Brief report

A partial loss-of-function variant in STAT6 protects against type 2 asthma

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Background: Signal transducer and activator of transcription 6 (STAT6) is central to type 2 (T2) inflammation, and common noncoding variants at the STAT6 locus associate with various T2 inflammatory traits, including diseases, and its pathway is widely targeted in asthma treatment.

Objective: We sought to test the association of a rare missense variant in STAT6, p.L406P, with T2 inflammatory traits, including the risk of asthma and allergic diseases, and to characterize its functional consequences in cell culture.

Methods: The association of p.L406P with plasma protein levels, white blood cell counts, and the risk of asthma and allergic phenotypes was tested. Significant associations in other cohorts were also tested using a burden test. The effects of p.L406P on STAT6 protein function were examined in cell lines and by comparing $CD4^+$ T-cell responses from carriers and noncarriers of the variant.

Results: p.L406P associated with reduced plasma levels of STAT6 and IgE as well as with lower eosinophil and basophil counts in blood. It also protected against asthma, mostly driven by severe T2-high asthma. p.L406P led to lower IL-4–induced activation in luciferase reporter assays and lower levels of STAT6 in $CD4^+$ T cells. We identified multiple genes with expression that was affected by the p.L406P genotype on IL-4 treatment of $CD4^+$ T cells; the effect was consistent with a weaker IL-4 response in carriers than in noncarriers of p.L406P.

Conclusions: A partial loss-of-function variant in STAT6 resulted in dampened IL-4 responses and protection from T2-high asthma, implicating STAT6 as an attractive therapeutic target. (J Allergy Clin Immunol 2024;■■■:■■■-■■■.)

Key words: *STAT6, partial loss of function, variant, protective, asthma, T2 endotype, $CD4^+$ T cells, RNA sequencing*

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INTRODUCTION

Signal transducer and activator of transcription 6 (STAT6) is the central transcription factor of IL-4/IL-13 signaling and contributes to thymic stromal lymphopoietin signaling, leading

Abbreviations used
DEG: Differentially expressed gene
DRG: Differentially responsive gene
FC: Fold change
FDR: False-discovery rate
LOF: Loss of function
OR: Odds ratio
pSTAT6: Phosphorylated signal transducer and activator of transcription 6
STAT6: Signal transducer and activator of transcription 6
T2: Type 2
WT: Wild type

to type 2 (T2) inflammation.[1-4] Therefore, STAT6 is thought to play a pivotal role in the pathogenesis of asthma and allergic diseases. On stimulation, STAT6 is phosphorylated by Janus kinases, allowing it to dimerize and enter the nucleus to induce a transcriptional response.[5] This leads to T_H2 differentiation of $CD4^+$ T cells and IgE class switching in B cells.[1,2,4] Several biologics targeting this pathway have been clinically validated to treat severe asthma.[6] Common noncoding variants at the STAT6 locus have been associated with the risk of asthma[7-9] and serum IgE levels.[10] In addition, individuals with gain-of-function variants in STAT6 have shown elevated IgE, eosinophilia, and severe atopic disease.[11] Here, we report a rare partial loss-of-function (LOF) missense variant in STAT6 that reduces T2 inflammatory responses and is protective against severe T2-high asthma.

RESULTS AND DISCUSSION

A rare missense variant (rs770378890-G, p.L406P; minor allele frequency = 0.67% in Iceland) was found to associate with reduced plasma levels of STAT6 in *cis* (effect, −1.10 SD; 95% CI, −1.20 to −0.99; $P = 1.1 \times 10^{-99}$) and IgE in *trans* (effect, −0.55 SD; 95% CI, −0.66 to −0.44; $P = 4.1 \times 10^{-24}$), as measured by Somascan in 36,136 Icelanders (Table I).[12] No associations between p.L406P and other traits, including proteins or diseases, reach genomewide significance ($P < 4.8 \times 10^{-8}$), but the fact that the variant is vanishingly rare outside of Iceland limits our power to detect such associations.

Given the importance of STAT6 in mediating T2 inflammation, we tested whether the variant associated with 20 different phenotypes of white blood cell counts and asthma and allergic diseases (P value cutoff, $0.05/20 = 2.5 \times 10^{-3}$). This revealed that p.L406P associated with lower eosinophil (effect, −0.13 SD; 95% CI, −0.18 to −0.08; $P = 9.0 \times 10^{-8}$) and basophil (effect, −0.09 SD; 95% CI, −0.14 to −0.05; $P = 6.0 \times 10^{-5}$) counts in blood and reduced asthma risk (odds ratio [OR], 0.75; 95% CI, 0.65 to 0.87; $P = 2.0 \times 10^{-4}$).

Because T2 inflammation is the defining hallmark of T2-high asthma endotypes, we further stratify asthmatic patients and controls into T2-high (≥250 eosinophils/μL) and T2-low (<250 eosinophils/μL) endotypes on the basis of maximum longitudinal blood eosinophil counts. p.L406P associated only with lower risk in the T2-high endotype (OR, 0.70; 95% CI, 0.58-0.87; $P = 4.7 \times 10^{-4}$) and not significantly with the T2-low endotype (OR, 0.89; 95% CI, 0.71-1.11; $P = .29$) although the ORs were not statistically different ($P = .11$; Table I). We defined moderately severe to severe asthma as those patients having prescriptions of asthma drugs corresponding to step 5 of controller treatment suggestions by the Global INitiative for Asthma. p.L406P was strongly associated with moderately severe to severe T2-high asthma, most significantly in patients who had blood eosinophil counts greater than or equal to 500 eosinophils/μL (OR, 0.27; 95% CI, 0.14-0.52; $P = 9.5 \times 10^{-5}$), whereas it did not associate significantly with moderately to severe T2-low asthma (OR, 0.77; 95% CI, 0.53-1.13; $P = .18$) (Table I).

