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Genome-wide association analysis of eosinophilic esophagitis provides insight into the tissue specificity of this allergic disease

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Contributions

M.E.R. and J.B.H. conceived the project and supervised the study. L.C.K. and M.E.R wrote the manuscript. L.C.K led and performed the genetic analysis. Statistical consultation and support was contributed by H.H. and L.J.M. B.P.D, J.D.S, and M.R. performed the experiments with assistance from E.M.S, and K.A.K. K.L, K.K, M.T.W., A.M.R., M.K., P.D, and S.V. provided analytical and bioinformatic support. S.L genotyped samples with assistance from A.M.. M.C., R.A.W., R.D.P., B.P.V, D.M.F., R.L, and H.A.S provided samples through the CoFAR consortium. V.M, P.E.P., and J.P.A, and M.E.R. provided samples for the CCHMC cohort. All of the authors discussed the results and commented on the manuscript.

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Abstract

Eosinophilic esophagitis (EoE) is a chronic inflammatory disorder associated with allergic hypersensitivity to food. We interrogated >1.5 million genetic variants in European EoE cases and subsequently in a multi-site cohort with local and out-of-study control subjects. In addition to replication of the *5q22* locus (meta-analysis $p = 1.9\times10^{-16}$), we identified association at 2p23 (encoding *CAPN14*, $p = 2.5\times10^{-10}$). *CAPN14* was specifically expressed in the esophagus, dynamically upregulated as a function of disease activity and genetic haplotype and after exposure of epithelial cells to IL-13, and located in an epigenetic hotspot modified by IL-13. There was enriched esophageal expression for the genes neighboring the top 208 EoE sequence variants. Multiple allergic sensitization loci were associated with EoE susceptibility ($4.8\times10^{-2} < p < 5.1\times10^{-11}$). We propose a model that elucidates the tissue specific nature of EoE that involves the interplay of allergic sensitization with an EoE-specific, IL-13–inducible esophageal response involving CAPN14.

Eosinophilic esophagitis (EoE) is a chronic, food antigen–driven, tissue-specific, esophageal, inflammatory disease characterized by marked mucosal eosinophil accumulation that is often associated with fibrosis, stricture formation, and impaired motility[1–4]. The disease remits after removal of specific food types, reoccurs upon food re-introduction, is associated with marked dysregulation of esophageal transcripts rich in elements involved in allergic inflammation and can be induced in mice by allergen exposure through IL-5– and IL-13–driven pathways[5–8]. Consistent with an allergic etiology, EoE frequently co-occurs with allergic diseases including asthma, eczema and food anaphylaxis[2–4]. Why EoE patients develop a tissue-specific response remains an enigma, as the currently identified inflammatory pathways and genes in EoE and other allergic diseases overlap.

The only EoE genome-wide association study (GWAS) reported to date identified a single significant susceptibility locus at 5q22, which harbors the gene for thymic stromal lymphopoietin, *TSLP*[9]. A candidate gene analysis confirmed the association of EoE with *TSLP*, as well as its receptor *CRLF2* (cytokine receptor-like factor 2)[10,11]. Candidate gene studies have shown that *CCL26* (eotaxin 3) and *FLG* (filaggrin)[12,13] are associated with EoE susceptibility. However, genetic variations in these genes and 5q22 have also been linked with other atopic disorders[14–16], highlighting the need to elucidate how genetics may contribute to the tissue specificity of this disorder.

Accordingly, we performed a GWAS of single-nucleotide polymorphisms (SNPs) from >1.5 million genetic markers. This GWAS greatly expanded the number of EoE cases (from 351 to 736) and controls (from 3105 to 9246) of the previous study. Combined genetic

association analysis identified 20 SNPs at 17 loci (threshold $p < 10^{-7}$). Ten of these loci were identified in the analysis of our Cincinnati Children's Hospital Medical Center (CCHMC) cohort ($p < 10^{-4}$) and in an analysis in which the external controls were excluded ($p < 5\times10^{-2}$) and also independently confirmed in the NIH Consortium of Food Allergy Research (CoFAR) cohort ($p < 5\times10^{-2}$; Table 1, Figure 1, Supplementary Tables 1 and 2, Supplementary Figure 1). This series of consistency checks was used to reduce the chance of false positives due to the inclusion of an external control cohort.

