 55, 56), thus we examined expression of
290 both forms. All subjects had detectable sfTSLP in NEC. Carriers had a significantly greater
291 proportion of detectable lfTSLP in NEC than non-carriers (82.8% vs. 55.0%, p=0.036). Detection
292 of total TSLP circulating in plasma did not significantly differ between carriers and non-carriers.
293 Both sfTSLP and lfTSLP mRNA expression were significantly correlated with each other among
294 carriers with the risk genotypes (rho=0.60, p=0.0005), but not among non-carriers (rho=0.31,
295 p=0.20). Median lfTSLP expression in NECs was significantly higher (p=0.031) and median
296 circulating total TSLP in plasma was nominally higher (p=0.056) in carriers than non-carriers
297 (Figure 3A). Median sfTSLP expression in NECs was non-significantly increased in carriers
298 compared to non-carriers.
299
300 ***TSLP* risk genotype is associated with increased asthma prevalence**
301 Since a recent genetic study found that *TSLP* SNPs increased the risk of asthma among
302 children with AD(20), we evaluated the associations of asthma and AD with *TSLP* risk
303 genotypes. Carriers with the risk genotypes were 3-times more likely to be diagnosed with
304 asthma compared with non-carriers after adjusting for race in logistic regression models
305 (p=0.06; OR 3.05; Table 3). A similar association between risk genotypes and ever having a
306 dual asthma and AD diagnosis was observed compared to non-carriers without the risk alleles
307 after adjustment (p=0.04; OR 3.33). Models adjusting for sex did not yield significantly different
308 effect estimates; thus, sex was not included as a covariate (data not shown).
309
310 **TSLP expression is increased among individuals with asthma**
311 Previous reports suggest that TSLP expression is increased in asthma(28, 29) and disease-
312 associated eQTL in *TSLP* correlate with TSLP expression(14). In our data, median sfTSLP and
313 lfTSLP expression in NECs were significantly higher in subjects with asthma compared to non-
314 asthmatics (p=0.003 and p=0.009, respectively; Figure 3B). Increased sfTSLP and lfTSLP

315 expression in NECs remained significantly associated with increased asthma diagnosis after
316 adjusting for race and sex in logistic regression models using all data (sfTSLP, p=0.011; lfTSLP,
317 p=0.028; Table 4).
318
319 **Individuals with the *TSLP* risk genotype and high TSLP expression are more likely to**
320 **have asthma**
321 Since (1) the *TSLP* risk genotypes were associated with increased asthma and (2) expression
322 of sfTSLP and lfTSLP in NECs were increased among asthmatics, we sought to determine
323 whether there was a combined effect of *TSLP* risk genotype and TSLP mRNA expression on
324 asthma prevalence. We performed analyses of sfTSLP and lfTSLP expression in NECs and
325 asthma prevalence stratified by *TSLP* risk genotypes (Figure 4). Among carriers with the
326 defined risk genotypes, those with high TSLP expression (top tertile) had significantly greater
327 asthma prevalence than those with low TSLP expression (bottom tertile; sfTSLP 100% vs. 29%,
328 p=0.002; lfTSLP 90% vs 30%, p=0.013; Figure 4). Among non-carriers, high sfTSLP and lfTSLP
329 expression were not associated with asthma prevalence. Carriers with high TSLP expression
330 had significantly increased asthma prevalence compared to non-carriers with low TSLP
331 expression (sfTSLP p=0.008; lfTSLP p=0.024). Multivariable logistic regression analysis using a
332 4-level variable for *TSLP* genotype and TSLP expression to account for combinatorial effects
333 showed that asthma prevalence was significantly higher among carriers with the risk genotypes
334 and high lfTSLP expression compared to non-carriers with low lfTSLP expression (aOR=3.415;
335 p=0.024). However, individuals with only one risk factor (being a carrier or having elevated
336 TSLP) were not appreciably different than those with neither risk factor. Similarly, multivariable
337 logistic regression models evaluating the interaction of continuous lfTSLP and sfTSLP
338 expression level with risk genotypes were significant (Table 4). Thus, Table 4 presents results
339 stratified by TSLP genotype that demonstrate carriers with increased TSLP NEC expression
340 had increased asthma prevalence (sfTSLP p=0.012; lfTSLP p=0.006; Table 4). Taken together,

these data suggest that *TSLP* risk genotypes act in combination with sfTSLP and lfTSLP expression to influence asthma prevalence.