A suite of common variants at the STAT6 locus have been reported to associate with T2 inflammatory phenotypes including asthma,[13] IgE levels,[14] and urticaria.[15] A variant in STAT6's 3′ untranslated region (rs1059513; minor allele frequency = 10.26%) represents the most significant association at the STAT6 locus with IgE levels and asthma risk in the Icelandic data set. However, the effects are much smaller than observed for p.L406P and the 2 variants represent 2 independent associations ($r^2 = 0.00069$; Table I).

Although p.L406P associated significantly with protection from only asthma and not other T2-mediated diseases, we note that asthma is by far the most common diagnosis in our data set and, thus, best powered to study the association of this rare protective variant. Therefore, we cannot exclude that STAT6 LOF may also protect from other T2 inflammatory diseases similar to the increased susceptibility of T2 diseases described for the STAT6 gain-of-function[11] and the common noncoding variants at the STAT6 locus.[13-15] However, evaluating the association between p.L406P and other T2 inflammatory diseases would require larger data sets for the other T2 diseases, preferably studying disease severity similar to what we have done here for asthma. Because LOFs in other STAT genes have been associated with susceptibility to infection,[16] we tested 16 most common infection-related phenotypes in Iceland, including bacterial and viral pneumonia and coronavirus disease 2019 (with all the lists tested having more than 1000 cases). We found no evidence for STAT6 p.L406P affecting susceptibility to infection (data not shown).

Among the approximately 250,000 individuals who have been whole-genome–sequenced at deCODE, p.L406P is found only in Iceland. By searching various databases, we find only 3 heterozygotes of 785,819 individuals in the gnomAD database of European (non-Finnish) ancestry and 1 heterozygote of 14,128 individuals in the dbSNP database of Japanese ancestry. On the basis of the available data, we therefore conclude that p.L406P is highly enriched in Iceland and vanishingly rare in other ancestries. Because this excludes direct replication of p.L406P associations in other cohorts, we ran a burden test, combining all rare predicted LOF variants with moderate impact variants with predicted deleterious effects in *STAT6* (LOF + missense), in 3 different data sets available to us (Table II). We observed a significant *STAT6* LOF + missense burden association with asthma (OR, 0.82; 95% CI, 0.76 to 0.88; $P = 2.8 \times 10^{-7}$) and eosinophil count (effect, −0.06 SD; 95% CI, −0.09 to −0.03; $P = 9.0 \times 10^{-6}$) with consistent direction of effects in all data sets. Looking at LOF only, we found 63 carriers of LOF variants in *STAT6*, and the burden of LOF variants in *STAT6* associated with eosinophil count (effect, −0.50 SD; 95% CI, −0.73 to −0.27; $P = 1.5 \times 10^{-5}$). These results support the association we observed with p.L406P, and the direction of effect is consistent with p.L406P being an LOF variant (Table II).

We performed functional experiments to determine whether p.L406P results in LOF. The variant is within STAT6's DNA

TABLE I. Association of STAT6 variants with quantitative traits and asthma endotypes in Iceland