Markers in 2p23, 5q22, 8p23, and 15q13 reached genome-wide significance ($p < 5\times10^{-8}$). The variants most highly associated with increased risk of EoE were found at 2p23 spanning the *CAPN14* gene (best SNP rs77569859, $p = 3.30\times10^{-10}$, odds ratio [OR]=1.98) (Table 1). In order to identify the commonly occurring variants (minor allele frequency [MAF] > 1%) on the risk haplotype that could be driving the genetic association, we imputed this region to a composite reference panel from 1,000 genomes[17,18]; no haplotype of continuous SNPs or haplotype constructed using the most associated variants in the region was more highly associated with EoE risk than rs77569859 alone (best haplotype $p = 3.5\times10^{-8}$, OR = 1.6; Figure 1b and data not shown). Variants at the other two newly discovered loci reaching genome-wide significance were located at the *XKR6* (XK, Kell blood group complex subunit-related family, member 6) gene (8p23) and in a gene desert (15q13). Very little is known about XKR6; however, public expression databases report expression in the immune compartment (Supplementary Figure 2). These four genome-wide susceptibility loci remained associated with EoE, and the effect size was not significantly influenced after correcting for atopy (Supplementary Table 3).

Using an independently ascertained cohort that did not overlap with the first EoE GWAS[9], there was strong replication of disease linkage with 5q22 (rs6594499, Fishers combined $p = 1.9\times10^{-16}$; Supplementary Table 4). After imputing the region, the most significant association with the development of EoE was downstream of *TSLP* and *WDR36* at rs1438672 (Figure 1C), with 12 variants having a $p < 10^{-2}$ (p between 0.001 and 0.05) after adjusting for the most significant variant. After accounting for multiple testing, we cannot reject the null hypothesis that there is one independent genetic effect at this locus.

Variants at 1p13, 5q23, 10p12, 11q13, 11q14, and 21q22 demonstrated suggestive genetic association with EoE ($p < 10^{-7}$) (Table 1, Figure 1, Supplementary Figure 5). After establishing statistical associations between genetic variants at these loci and EoE, we performed fine-mapping studies starting with imputation variants not captured in the combined GWAS dataset (Figure 1, Supplementary Figure 3). The 11q13 region has been associated with asthma[19,20], atopic dermatitis[21–24], inflammatory bowel disease[25], allergic rhinitis[26], and sensitization to grass[26]. The EoE-associated variants at 11q13 are between *C11orf30* (chromosome 11 open reading frame 30) and *LRRC32* (leucine-rich repeat containing 32, also known as GARP). LRRC32 has a role in latent transforming growth factor beta (TGF)-β surface expression[27], and *LRRC32* mRNA is highly expressed in activated forkhead box P3 $(FOXP3)^+$ T regulatory cells. Notably, TGF-β and $FOXP3^+$ T regulatory cells have been implicated in EoE[28–32].

CAPN14 encodes for calpain 14, a calcium-activated cysteine protease. A survey of 130 tissues revealed that *CAPN14* was most highly expressed in the esophagus (Figure 2a, Supplementary Figure 5). When we assessed the expression of the calpain family members in biopsies from subjects with and without EoE, we found a distinct pattern for control, treated EoE, and untreated EoE subjects; Importantly, *CAPN14* was dynamically expressed as a function of disease activity (Figure 2b). *CAPN14* showed the largest upregulation compared with all members of the CAPN family (Figure 2b), but three of the other fifteen family members, *CAPN3*, *CAPN5*, and *CAST* (calpastatin), were also dysregulated in EoE esophageal biopsies (Figure 2b). CAST is a calpain inhibitor and was downregulated (29%, $p < 10^{-4}$). We found a >2-fold increase in *CAPN14* expression in the esophageal biopsies of patients with active EoE (Figure 2c). Furthermore, IL-13 stimulation of primary esophageal epithelial cells and an esophageal epithelial cell line grown at the air-liquid interface with IL-13 resulted in a 4-fold and >100-fold increase in *CAPN14* expression, respectively (Figure 2d, g). Patients with the risk haplotype expressed 30% lower *CAPN14* mRNA than those without the risk allele ($p < 10^{-2}$) (Figure 2e).