DISCUSSION

Our study is distinct from previous studies because it simultaneously examines *TSLP* SNPs, TSLP local tissue mRNA expression (sfTSLP and lfTSLP), and circulating protein to determine their independent and combined impact on asthma prevalence. Further, our study used a novel algorithm to prioritize SNPs to define risk genotypes and then recruited a cohort based on selected *TSLP* genotypes, which served as the basis for our studies. Our data demonstrate that TSLP SNPs and local airway expression of TSLP have synergistic contributions to asthma. The increase in asthma risk is likely manifest only when genetic variation enables eQTL in the *TSLP* locus to elevate lfTSLP. First, our data demonstrate that children carrying one or more alleles for rs2289277 and/or rs11466750 have increased prevalence of detectable TSLP expression, correlation between TSLP isoforms in the upper airway, and marginally increased childhood asthma in carriers compared to non-carriers. Second, we demonstrate that TSLP expression in the upper airway, but not circulating total TSLP in plasma, is increased among children with asthma, suggesting that pathogenesis of asthma is driven by local rather than systemic TSLP secretion. Third, we identify the combined effect of *TSLP* rs2289277 and rs11466750 genotypes and TSLP (sfTSLP and lfTSLP) expression in NEC that associates with increased childhood asthma thereby demonstrating a novel genotype-dependent contribution of TSLP expression to asthma risk. This indicates that *TSLP* rs2289277 and rs11466750 genotypes and TSLP expression have non-redundant contributions to asthma.

This study adds several new findings to the current knowledge. Previous studies demonstrated that increased TSLP expression is linked to the pathogenesis of Th2 inflammatory diseases,

including asthma and AD(32, 57-59). TSLP is necessary and sufficient for the development of Th2 cytokine-associated inflammation of the airways in mice(1, 60). In human studies, airway biopsy samples of asthmatic patients reveal significantly increased expression of TSLP and Th2 cytokines in the airway epithelium, lamina propria, and bronchial submucosa(28, 61). A bronchial allergen challenge study in mild asthmatics resulted in substantially increased IL-25, IL-33, and TSLP expression in the bronchial epithelium and submucosa(59). Importantly, expression of lfTSLP, but not sfTSLP, is highly inducible by polyI:C, proinflammatory cytokines (IL-1B and TNF-a), and Th2 cytokines (IL-4 and IL-13) in bronchial epithelial cells, underscoring the importance of lfTSLP expression in patients with allergic inflammation(30). While lfTSLP is proinflammatory and expressed during inflammation, sfTSLP demonstrates anti-inflammatory and antimicrobial activities by affecting the capacity of PBMCs and dendritic cells to produce inflammatory cytokines(55, 56). sfTSLP mRNA is constitutively expressed in a variety of tissues, including bronchial and colonic epithelial cells, keratinocytes, and lung fibroblasts(30, 32, 55, 56), but its function *in vivo* remains unclear. One report demonstrated a modest biological effect of administered sfTSLP *in vivo* in a mouse house dust mite-induced airway inflammation model(62); however, there is no known receptor for the putative sfTSLP protein so it is less likely to be relevant and translated into a biologically active protein *in vivo*(32). Consistent with previous studies on small airway epithelial cells and NECs(29, 30), our data demonstrate that lfTSLP expression is increased in the nasal epithelium of asthmatic patients. We also demonstrate a simultaneous increase in sfTSLP expression that is correlated with lfTSLP, and context and genotype dependent despite their differing biological properties.

Genetic studies demonstrated associations between *TSLP* SNPs and asthma prevalence, including meta-analyses of GWAS in ethnically diverse populations(10, 21, 23, 24, 63-65). Our study confirmed an association between *TSLP* rs2289277 and/or rs11466750 risk genotypes and asthma. Previous studies also demonstrated associations of *TSLP* variants with TSLP

mRNA expression and asthma, including *TSLP* rs2289277 and rs11466750 with AD(13), asthma(10), EoE(66, 67), IgE(13, 68), and regulation of TSLP expression in allergic disease(49). Other studies demonstrated associations of *TSLP* rs3806933, rs3806932, and rs11466749 with asthma(2, 10, 69) and EoE(66, 70, 71). *TSLP* rs1837253 was associated with asthma risk in three ethnic groups(24) and identified as a susceptibility locus in a Japanese cohort(22). We found that individuals with defined *TSLP* risk alleles and high levels of NEC sfTSLP and lfTSLP expression had increased odds of asthma. Other studies have illustrated cell-type-specific regulation of expression of *TSLP* and other asthma-related genes(14, 29), which taken with our results and the importance of local secretion of lfTSLP(31), may explain the inconsistent associations reported with circulating TSLP in plasma and asthma, recurrent wheeze or AD(27, 31-35). By using orthogonal pieces of evidence, surprisingly, the variants we identified with the strongest evidence of contributing to etiology were not the top variants from GWAS studies. This may be is due to the fact that GWAS is a marker for a region that harbors genetic association, but the strength of this association is likely multifactorial including the allele frequency and LD with causal variant(s). GWAS data is dynamic and changes over time. Our study reflects GWAS data that were available at the date of accession; however, new data have since been added. Thus, future studies may benefit from inclusion of TSLP expression in addition to *TSLP* genotype. Combined, these results suggest increased asthma risk is manifest only when genetic variation enables eQTL in the *TSLP* locus to elevate lfTSLP expression. There may be additional genetic variants not evaluated in the present study outside of *TSLP* that also impact TSLP expression and impact asthma.