rs no./chr location (hg38)			rs770378890/chr12:57102917		rs1059513/chr12:57095926		rs770378890 adjusted for rs1059513	
Coding effect			Missense p.L406P		3_prime_UTR_variant		Missense p.L406P	
Effect allele/other allele			G/A		C/T		G/A	
Effect allele frequency (%)			0.67		10.26		0.67	
Quantitative traits	No. of cases		Effect (95% CI)	P value	Effect (95% CI)	P value	$Effect_{adj}$ (95% CI)	P_{adj} value
STAT6 (*cis*-pQTL)	36,136		−1.10 (−1.20 to −0.99)	1.05×10^{-99}*	0.02 (−0.01 to 0.05)	.16	1.09 (−1.20 to −0.99)	2.07×10^{-99}*
IgE (*trans*-pQTL)	36,136		−0.55 (−0.66 to −0.44)	4.14×10^{-24}*	−0.19 (−0.21 to −0.16)	1.85×10^{-42}*	−0.57 (−0.68 to −0.46)	5.06×10^{-26}*
Eosinophil count	282,744		−0.13 (−0.18 to −0.08)	9.01×10^{-8}†	−0.03 (−0.04 to −0.02)	2.74×10^{-6}†	−0.14 (−0.19 to −0.09)	4.024×10^{-8}†
Basophil count	279,371		−0.09 (−0.14 to −0.05)	5.96×10^{-5}†	−0.01 (−0.02 to 0.01)	.38	−0.09 (−0.14 to −0.05)	5.29×10^{-5}†
Lymphocyte count	282,069		0.03 (−0.02 to 0.08)	.21	−0.00 (−0.02 to 0.01)	.80	0.03 (−0.02 to 0.08)	.216
Neutrophil count	282,015		−0.02 (−0.07 to 0.02)	.31	0.01 (−0.00 to 0.02)	.19	−0.02 (−0.07 to 0.02)	.330
Monocyte count	282,068		−0.00 (−0.06 to 0.03)	.54	0.03 (0.01 to 0.04)	7.64×10^{-5}†	−0.01 (−0.06 to 0.04)	.622
Diseases	**No. of cases**	**No. of controls**	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**	**OR_{adj} (95% CI)**	**P_{adj} value**
Asthma	37,840	315,018	0.75 (0.65 to 0.87)	2.02×10^{-4}†	0.91 (0.87 to 0.94)	1.60×10^{-6}	0.73 (0.62 to 0.86)	1.43×10^{-4}†
T2-low asthma (<250 eosinophils/μL)	10,965	105,156	0.89 (0.71 to 1.11)	.29	0.94 (0.88 to 0.99)	.03	0.88 (0.70 to 1.11)	.29
T2-high asthma (≥250 eosinophils/μL)	19,748	104,103	0.70 (0.58 to 0.86)	4.70×10^{-4}†	0.91 (0.86 to 0.94)	8.03×10^{-5}†	0.69 (0.57 to 0.85)	4.15×10^{-4}†
Severe T2-low asthma (<250 eosinophils/μL)	2,792	274,757	0.77 (0.77 to 1.13)	.19	0.95 (0.87 to 1.05)	.34	0.79 (0.54 to 1.16)	.23
Severe T2-high asthma (≥250 to <500 eosinophils/μL)	2,274	263	0.56 (0.34 to 0.91)	2.10×10^{-2}	0.90 (0.81 to 1.00)	.06	0.53 (0.31 to 0.91)	.02
Severe T2-high asthma (≥500 eosinophils/μL)	2,028	259,729	0.27 (0.14 to 0.52)	9.50×10^{-5}†	0.90 (0.80 to 1.01)	.08	0.27 (0.14 to 0.51)	7.90×10^{-5}†
Atopic dermatitis	19,694	318,446	0.79 (0.63 to 0.99)	.04	0.93 (0.88 to 0.98)	7.36×10^{-3}†	0.78 (0.60 to 1.00)	.05
Urticaria	21,810	330,854	0.88 (0.74 to 1.04)	.14	0.93 (0.89 to 0.97)	1.00×10^{-3}†	0.87 (0.72 to 1.04)	.13
Allergic rhinitis	13,560	322,983	0.92 (0.74 to 1.15)	.47	0.87 (0.82 to 0.92)	1.95×10^{-6}†	0.92 (0.73 to 1.17)	.50
Chronic rhinosinusitis	29,005	314,620	0.96 (0.84 to 1.10)	.56	0.97 (0.94 to 1.01)	.15	0.95 (0.82 to 1.10)	.47
Nasal polyps	3,510	339,902	0.65 (0.41 to 0.98)	.04	0.91 (0.83 to 1.00)	.04	0.62 (0.41 to 0.93)	.02
Chronic rhinosinusitis with nasal polyps	1,610	274,558	0.52 (0.28 to 0.97)	.04	0.91 (0.80 to 1.04)	.15	0.49 (0.26 to 0.93)	.03
Chronic rhinosinusitis without nasal polyps	27,395	314,620	0.99 (0.85 to 1.14)	.85	0.98 (0.94 to 1.02)	.25	0.98 (0.84 to 1.13)	.75

pQTL, Protein quantitative trait locus; *UTR*, untranslated region.
*Association between a given trait and the rare missense variant rs770378890 that reached genomewide significance ($P < 4.8 \times 10^{-8}$).
†Associations that reached the *P* value cutoff ($P < 1.3 \times 10^{-3}$) when correcting for the 2 variants and 20 relevant T2 traits and diseases. Effect for quantitative traits is given in SD. $Effect_{adj}$, OR_{adj}, and P_{adj} represent the association result for the rare missense variant rs770378890 when adjusted for the common 3′ UTR variant rs1059513.

binding domain (Fig 1, *A*) and in a position that is conserved across vertebrates.[17-19] Therefore, we hypothesized that the p.L406P substitution adversely affected either STAT6's stability or its ability to regulate gene expression.

To test whether p.L406P affects STAT6's ability to induce expression through its canonical binding site, we performed dual luciferase reporter assays with and without IL-4 treatment. We transfected a luciferase reporter plasmid containing 4 STAT6 binding sites and plasmids overexpressing either the reference (wild-type [WT]) or the p.L406P version of DYKDDDDK-tagged (DDK) STAT6. We included a STAT6 overexpression plasmid with an experimental mutation previously shown to disrupt DNA binding (histidine 415 to alanine, p.H415A) as a baseline for STAT6 LOF.[20] We observed a significant drop in the IL-4–responsive luciferase activity when the reporter was coexpressed with the STAT6 p.L406P variant compared with WT in both HEK293T (log_2 fold change [log_2FC], −1.0; 95% CI, −1.4 to −0.53; $P = 2.2 \times 10^{-3}$; n = 6 triplicates) and HeLa (log_2FC, −1.9; 95% CI, −2.3 to −1.3; $P = 7.9 \times 10^{-3}$; n = 5 triplicates) cells (Fig 1, *B*). The reduction in activity for p.L406P was not as great as for p.H415A (for HEK293T: log_2FC, −4.8; 95% CI, −5.1 to −4.4; and for HeLa: log_2FC, −4.7; 95% CI, −5.1 to −4.3).