To identify the genomic mechanisms that may underlie the 2p23 association with EoE, we performed chromatin immunoprecipitation sequencing (ChIP-seq) on esophageal epithelial cells treated with IL-13. Of the six SNPs most highly associated at the *CAPN14* locus after imputation, two (rs76562819 and rs75960361) were located in putative regulatory regions on the basis of the IL-13–induced H3K27Ac ChIP-seq marks of esophageal epithelial cells, as well as publically available ENCODE functional genomics data (including histone marks, DNaseI hypersensitivity data, and ChIPseq data)[33–35] (Figure 2h).

rs76562819 is located proximal to the 5′ of the *CAPN14* transcription start site (Figure 2h), lies within a region of elevated H3K4Me1 histone marks in multiple cells lines, and intersects with open chromatin regions in 34 cell types on the basis of DNaseI hypersensitivity site mapping data. We subsequently performed an electrophoretic mobility shift assay (EMSA) using a capture probe from this region and found that the risk allele (rs7462819) preferentially bound to a nuclear protein complex compared to the non-risk allele (Figure 2i).

Importantly, *CAPN14* was the only CAPN family member to be upregulated as measured by microarray and RNA sequencing (RNA-seq) in either primary epithelial cells from esophageal biopsies and in the organotypic culture after treatment with IL-13 (Figure 3a), consistent with previous preliminary findings in other cell types[36,37]. IL-13 increased calpain activity in esophageal epithelial cells treated with IL-13, and this activity was inhibited by a calpain specific inhibitor (Figure 3b).

We searched for proximal (cis-acting) expression quantitative trait loci (eQTLs) using gene expression data obtained from six cell types or tissues (white blood cells [WBCs], lymphoblastoid cell lines, whole blood, adipose tissue, B cells, and monocytes). At two of the ten most highly associated EoE risk loci, the sentinel SNP was associated ($p < 10^{-3}$) with the expression of one or more nearby genes. Specifically, the most highly associated variants at 1p13 and 8p23 were strongly associated with the expression of the nearest genes *SLC25A24* (solute carrier family 25 [mitochondrial carrier; phosphate carrier], member 24; p

= 1.25×10^{-9}) and *XKR6* (p = 1.02×10^{-7}) (Supplementary Table 5). From our own expression databases, we have previously reported that *TSLP* expression is upregulated in the biopsies of EoE patients in an allele-dependent manner and in esophageal epithelial cell lines treated with polyinosinic:polycytidylic acid[9,10].

We measured the RNA-seq expression of all genes within 25 kb of variants with combined p < 10^{-4}. Of these 208 genes, 48% were expressed in the esophagus at appreciable levels[38]; this represented an enrichment compared with the expression of the whole genome in the esophagus ($p < 10^{-4}$ using permutation testing). Furthermore, differential expression of these genes was sufficient to segregate EoE cases from controls, and 8% of the genes near the most highly associated EoE risk loci were differentially expressed (>2-fold average change, Bonferroni adjusted $p < 5\times10^{-2}$) in EoE patient vs. control biopsies (Figure 4, Supplementary Figure 6). Therefore, these data demonstrate a concentration of EoE susceptibility loci in the neighborhood of genes expressed and/or dysregulated in the esophagus of diseased patients, suggesting an esophageal functional role for the implicated gene. We assessed the GWAS associations for differential H3K27Ac marks in epithelial cells after IL-13 treatment. Notably, *CAPN14* was one of two genes with these IL-13–responsive epigenetic characteristics (Supplementary Figure 7). This analysis further underscores the potential centrality of *CAPN14* in the etiology of EoE. These data are consistent with the mechanistic model in which *CAPN14* is dynamically expressed in the esophagus in response to inflammatory stimuli, a regulatory mechanism disrupted by the decreased expression associated with the risk haplotype (Supplementary Figure 8).