To prioritize *TSLP* SNPs for this study, we developed a novel algorithm to identify SNPs that can be applied to other genes of interest. By using a targeted approach to select subjects enriched for specific *TSLP* genotypes, we ensured sufficient genetic variability and disease homogeneity(23, 72) that enabled us to rigorously examine the combined effects of *TSLP*

genotype, TSLP secretion, and asthma prevalence. This is a highly innovative feature of this study as the subjects included were carefully phenotyped and genotyped. This study design may have value over larger population studies where the subject heterogeneity and population substructure can reduce power and lead to spurious results.

Our observation that *TSLP* rs2289277 and rs11466750 genotypes and TSLP mRNA (sfTSLP and lfTSLP) expression act in combination to confer asthma risk has important clinical implications. *TSLP* genotype alone is not sufficient and requires a modifier that enables expression from the allele, i.e. the eQTL to express. Indeed, we did not observe increased asthma prevalence among subjects without the *TSLP* risk genotypes when comparing those with high and low sfTSLP and lfTSLP expression. It is possible that NEC expression of TSLP is a surrogate for lfTSLP status in other tissues critical for promoting asthma when combined with the presence of risk alleles and a genome permissive for their effect. In any case, TSLP expression alone is not a suitable biomarker for predicting asthma risk despite the associations observed in the present study. Additional studies to identify other risk factors are warranted because the failure to carry the *TSLP* risk genotypes or to have high TSLP expression does not rule out asthma.

The observed combined effects between *TSLP* genotypes and TSLP (sfTSLP and lfTSLP) expression might elucidate differences in therapeutic effects of anti-TSLP therapy. Clinical trials of Tezepelumab demonstrated the therapeutic potential of inhibiting TSLP in patients with asthma and with AD. In a small proof-of-concept study of patients with moderate-to-severe AD, treatment with Tezepelumab resulted in a numerical but not statistically significant improvement in eczema-severity scores, potentially due to the use of ‘background medication’ during the trial(73). Based on our data, perhaps the efficacy would be most evident amongst those with both AD and asthma. In a bronchial allergen challenge study, treatment with Tezepelumab in

patients with mild allergic asthma resulted in significant reductions of blood and sputum eosinophils and exhaled nitric oxide indicating TSLP has a key upstream role(8, 32). Treatment with Tezepelumab in a phase 2 randomized placebo-controlled trial of moderate-to-severe uncontrolled asthma was associated with a reduction in the annualized asthma exacerbation rate in patients with and without type 2 asthma(6, 7, 32). The observed improvements in disease control in patients who received Tezepelumab highlights the potential pathogenic role of TSLP across different asthma phenotypes(6, 7). The combined effects of *TSLP* genotypes and nasal TSLP expression in airway tissues, which are sensitive to increased TSLP secretion to drive allergic disease pathogenesis, might explain how treatments can be broadly effective against a variety of asthma and atopic diseases. TSLP may affect disease activity more broadly than inhibition of a single downstream pathway. Further, because TSLP expression acts in combination with *TSLP* genotypes, it is critical to genotype carrier and non-carrier subjects to minimize underlying heterogeneity that may impact the therapeutic response and disease pathogenesis.

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Table 1. SNP algorithm analysis to calculate a cumulative score to select functionally important SNPs (n=14).

TSLP SNP ID:	rs1837253	rs10061842	rs10455025	rs3806932	rs3806933	rs1898671	rs2289277	rs11466749	rs11466750	rs10073816	rs11466754	rs56390548	rs6594497	rs55887250
GWAS[1]	1		1			1								
eQTL[2]		1		1	1		1	1	1	1	1	1	1	1
TSLP transcription regulatory regions[3]				1	1		1	1	1					
DNase I-hypersensitive sites[4]				1			1							
Transcription factor binding motifs[5]	1			1			1			1	1	1		
Chromatin state histone marks[6]		1	1	1	1	1	1	1	1					
Total Score (out of 6)	2	2	2	**5**	**3**	2	**5**	**3**	**3**	2	2	2	1	1

Includes all SNPs with MAF >0.10 in the human *TSLP* gene (within 5.6 kb of the 5' flanking region of the promoter and 5.6 kb of the 3' flanking region of the last exon) that were associated with allergic diseases (including asthma, allergy, allergen sensitization, atopic dermatitis, and allergic rhinitis) in a GWAS with a *P*-value $<5x10^{-8}$ or that were eQTL in lung, skin (not sun-exposed), and esophageal mucosal tissues (n=14) as of March 1, 2019. SNP matrix scores ranged from 1-5, with a mean of 2.5 points (standard deviation=1.2) out of 6 points possible. rs2289277 and rs3806932 had the highest score of 5 points; rs11466749, rs11466750, rs3806933 had the second highest score of 3 points as noted with bold font.