We hypothesized that the reduced function of STAT6 was, at least in part, mediated through decreased levels, as indicated by the *cis*–protein quantitative trait locus association observed in plasma. However, there is a risk of epitope effects, wherein a missense variant such as p.L406P directly affects binding of the Somascan aptamer to the protein, rather than reflecting actual variation in STAT6 plasma levels. Therefore, we compared STAT6 protein levels in anti-DDK Western blots in HEK293T cells and found significantly lower STAT6 levels in lysates from cells expressing STAT6 p.L406P than those expressing WT STAT6 (log_2FC, −1.0; 95% CI, −1.6 to −0.42; $P = .013$) (Fig 1, *C*). The same was not true of H415A, whose LOF mechanism is through a defect in DNA binding (log_2FC, 0.56; 95% CI, −1.1 to 2.1; $P = .27$).

We also performed immunofluorescence staining for phosphorylated STAT6 (pSTAT6) in HeLa cells overexpressing WT or p.L406P STAT6 after 0, 15, 30, and 60 minutes of treatment with IL-4 to determine the nuclear/cytoplasmic ratio of pSTAT6 (Fig 1, *D*). We did not see a significant reduction in nuclear translocation of the variant protein compared with the WT (corrected *P* value threshold, 0.05/4 = .0125), although both WT and p.L406P showed significant associations between nuclear/cytoplasmic ratios and IL-4 treatment ($P = 3.2 \times 10^{-3}$ and $P = 2.9 \times 10^{-2}$ for WT and p.L406P, respectively). Together, these results indicate that p.L406P leads to a partial LOF of STAT6 that may be mediated through reduced STAT6 levels.

To evaluate the effect of the variant in relevant cells, we measured STAT6 levels in cell lysates of $CD4^+$ T cells isolated from PBMCs of p.L406P carriers and noncarriers (matched to carriers based on age and sex) with an orthogonal method, Simple

TABLE II. Burden test association of rare predicted LOF and deleterious missense variants in STAT6 with asthma and eosinophil count*

Gene	Selected variants	Data set	No. of variants	MAF (%)	No. of carriers/ noncarriers	Asthma			Eosinophil count		
						OR	95% CI	*P* value	Effect (SD)	95% CI	*P* value
STAT6	LOF + missense	Meta-analysis				0.82	0.76 to 0.88	2.7×10^{-7}	−0.06	−0.09 to −0.03	9.0×10^{-6}
		Iceland	19	0.76	2,547/167,902	0.82	0.73 to 0.92	9.6×10^{-4}	−0.10	−0.15 to 0.05	5.7×10^{-5}
		UK Biobank	284	0.30	2,499/426,427	0.84	0.74 to 0.94	.002	−0.07	−0.11 to −0.03	9.1×10^{-4}
		Denmark	10	0.22	1,783/381,719	0.75	0.60 to 0.93	.0088	0.00	−0.10 to 0.11	.94
STAT6	LOF	Meta-analysis				0.62	0.28 to 1.38	.24	−0.50	−0.73 to −0.27	1.5×10^{-5}
		Iceland	3	0.0011	5/170,418	2.35	0.22 to 25.2	.48	0.39	−0.99 to 1.77	.58
		UK Biobank	40	0.0066	58/428,868	0.52	0.22 to 1.24	.14	−0.53	−0.76 to −0.30	7.4×10^{-6}
		Denmark	0	0	0/383,502						

MAF, Minor allele frequency.
*The burden association was tested in 3 different data sets: Iceland, UK Biobank, and Denmark.

Western, using an antibody that recognizes a peptide of STAT6 distal from the variant. Total STAT6 levels were lower in carriers, both homozygote (log_2FC, −2.65; 95% CI, −2.9 to −2.3; $P = 4.5 \times 10^{-13}$; n = 7) and heterozygote (log_2FC, −1.14; 95% CI, −1.5 to −0.81; $P = 2.4 \times 10^{-6}$; n = 8), than in noncarriers (n = 18) (Fig 1, *E*).

To determine whether p.L406P also affects STAT6 activation, we estimated the abundance of pSTAT6 in samples treated with IL-4 (Fig 1, *F*). Similar to total STAT6, we saw a reduction in pSTAT6 levels in both homozygotes (log_2FC, −2.75; 95% CI, −3.0 to −2.3; $P = 5.2 \times 10^{-13}$) and heterozygotes (log_2FC, −1.26; 95% CI, −1.6 to 0.83; $P = 6.5 \times 10^{-6}$). However, the pSTAT6/total STAT6 ratio was not affected by p.L406P in either heterozygotes (log_2FC, −0.06; 95% CI, −0.45 to 0.27; $P = .59$) or homozygotes (log_2FC, −0.08; 95% CI, −0.29 to 0.17; $P = .57$) (Fig 1, *G*), indicating that in $CD4^+$ T cells, the variant mainly affects the total amount of STAT6 available for activation, without directly affecting its phosphorylation.