The previous genome-wide study of EoE did not assess the most significant variant in *CAPN14* but did identify suggestive association ($p < 10^{-4}$) from variants in the region[9]. With additional EoE cases and controls, the current study was better powered to identify statistically significant association of genetics variants with the development of EoE. A limitation of this study is the lack of a replication stage for those loci that are most highly associated with EoE in the combined analysis of the two independent cohorts that were assessed. As such, apart from the association at TSLP/WDR36 and CAPN14, other highly associated loci remain suggestive until confirmed in subsequent studies.

Two recent GWAS reported 22 loci associated with allergic sensitization[39,40]. Remarkably, we found EoE association at 9 of these 22 loci (Supplementary Table 6), underscoring the key role of atopy in EoE, and 8 of these SNPs were associated with comparable disease risk effects. The atopic sensitization loci with the greatest association with EoE were at *CLEC16A*, *LRRC32*, *LPP* (C-type lectin domain family 16, member A, Leucine rich repeat containing 32, LIM domain containing preferred translocation partner in lipoma), and *TSLP/WDR36* (Figure 1, Supplementary Figure 9). However, of the ten loci that were linked with EoE in our study, only two overlapped with the 22 allergic sensitization loci, highlighting that non-atopy–related processes may be operational.

In conclusion, herein we have increased the number of putative susceptibility loci for EoE from one to four and present compelling evidence for six other loci. Importantly, our data substantiate a mechanism to elucidate the tissue-specific manifestations of this prototypic allergic disease. In particular, we provide additional evidence of shared genetic and

molecular pathways between general atopy risk factors (e.g. *TSLP/WDR36, LRR32, IL-33, LPP*) and EoE disease–specific elements, most notably genetic risk factors present at 2p23 where *CAPN14* is located. Consistent with this model, the *CAPN14* gene is located in a baseline epigenetic hotspot that is modified by IL-13, and CAPN14 is specifically expressed in esophageal epithelium and is dynamically upregulated as a function of disease activity and genetic haplotype and after exposure of epithelial cells to IL-13. It has not escaped our attention that mutations in *CAPN3*, whose gene product is specifically expressed in skeletal muscle, have been associated with susceptibility to another tissue-specific eosinophilic disorder (eosinophilic myositis)[41–46]. Although we do not yet know the molecular steps that link calpainopathy to eosinophilic responses, it is notable that CAPN14 belongs to the classical calpain sub-family that comprises one of the major proteolytic systems that mediate protein cleavage (in addition to the proteasome, lysosome, and caspase systems)[47]. Classical calpains are calcium regulatory proteases and their substrates include structural proteins, signaling molecules, transcription factors[48,49], and inflammatory mediators that are germane for allergic responses, such as STAT-6 (signal transducer and activator of transcription 6) and IL-33[50,51], the latter of which shows some linkage with EoE (Supplementary Figure 7, Table 2). On the basis of the collective data, we propose a model that links the interplay of allergic sensitization with an EoE-specific, IL-13–inducible esophageal response involving *CAPN14*.

Data access

The genotyping data from this study have been submitted to dbGAP under the accession number phs000494.v1.p1. The expression data from this study have been submitted to the NCBI GEO database accession number #.

Methods

Genotyping

Genotyping was performed on the Illumina OMNI-5 and OMNI-2.5 genotyping arrays (Illumina) using Infinium2 chemistry. Genotypes were called using the Gentrain2 algorithm within Illumina Genome Studio.