[1]**GWAS;** SNPs identified with genome-wide association with allergic disease phenotype in GWAS catalogue, Phenotype-Genotype Integrator (PheGenI), and NHLBI GRASP catalog at significance of *P*-value $<5x10^{-8}$.
[2]**eQTL**; eQTL effects with a *P*-value <0.001 in GTex for lung, skin (not sun-exposed), and esophageal mucosal tissues.
[3] **TSLP transcription regulatory regions;** SNPs identified as being in accessible genomic regions and dynamic FAIRE-seq chromatin peaks within the TSLP gene during epidermal development and dysfunction(49, 50), and allergic disease associated SNPs in high LD (r^2 = 0.8-1.0) with SNPs located in dynamic FAIRE peaks.
[4]**DNAse I hypersensitive sites;** SNPs located in regions of DNase I sensitivity identified using HaploReg v4.1.
[5]**Transcription factor binding and regulation motifs;** Assessment of SNPs that may modify or disrupt transcription factor regulation motifs using HaploReg v4.1 and SNP2TFBS databases.
[6]**Chromatin state histone modification;** Chromatin state analysis to determine whether the identified SNPs overlapped with the major histone modifications (H3K4me1 and H3K27ac for enhancer; H3K4me3 and H3K9ac for promoter) in asthma relevant tissues using HaploReg v4.1.

Table 2. Demographic and clinical characteristics of the TSLP cohort (n=51).

Characteristics	All (n=51)	Non-Carriers (n=21)	Carriers (n=30)	P-value
	n (%)	n(%)	n(%)	
Age (years), Median [IQR]	16.1 [14.4-17.0]	16.2 [13.8-17.0]	16.0 [14.4-16.6]	0.716
Weight (kg), Median [IQR]	65.8 [54.1-75.1]	62.4 [53.8-70.7]	68.8 [54.4-86.4]	0.141
Sex				
M	28 (54.9%)	10 (47.6%)	18 (60.0%)	0.387
F	23 (45.1%)	11 (52.4%)	12 (40.0%)	
Race				
White	30 (58.8%)	11 (52.4%)	19 (63.3%)	0.439
Black	21 (41.2%)	10 (47.6%)	11 (36.7%)	
Ever diagnosed with asthma				
Asthma-	19 (37.3%)	11 (52.4%)	8 (26.7%)	**0.064**
Asthma+	32 (62.7%)	10 (47.6%)	22 (73.3%)	
Ever diagnosed with AD				
AD-	8 (15.7%)	5 (23.8%)	3 (10.0%)	0.186
AD+	43 (84.3%)	16 (76.2%)	27 (90.0%)	
Ever diagnosed with Asthma and AD				
Asthma- AD-	2 (3.9%)	2 (9.5%)	0 (0.0%)	**0.022**
Asthma+ or AD+	23 (45.1%)	12 (57.1%)	11 (36.7%)	
Asthma+ and AD+	26 (51.0%)	7 (33.3%)	19 (63.3%)	

*P-value comparing carriers with TSLP risk genotype and non-carriers using fisher's exact test for categorical variables and the nonparametric Wilcoxon rank-sum test for continuous variables. Boldface indicates a p-value of less than 0.10.

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Table 3. Association of *TSLP* risk genotype with clinical outcomes.

Outcome	All (n=51)	
	Adjusted OR	P-value
Ever diagnosed with Asthma	3.05 (0.93 - 9.98)	**0.06**
Ever diagnosed with AD	2.60 (0.53 - 12.70)	0.25
Ever diagnosed with Asthma and AD*	3.33 (1.02 - 10.86)	**0.04**

Multivariable logistic regression models to calculate odds ratios (OR) of the clinical outcome comparing carriers with risk genotype and non-carriers adjusted for race. Empiric p-values were derived by randomly permuting TSLP risk genotype 10,000 times. Due to sparseness of the data, confidence intervals from the regression model are reported. Boldface indicates a p-value of less than 0.10. *Subjects with asthma and AD were compared to all other subjects (includes individuals who had only asthma, only AD, or neither condition).

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Table 4. Association of TSLP levels with asthma stratified by *TSLP* risk genotype.

