Supplementary Material

Targeted resequencing and functional testing identifies low-frequency missense variants in the gene encoding GARP as significant contributors to atopic dermatitis risk

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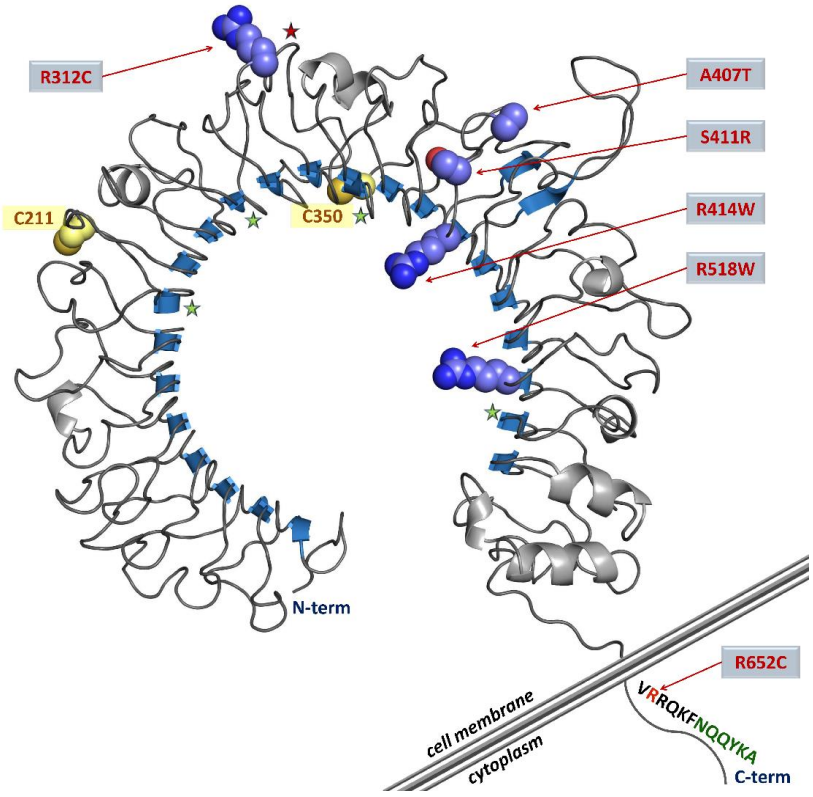
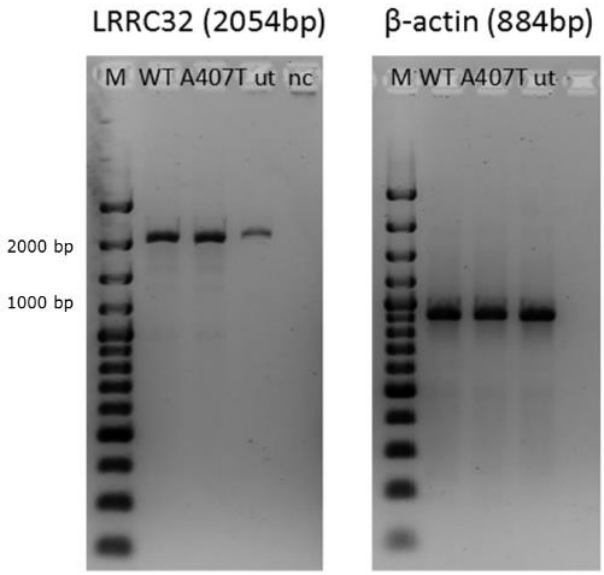
Supplementary Figures

Figure S1: Structural model of GARP.



The extracellular LRR repeat domain is visualized in cartoons. The cell membrane and intracellular domain of GARP are depicted schematically. Variant positions in the extracellular domain are shown as blue spheres. Cysteines forming disulfid bridges with TGFβ are shown as yellow spheres. N-glycosylation sites are marked with asterisks (green: predicted, red: verified). In the cytoplasmic tail, R652C is highlighted in red, a potential PDZ binding motif in green letters

Figure S2: mRNA expression of wildtype LRRC32 (WT), LRRC32 including SNP rs79525962 (A407T) and human ß-actin in $CD4^+CD25^-$ T cells.



RNA was prepared from wildtype LRRC32-, A407T LRRC32-overexpressing, and untransfected cells (ut), reverse transcribed and amplified by PCR. Amplification of ß-actin was used as internal control. nc=negative control (water instead of cDNA), M= 100bp Plus DNA Ladder (Fermentas, Vilnius, Lithuania)

Supplementary Tables

Table S1: Alleles, amino acid changes and minor allele frequencies of 6 identified missense variants in *LRRC32* in 2193 AD cases and 2197 controls.