Next, we explored downstream effects of p.L406P on the transcriptomes of activated $CD4^+$ T cells from carriers and the age- and sex-matched noncarriers by RNA sequencing. $CD4^+$ T cells were stimulated with either anti-CD3 and anti-CD28 antibodies alone (direction-neutral activation) or in combination with IL-4 and an anti–IFN-γ antibody to induce STAT6 signaling (IL-4 activation). Cells were collected either after 4 hours of treatment ($n_{AA} = 20$; $n_{AG} = 8$; $n_{GG} = 7$) to capture primary targets or after 24 hours of treatment ($n_{AA} = 17$; $n_{AG} = 3$; $n_{GG} = 6$) for more downstream effects (Fig 2, *A*).

We examined the effect of p.L406P carrier status on gene expression using a linear model adjusting for sex and age. We also determined the fraction of naive cells and adjusted for it in our model, because naive and memory T cells have distinct responses to IL-4.[21,22] The full list of differentially expressed genes (DEGs) is provided in Tables E1 and E2 (in the Online Repository available at www.jacionline.org). Of note, p.L406P does not associate with lower STAT6 transcript levels in any of the conditions (false-discovery rate [FDR], >0.1; Fig 2, *B*), indicating that the lowered STAT6 protein levels are mediated through either reduced translation or, more likely, protein degradation. However, differences in ratios of $CD4^+$ T-cell subtypes, such as T_H1 and T_H2, within the samples could also, at least partly, explain the difference in STAT6 levels. Further study is therefore needed to determine the mechanism by which the variant reduces STAT6 in primary cells.

On IL-4 activation, we found 307 DEGs by p.L406P genotype at 4 hours (FDR < 0.05) and 951 DEGs at 24 hours (Fig 2, *C*). Of these, 138 were DEGs at both time points. In contrast, we found only 1 DEG by genotype after 4 hours of direction-neutral activation. At 24 hours, however, we found 158 DEGs, indicating endogenous activation of STAT6 on direction-neutral activation. Indeed, we found evidence of increased *IL4* expression ($P = 4.5 \times 10^{-7}$; Fig 2, *D*) and STAT6 activation (Fig 2, *E*) after 24 hours of direction-neutral activation.

Because IL-4 activates STAT6, we hypothesized that p.L406P would counteract the effect of IL-4 on STAT6 target genes. We first identified genes that were induced by IL-4 in noncarrier cells by comparing cells that were treated with direction-neutral or IL-4 activation and found 2641 and 625 IL-4 DEGs (FDR < 0.05) at 4 and 24 hours, respectively (Tables E1 and E2). Of note, most of the p.L406P DEGs in the IL-4 activation at 4 hours were also IL-4 DEGs in noncarriers (283 of 307; Fig 2, *B*). Next, for each p.L406P DEG, we compared the effect of carrying the variant with the effect of IL-4 on noncarrier cells. Strikingly, we observed an inverse relationship between the effect of IL-4 and the effect of p.L406P on the expression of p.L406P DEGs (Pearson correlation coefficient, $r = -0.915$), with genes that were induced by IL-4 being expressed at lower levels in p.L406P carriers and *vice versa* (Fig 2, *F*). We observed the same trend at 24 hours ($r = -0.842$; Fig 2, *G*), but, in line with the endogenous STAT6 activation in the direction-neutral condition at 24 hours, we saw fewer p.L406P DEGs overlapping IL-4 DEGs (281 of 951). Among the DEGs, we observed 2 classic targets of STAT6: IL-4R and GATA-3. However, we note that we did not detect differences in the expression of any of the classical T_H2 cytokines at either time point, perhaps illustrating their time-specific response to stimulation.

Next, to quantify the difference in the transcriptional response to IL-4, we used a linear model correcting for the same covariates as earlier but modeling the log_2FC between the IL-4 and direction-neutral activation condition for each individual. We found 320 differentially responsive genes (DRGs) at 4 hours (FDR < 0.05; Fig 3, *A*; see also Table E1). The DRG effect was strongly inversely correlated with the log_2FC in noncarriers ($r = -0.932$) (Fig 3, *B*), consistent with the effect of the genotype dampening the effect of IL-4, which we further illustrated for the top 40 most significant DRGs (Fig 3, *C*). An example of this is the E3 ubiquitin ligase ring finger protein 125, a known target of STAT6[23] (Fig 3, *D*).