Asthma Outcome	All (n=51)		Carriers with TSLP risk genotype (n=30)		Non-carriers without risk genotype (n=21)		Interaction term
	Adjusted OR	P-value	Adjusted OR	P-value	Adjusted OR	P-value	P-value
Short form TSLP expression*	1.33 (1.01 – 1.93)	**0.011**	2.53 (1.11 -6.16)	**0.012**	1.02 (0.56 - 1.83)	0.91	**0.005**
Long form TSLP expression	1.47 (1.02 – 3.35)	**0.028**	3.12 (1.19 – 70.3)	**0.006**	1.20 (0.06 – 5.13)	0.52	**0.073**
Circulating Total TSLP	1.00 (0.98 – 1.03)	0.55	1.01 (0.94 - 1.08)	0.68	0.99 (0.85 - 1.09)	0.76	0.59

Multivariable logistic regression models to calculate odds ratios (OR) for asthma diagnosis using continuous TSLP expression data adjusted for race and sex. Models consider the full dataset as well as the data stratified by risk genotype. Given the differences in association between asthma and TSLP in carriers with the risk genotype and non-carriers, we performed multivariable logistic regression including a continuous TSLP level*TSLP genotype (carrier/non-carrier) interaction term (other variables in the model include continuous TSLP expression, genotype, race, and sex). TSLP levels measured long and short form TSLP expression (relative to B-actin) in nasal epithelium, and circulating total TSLP concentration (pg/ml) levels in plasma and were analyzed as continuous variables. Empiric p-values were derived by randomly permuting asthma diagnosis 10,000 times. Bootstrapping was used to estimate the confidence intervals for the odds ratio. Boldface indicates a p-value of less than 0.10. *Data for short form TSLP were rescaled per 100 units to calculate the adjusted odds ratio for ease of interpretation.

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Figure Legends

Figure 1. *TSLP* locus and SNP prioritization strategy. A) Flow chart of the SNP selection strategy. B) Schematic of *TSLP* locus and 14 SNPs of interest analyzed in discrete genomic functional annotation analyses. Genomic localization (GRCh38/hg38) of the human *TSLP* gene on chromosome 5q22.1 with mapped SNPs (MAF≥10%).

Figure 2. Selection and use of disease-associated *TSLP* SNPs to define risk genotype carriers for TSLP cohort. A) Linkage disequilibrium (r^2) among 5 SNPs with highest scores in the SNP matrix discrete analysis and GWAS associations performed in the human *TSLP* gene in European descent (CEU) populations. Among these 8 SNPs, the median D'=1.0**.** Table includes data from dbSNP and MAF reported from 1000 Genomes. Gray shaded SNPs in the table indicate those that scored the highest in our SNP matrix analyses. B) Disease-associated *TSLP* SNPs rs2289277 and rs11466750 define carriers with the risk genotype associated with TSLP expression in the TSLP cohort (n=51). Of these, non-carriers (n=21) were defined using GTex data for each genotype such that rs2289277 'GG' (alternate allele) and rs11466750 'GG' (reference allele) have the lowest expected TSLP expression. There were n=30 carriers with a TSLP risk genotype. Carriers with the risk genotype had at least one copy of the rs2289277 ('C') reference allele and at least one copy of the rs11466750 ('A') alternate allele. The rs2289277 'CC' genotype allele is associated with increased risk of allergic disease; the rs11466750 'AA' genotype is associated with increased risk of allergic disease.

Figure 3. Median TSLP expression in nasal epithelial cells and median circulating TSLP concentration in plasma. A) Median TSLP level by TSLP risk genotype. Differences in median long and short form TSLP expression (relative to B-actin) in nasal epithelium and total circulating TSLP concentration (pg/ml) levels in plasma comparing carriers with the *TSLP* risk genotype to non-carrier subjects without. P-value comparing TSLP levels in carriers and non-

carriers using nonparametric Wilcoxon rank-sum test. Outliers (defined as 1.5 times the interquartile range above the upper quartile and below the lower quartile) are not shown for visualization purposes in this figure. Outliers were not removed from the analyses. B) Median TSLP level by asthma status. Differences in median short form and long form TSLP expression (relative to B-actin) in the nasal epithelium, total circulating TSLP concentration (pg/ml) levels in plasma comparing asthmatic and nonasthmatic children. P-value comparing asthmatic and nonasthmatic children using nonparametric Wilcoxon rank-sum test. Outliers (defined as 1.5 times the interquartile range above the upper quartile and below the lower quartile) are not shown for visualization purposes in this figure. Outliers were not removed from the analyses.