Variant	Alleles	aa change	Cases freq/n	Controls freq/n
rs371900727	T<C	R652C	0.0002/1	0.0000/0
rs142940671	A<G	R518W	0.0007/3	0.0002/1
-	C<C	S411R	0.0002/1	0.0000/0
rs201431152	T<C	R414W	0.0005/2	0.0007/3
rs79525962	T<C	A407T	0.0284/123	0.0195/85
rs143082901	T<C	R312C	0.0009/4	0.0007/3

aa: amino acid; cases freq/n: minor allele frequency and absolute number of carriers in affected cases; controls freq/n: minor allele frequency and absolute number of carriers in unaffected controls; variants are sorted by position

Table S2: Linkage Disequilibrium for A407T/rs79525962 and previously reported GWAS SNPs for AD and other inflammatory traits within the susceptibility locus on chromosome 11q13.5.

SNP (Ref.)	**A407T/ rs79525962**	rs7130588 (Ferreira *et al.*, 2011; Weidinger *et al.*, 2013)	rs2155219 (Anderson *et al.*, 2011; Betz *et al.*, 2015; Bonnelykke *et al.*, 2013; Kottyan *et al.*, 2014; Ramasamy *et al.*, 2011)	rs7927894 (Barrett *et al.*, 2008; Esparza-Gordillo *et al.*, 2009)	rs7927997 (Franke *et al.*, 2010)
A407T/ rs79525962	**1**	0.015	0.010	0.014	0.015
rs7130588	1.000	**1**	0.708	0.965	1.000
rs2155219	1.000	1.000	**1**	0.733	0.708
rs7927894	1.000	1.000	1.000	**1**	0.965
rs7927997	1.000	1.000	1.000	1.000	**1**

Upper right triangle displays r2-values (black) and lower left triangle D'-values (red). LD was derived from 1000 genomes (release March 2012).

Table S3: Flow cytometric (FACS) data of GARP surface expression on transiently transfected $CD4^+CD25^-$ T cells of four independent donors.

	log (% GARP positive cells)			
Donor	Unstained	Control	Wildtype	Mutant (A407T)
1	0	3.8918	5.9865	5.4072
2	0	3.6376	6.2265	5.9081
3	0	4.6151	6.7581	5.9026
4	0	3.5264	6.1377	5.3132
Mean		3.9177	6.2772	5.6328
Std Dev		0.4894	0.3356	0.3171
ANOVA		$P=3.53x10^{-5}$		
Post hoc: wildtype vs. control		$P_{BH}=3.7x10^{-5}$		
Post hoc: mutant vs. control		$P_{BH}=0.0003$		
Post hoc: mutant vs. wildtype		**$P_{BH}=0.0436$**		

Table shows log-transformed percentage of GARP-positive cells normalized by the percentage of unstained cells. Statistical analysis was performed using ANOVA following a Bonferroni-Holm (BH) corrected post-hoc analysis.

Table S4: Flow cytometric (FACS) data of intracellular GARP expression in transiently transfected CD4+CD25- T cells of three independent donors.

	log(% GARP positive cells)			
Donor	Unstained cells	Control	Wildtype	Mutant (A407T)
1	0	3.9493	1.6677	2.2192
2	0	3.7955	2.1972	2.4681
3	0	3.8670	1.9315	2.2407
Mean		3.8706	1.9322	2.3093
Std Dev		0.0770	0.2648	0.1379
ANOVA		$P= 2.47x10^{-5}$		
Post hoc: wildtype vs. control		$P_{BH}= 3.3x10^{-5}$		
Post hoc: mutant vs. control		$P_{BH}= 7.7x10^{-5}$		
Post hoc: mutant vs. wildtype		**$P_{BH}= 0.041$**		

Table shows log-transformed percentage of GARP-positive cells, normalized by the percentage of unstained cells. Statistical analysis was performed using ANOVA following a Bonferroni-Holm (BH) corrected post-hoc analysis.

Table S5: PCR primers used for validation of A407T/rs79525962 and R518W/rs142940671 and resequencing of the *LRRC32* coding DNA sequence.

	Forward 5‘-3‘	Reverse 5‘-3‘
NGS Validation		
A407T/rs79525962	tatctcattatccaccaggctca	gggagaacaaactgctccattt
R518W/rs142940671	aaggtgaggatgatgatgaggttga	ggaaccgagtcagcccctgt
LRRC32 CDS		
LRRC32_Exon2	ggtacattccctttcctctcctg	catttcccacctggctctctg
LRRC32_Exon3.1	gagatgcaggtgagggaattcc	acatgtctggtttcatcccctac
LRRC32_Exon3.2	cagtgtctccagggcattgtg	gaacagtctgactcgcctcac
LRRC32_Exon3.3	gaagctgttgtttcgcaggtc	gaaactgcttgcggacctttg
LRRC32_Exon3.4	cctgaaaccgcccaacttctg	ccctgcttcatctgcctcaag

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