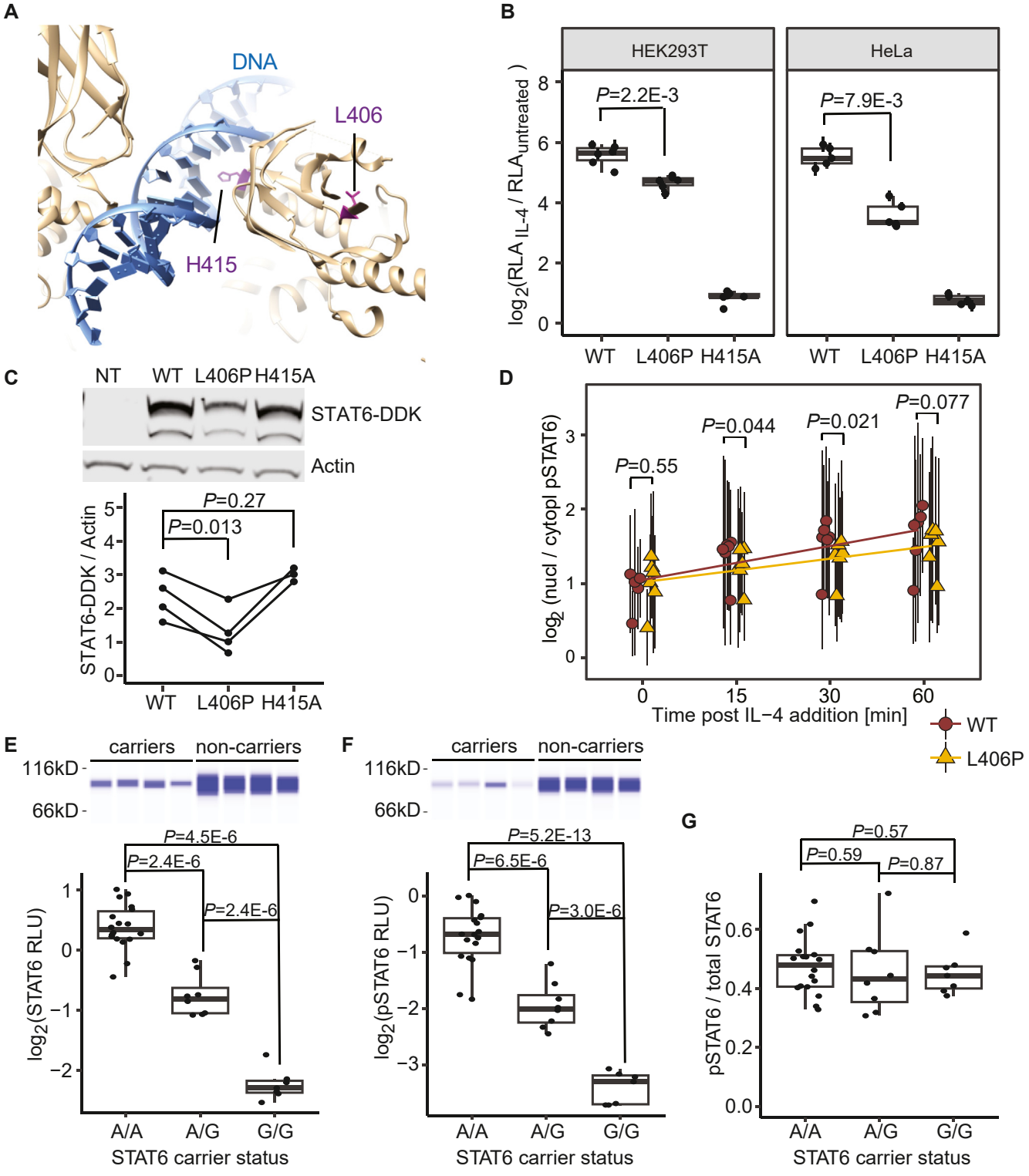

FIG 1. p.L406P is a partial LOF variant. **A,** Crystal structure of STAT6 DNA binding domain in interaction with DNA (Protein Data Bank code: 4y5w (20)), showing the location of relevant amino acids. Figure generated using UCSF Chimera, University of California, San Francisco. **B,** RLA upon overexpression of STAT6 and activation with IL-4 for 2 hours. *P* values from Wilcoxon rank-sum test (n = 6 and n = 5 transfections in technical triplicates for HEK293T and HeLa, respectively). **C,** Representative western of STAT6 overexpression in HEK293T cells with quantification of 4 independent replicates. *P* values from paired Student *t* test. **D,** Median $\log_2$(nuclear/cytoplasmic) pSTAT6 immunofluorescence staining with interquartile ranges for 6 individual replicates in HeLa cells overexpressing WT or p.L406P STAT6. *P* values from paired Student *t* test. **E** and **F,** Relative signal intensity of Simple Western peaks using anti-STAT6 (Fig 1, *E*) or anti-pSTAT6 (Fig 1, *F*) antibodies. *P* values from Student *t* test; noncarriers (A/A, n = 18), heterozygote carriers (A/G, n = 8), and homozygote carriers (G/G, n = 7). Pseudoblots of 4 representative homozygote carriers and matched noncarrier samples shown. **G,** Ratio of pSTAT6 over total STAT6 signal intensities. *DDK*, DYKDDDDK-tag; *NT*, nontransfected (HEK293T cells); *RLA*, relative luciferase activity; *RLU*, relative light unit.