Figure 4. Association of asthma and TSLP mRNA expression stratified by *TSLP* risk genotype. A) Short form TSLP. B) Long form TSLP. *TSLP* SNPs rs2289277 and rs11466750 define carriers with the risk genotypes and non-carriers without the risk genotype. Prevalence of asthma diagnosis among children with high and low (A) short form and (B) long form TSLP expression (High TSLP+ = high TSLP $\geq$ 66th percentile; TSLP- = low TSLP < 33th percentile) stratified by TSLP risk genotype (Risk genotype+ = Carriers with risk genotype; Risk genotype- = Non-carriers without risk genotype). P-values for pairwise comparisons using Fisher's exact test.

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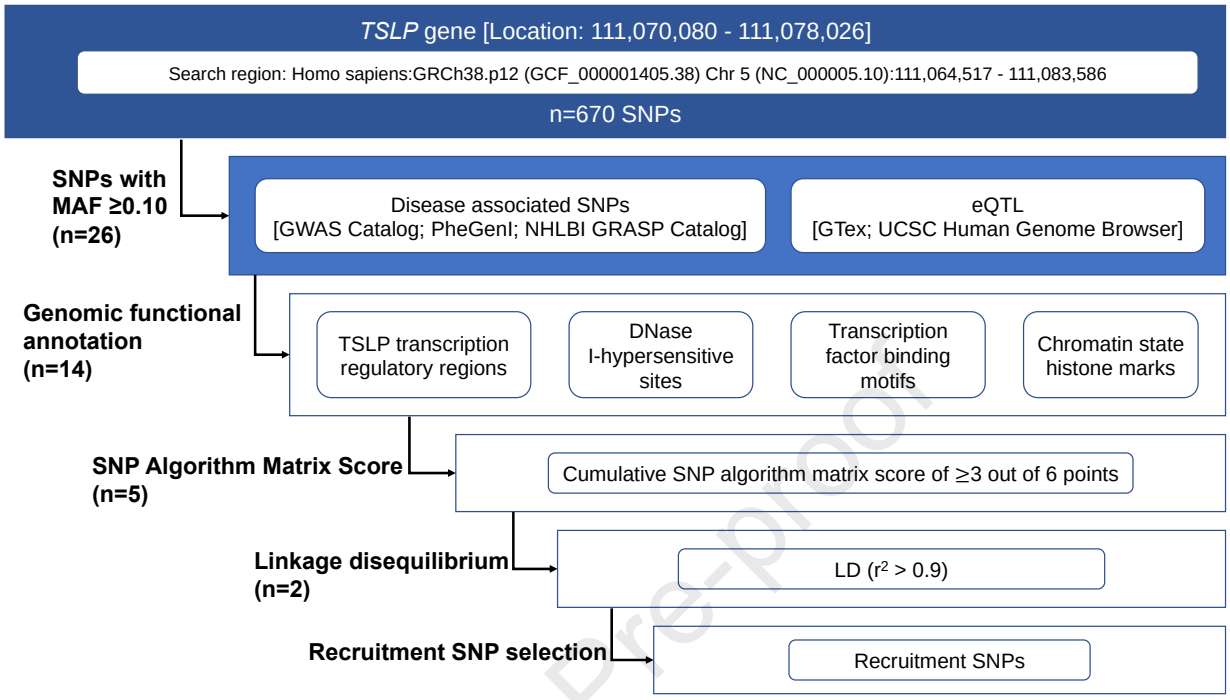
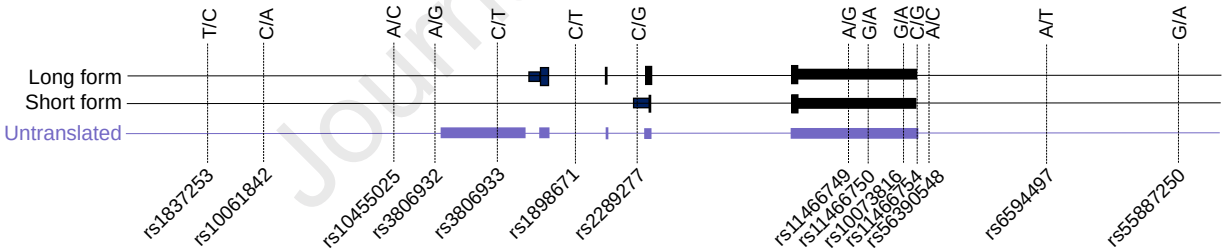
A.

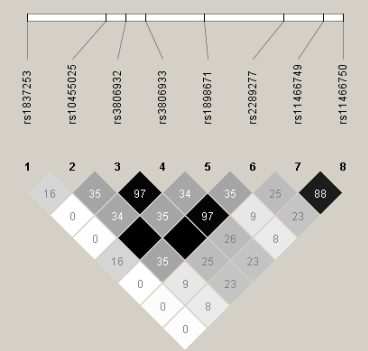


B.