Subjects

The study was approved by the Institutional Review Boards at Cincinnati Children's Hospital Medical Center (CCHMC) and all participating sites that were part of the NIH Consortium of Food Allergy Research EoE Cohort (Mount Sinai Medical Center, University of North Carolina, Johns Hopkin's University, University of Colorado Health Center/ National Jewish Research Center, and Arkansas Children's Hospital). Parental informed consent was obtained from all participants in this study for the purpose of DNA collection and genotyping. Cases were confirmed by a physician to fulfill the diagnostic criteria for EoE. EoE is defined as peak eosinophil count ≥15 eosinophils / high-power field in esophageal biopsy sections; 30% of CCHMC and 51% of CoFAR patients had PPI therapy before the diagnostic endoscopy. Control subjects (non-EoE) included the self-reported Caucasian subjects in the Cincinnati Genomic Control Cohort CCHMC (n = 831, age range

2–18 years)[54], and an external control cohort (non-EoE) acquired from a database of Genotypes and Phenotypes (dbGAP) University of Michigan study (n = 8,580). In the CCHMC and CoFAR cohorts, 73% and 62% of EoE patients were male, respectively, and EoE patients had an age range of 2–52 years. The external control cohort was collected through an aging and retirement study; these subjects were significantly older than the cases at the time of DNA collection. The subjects in the external control cohort were randomly assigned to the CCHMC or CoFAR analysis with the goal of equivalent case:control ratios in each cohort.

Population Stratification

Genome-wide data were used to infer the top six principal components of genetic variation and correct for possible population stratification using Eigensoft. All local cases and controls were self-identified as having European ancestry, and principal component analysis was used to exclude all subjects (n = 271) who segregated more than 4 standard deviations outside of the mean of the first 4 principal components. Specifically, we removed 34 genetic outliers from 736 EoE cases, 13 from 235 CoFAR cases, 71 of 831 CCHMC controls, and 166 of 8,652 dbGAP University of Michigan controls. The resulting genomic inflation factor was 1.001 in a set of ancestral informative markers not including the associated loci. The genome-wide genomic inflation seen in our study for the CCHMC cohort and CoFAR cohorts were 1.04 and 1.05, respectively.

Genotyping Quality Control

To confirm accurate genotyping and sample identification, we performed an identity-by-state analysis on a subset of 10,732 SNPs that were genotyped on both the Illumina OMNI-5 and the previous GWAS platform (Illumina 550)[9] and found 99.998% concordance between the 134 samples that were genotyped on both platforms. The genome-wide genotyping datat was deposited into dbGAP (study Accession: phs00494.v1.p1). Of the original 4,301,332 markers on the OMNI-5, 2,512,766 were derived from rare variants (<1% MAF). Notably, 87,539 markers had a suboptimal call rate (e.g. <96%), and 44,246 were not in Hardy Weinberg Equilibrium in the controls ($p < 10^{-4}$). We assessed the most significant 10,000 variants in Genome-Studio projects that included the locally genotyped cases and controls and the external controls from dbGAP; upon visual inspection, 124 of these SNPs did not cluster properly and were removed from further analysis. rs8041227 reached genome-wide significance; however, no other SNPs around this variant in the dataset were strongly associated. The Genome-Studio cluster plot of this particular SNP is given in Supplementary Figure 11. External controls were genotyped on the OMNI-2.5, and overlapping variants that passed the quality measures described above in both datasets were analyzed. We controlled for the presence of potential batch effects in the analysis of the CoFAR samples, by removal of SNPs that exhibited outlier fluorescence associated with deviation between plates (P<10-4), as per the manufacturer's recommendation. The final genotyping rate for all SNPs was 99.7%. After applying the above filters, we used genotypes from 1,468,075 autosomal SNPs in a CCHMC cohort of 435 cases and 716 well-phenotyped local controls from CCHMC and 5,776 controls from the dbGAP study and a CoFAR cohort of 222 cases from the NIH CoFAR EoE cohort and 2,804 controls from the University of Michigan Health and Retirement System (obtained from dbGAP) (Supplementary Table 1).

Association and Linkage Disequilibrium Analysis

After removing genetic outliers, we performed a logistic regression to calculate p values and odds ratios for each SNP using PLINK[55] with gender as a co-variant. For some analyses, atopy and the most significant SNP in the region were also used as covariates. For analyses that used atopy as a covariate, only subjects with known atopic status (EoE cases and local Cincinnati controls) were included. For cases, atopy was defined by a physician-documented history of positive skin-prick test, allergic rhinitis, allergic dermatitis/eczema, asthma, or food allergy. The prevalence of atopy for the local CCHMC and CoFAR EoE cohorts was 96.2% and 91.3%, respectively. For the Cincinnati Genomic Control Cohort, atopy was defined as parent-reported history of allergic rhinitis, eczema, asthma, or food allergy; the atopy prevalence of this cohort was 28.6%. LocusZoom[56] and R were used to map the associated loci in the context of chromosomal recombination and nearby genes.