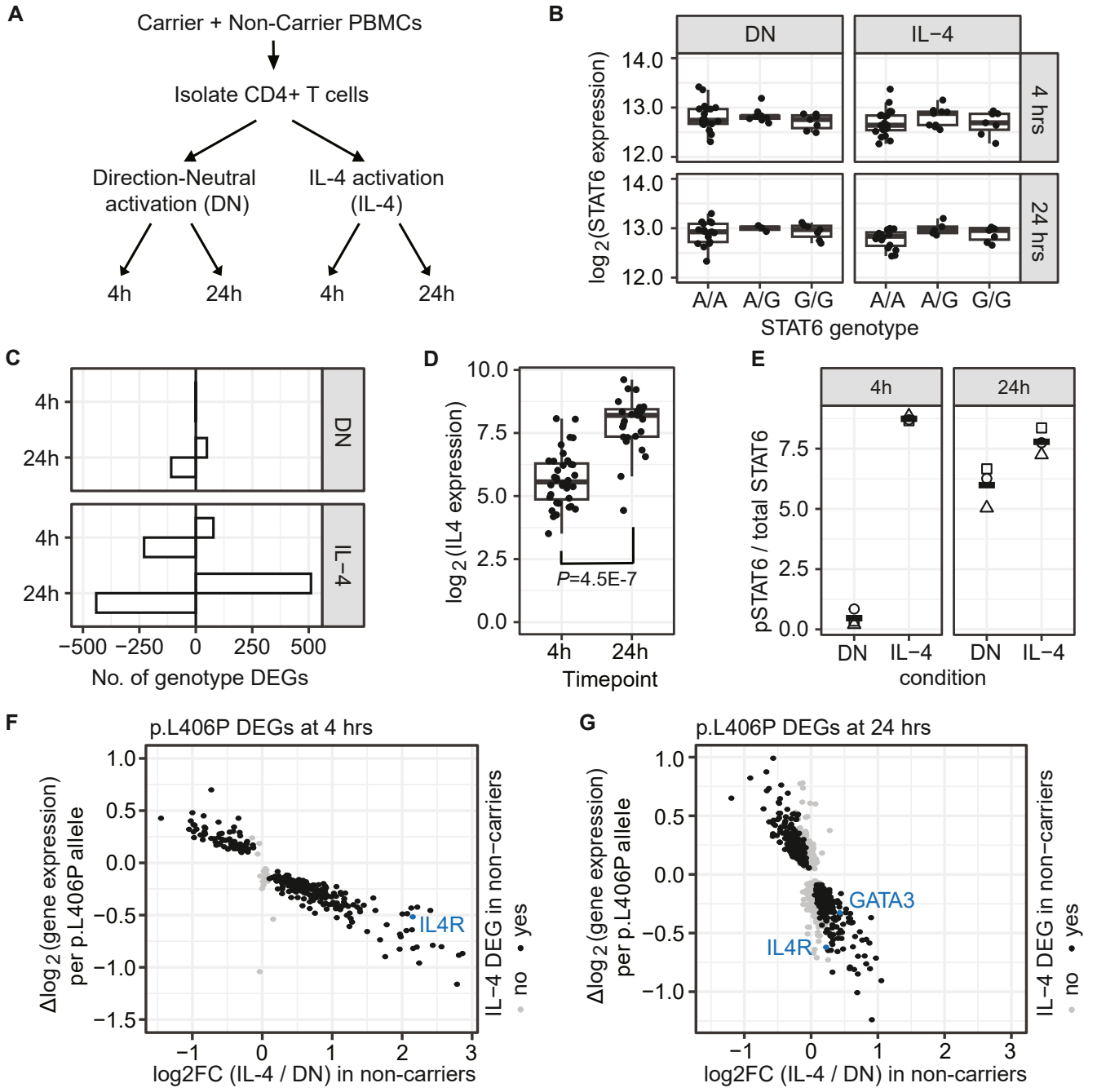

FIG 2. Multiple DEGs by p.L406P genotype. **A,** Schematic of experiment. **B,** STAT6 expression in each genotype in each condition. Expression measured by $\log_2$(adjusted read counts). **C,** Number of DEGs by p.L406P genotype in DN and IL-4 activation at 4 and 24 hours, separated by direction of differential expression. **D,** *IL4* expression, in $\log_2$(adjusted read counts), at 4 and 24 hours of CD3/CD28 treatment. *P* values from Student *t* test. **E,** Relative pSTAT6 over total STAT6 signal in Simple Western at different times post activation, with (IL-4) and without (DN) additional IL-4 (n = 3; individual indicated by shape). **F,** Per-allele p.L406P genotype effect on expression (beta in linear regression model), for all genotype DEGs in the IL-4 activation at 4 hours, as function of the median $\log_2$(IL-4 activation/DN activation) in noncarriers at the same time point. Genes that are also IL-4 DEGs in noncarriers are indicated in *black* and IL-4R is highlighted in *blue*. **G,** Same as in Fig 2, *F*, for cells treated for 24 hours. IL-4R and GATA-3 are highlighted in *blue*. *DN*, Direction-neutral.

We associate a partial LOF in STAT6 with protection against severe asthma, mediated through reduced protein levels causing a weakened IL-4 response and lower IgE levels and eosinophil counts in blood. The pathway to which STAT6 belongs is highly targeted by asthma biologics. Upstream of STAT6, tezepelumab inhibits thymic stromal lymphopoietin,