Journal Pre-proof

A.



SNP	Position relative to *If*TSLP	Alleles	Minor Allele Frequency in CEU
rs1837253	Promoter region	T>C	T = 0.382
rs10455025	Promoter region	A>C	C = 0.132
rs3806932	Promoter region	A>G	A = 0.467
rs3806933	Non-coding transcript variant	C>A / C>T	T = 0.426
rs1898671	Intron 1	C>T	T = 0.142
rs2289277	Intron 2	C>A / C>G / C>T	G = 0.476
rs11466749	Untranslated 3-prime	A>G	G = 0.148
rs11466750	Untranslated 3-prime	G>A	A = 0.173

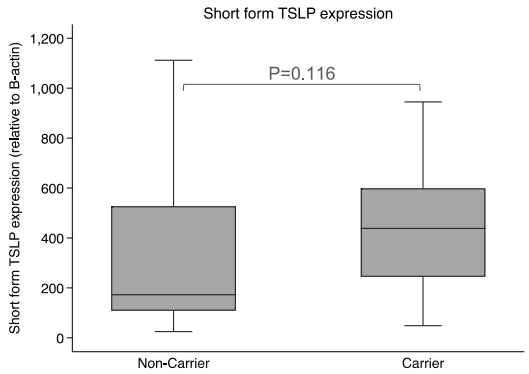
B.

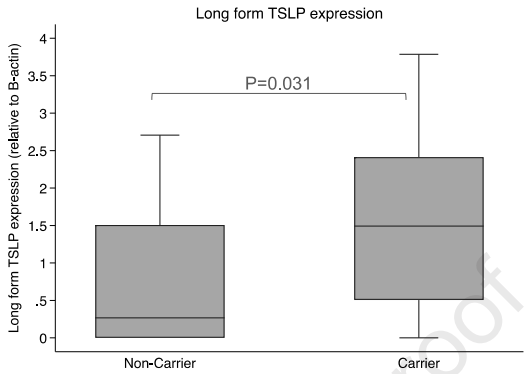
	rs11466750 (n, alternate alleles)		
rs2289277 (n, alternate alleles)	**GG (0)**	**AG (1)**	**AA (2)**
CC (0)	Carrier (n=9)	Carrier (n=1)	Carrier (n=0)
GC (1)	Carrier (n=13)	Carrier (n=2)	Carrier (n=0)
GG (2)	Non-Carrier (n=21)	Carrier (n=4)	Carrier (n=1)

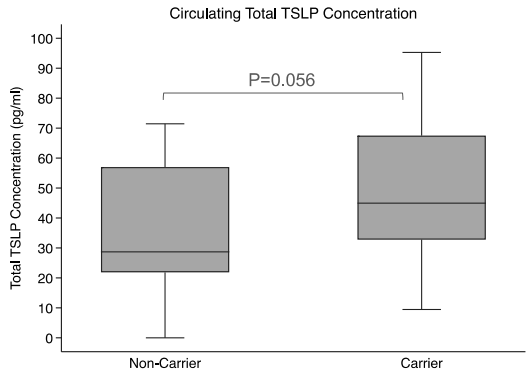
Journal Pre-proof

Journal Pre-proof

A.