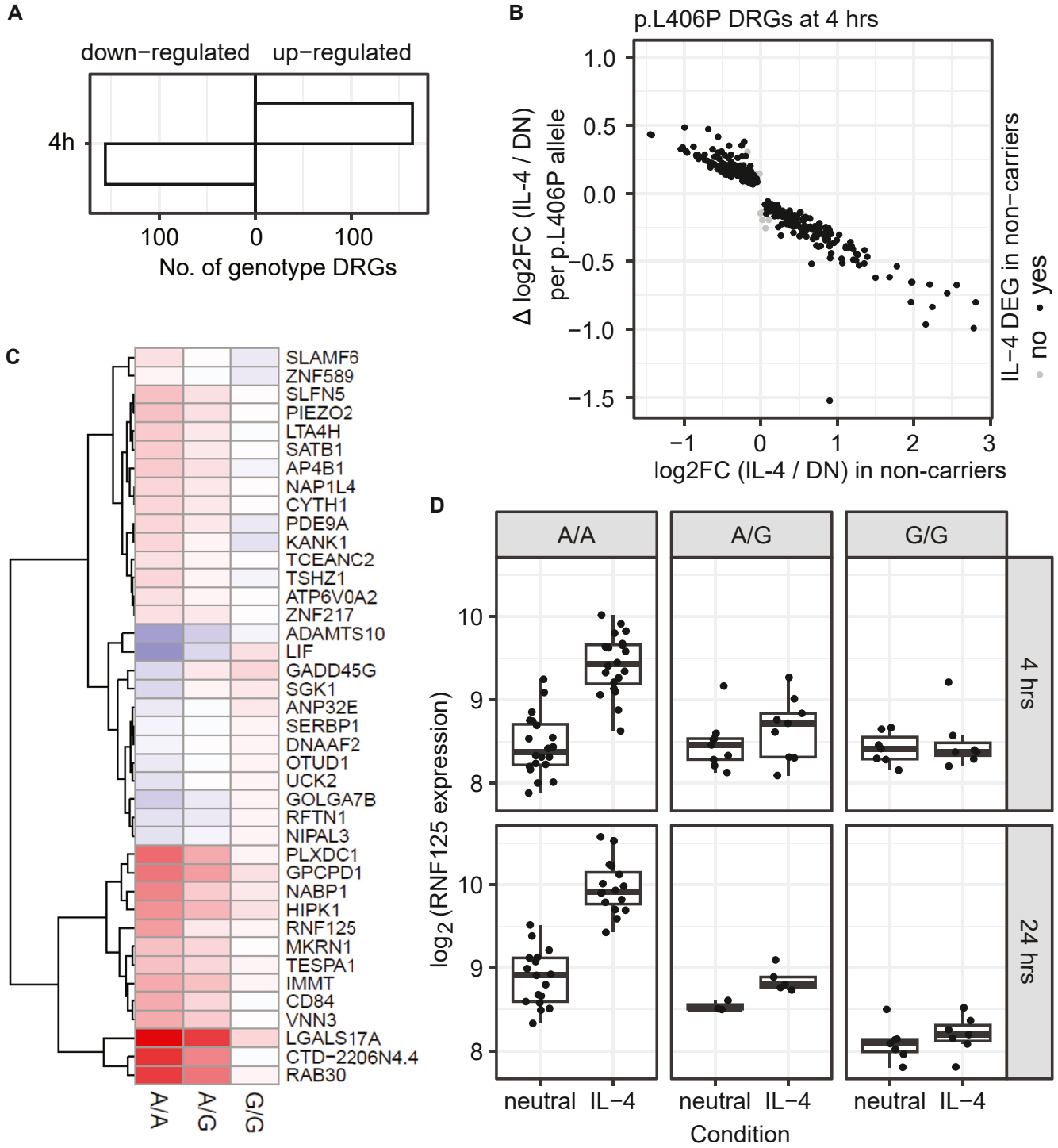

FIG 3. Response to IL-4 is dampened by p.L406P. **A,** Number of DRGs by p.L406P genotype at 4 hours, separated by direction of differential response. **B,** Per-allele effect of p.L406P genotype on $\log_2$FC at 4 hours as function of median $\log_2$(IL-4 activation/DN activation) in noncarriers. Genes that are also IL-4 DEGs in noncarriers are indicated in *black*. **C,** Heatmap of $\log_2$FC(IL-4 activation/DN activation) for the top 40 most significant DRGs separated by genotype. **D,** Expression of RNF125 in all conditions, separated by genotype. *DN*, Direction-neutral; *RNF125*, ring finger protein 125.

dupilumab blocks IL-4 and IL-13 signaling through IL-4Rα, and several inhaled Janus kinase inhibitors are in development.[6,24] Downstream, omalizumab inhibits IgE, and mepolizumab, reslizumab, and benralizumab target IL-5 and its receptor.[6] Downregulating STAT6 in a clinical setting may, therefore, combine the effects of many of these drugs, making it an attractive target for drug development to treat severe, uncontrolled T2-high asthma.

DISCLOSURE STATEMENT

Disclosure of potential conflict of interest: K. Kristjansdottir, G. L. Norddahl, E. V. Ivarsdottir, G. H. Halldorsson, G. Einarsson, K. Bjarnadóttir, G. Rutsdottir, A. O. Arnthorsson, S. Gudmundsdottir, K. Gunnarsdottir, B. V. Halldorsson, H. Holm, S. Saevarsdottir, A. S. Snaebjarnarson, G. Sveinbjornsson, G. E. Thorlacius, G. Thorleifsson, V. Tragante, P. Sulem, D. F. Gudbjartsson, P. Melsted, I. Jonsdottir, T. A. Olafsdottir, and K. Stefansson are employees of deCODE genetics, a subsidiary of Amgen.

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Key messages

- **STAT6 p.L406P associated with lower plasma levels of STAT6 and IgE, lower eosinophil and basophil counts, and reduced risk of asthma.**
- **STAT6 p.L406P led to a weakened IL-4 response in $CD4^+$ T cells.**

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