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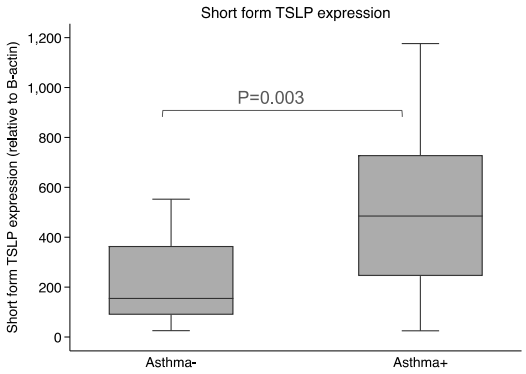
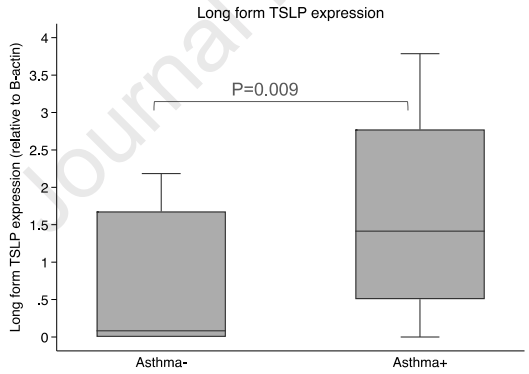
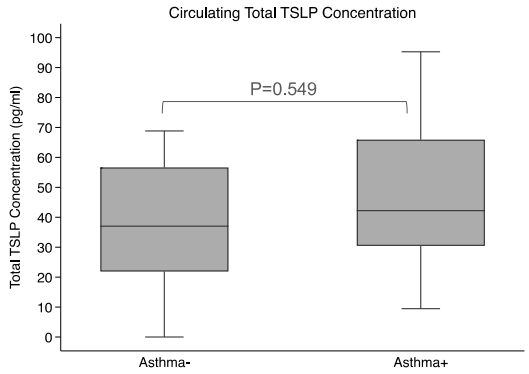
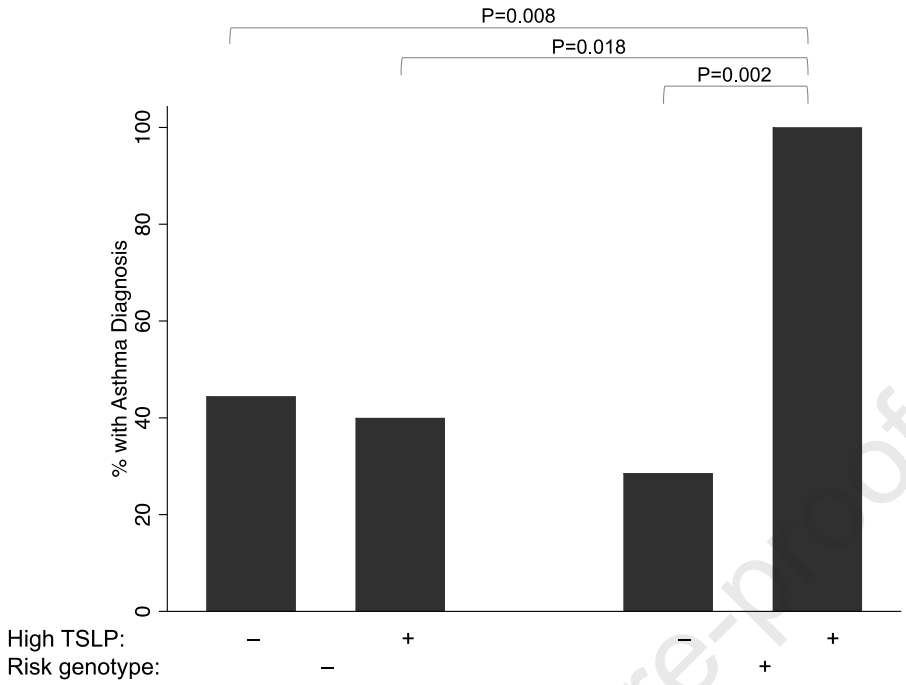






Journal Pre-proof

A.



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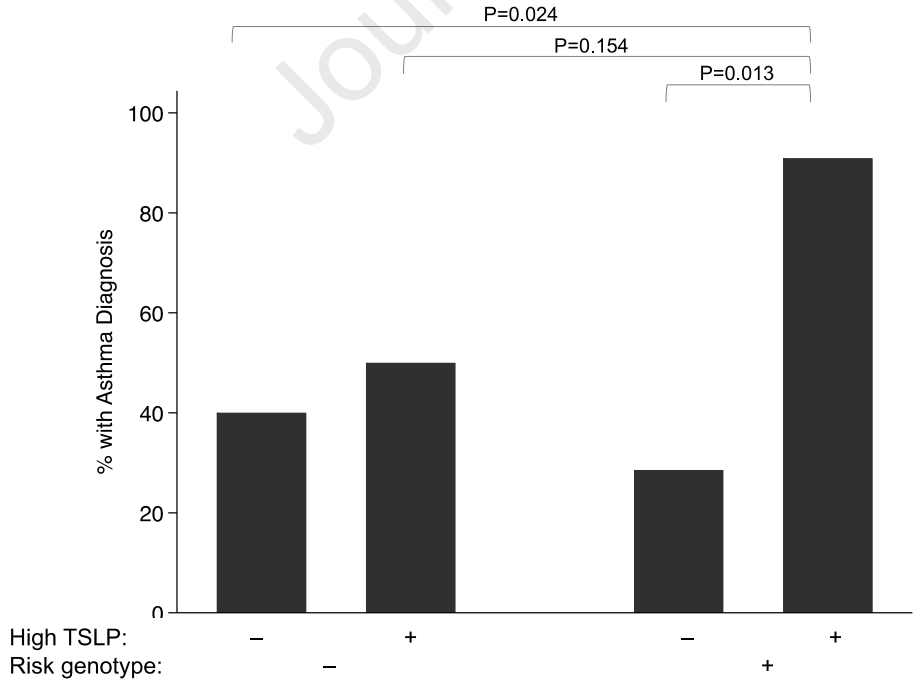

Journal Pre-proof