Imputation to the 1,000 Genomes Reference Panel

To detect associated variants that were not directly genotyped, we imputed highly associated regions with IMPUTE2 and used a composite imputation reference panel of integrated haplotypes from the 1,000 Genomes Project sequence data freezes from March 2012 produced using SHAPIT2[17,18]. Imputed genotypes were required to meet or exceed a probability threshold of 0.9, an information measure of >0.4, and the same quality-control criteria threshold described for the genotyped markers.

Expression Analysis

Quantitative real-time PCR analysis was performed on cDNA from distal esophageal biopsy RNA. EoE biopsies showed active disease pathology at the time when they were taken, and all patients reported no glucocorticoid treatment at the time of biopsy, except for the analysis of gene expression as a function of disease activity in which remission was defined after glucocorticoid therapy. Statistical testing for mRNA expression normalized to *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) was determined by Mann-Whitney U test using GraphPad Prism software.

Expression Microarray Analysis

For each patient, 1 distal esophageal mucosal biopsy sample was immersed in RNAlater RNA stabilization reagent (QIAGEN) and stored at 4°C for less than 15 days. Total RNA was extracted using RNeasy Mini Kit (QIAGEN) according to the manufacturer's recommendations. Hybridization to DNA microarray was performed by the Microarray Core at CCHMC, as previously reported[13,57]. The genome-wide human Affymetrix U133 Plus 2.0 GeneChip was used, and gene transcript levels were determined using algorithms in the Microarray Analysis Suite and GeneSpring software (Silicon Genetics).

RNA Sequencing

Esophageal biopsy RNA was isolated from EoE patients with active disease and from unaffected controls as previously described[13,38]. RNA sequencing acquiring 10 million mappable 100 base-pair reads from paired-end libraries was performed at the Genetic Variation and Gene Discovery Core Facility at CCHMC. Data were aligned to the GrCh37

build of the human genome using the Ensembl[58] annotations as a guide for TopHat[59]. Expression analysis was performed using Cufflinks[60]. Data were visualized using the Integrative Genomics Viewer (Broad Institute). Enrichment analysis was performed using permutation testing by randomly selected SNPs from the chip, identified the nearest gene and assessed the number of genes expressed in the esophagus. Only 10 permutations out of 100,000 total permutations had more than or equal to 99 genes expressed in the esophagus.

Electrophoretic Mobility Shift Assay (EMSA)

Pairs of single-stranded 5′ IRDye infrared dye labeled and unlabeled oligonucleotides (obtained from IDT Inc, Coralville, Iowa, USA) were annealed to generate double-stranded probes. Twenty-five to 50 fmoles of labeled probes was incubated with 8 or 10 μg of nuclear extract prepared from esophageal cell line TE-7, 6 μg poly (deoxyinosinic-doxycytidylic) acid, and 1 μl salmon sperm provided along with the buffers and protocols supplied with the Odyssey Infrared EMSA kit (LI-COR Biosciences, Lincoln, Nebraska, USA). The binding reactions were analyzed using electrophoresis on 6% Tris-Borate-EDTA polyacrylamide gels and detected by an infrared fluorescent procedure using the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, Nebraska, USA)

Organotropic Cultures

For the air-liquid interface (ALI) culture system, the esophageal epithelial cell line (hTERT-immortalized EPC2 line from Dr. Anil Rustgi [University of Pennsylvania]) was grown to confluence on 0.4 μm pore–size polyester permeable supports (Corning Incorporated, Corning, NY) in keratinocyte serum-free media (K-SFM) (Life Technologies, Grand Island, NY) supplemented with 1.8 mM calcium. Epithelial differentiation was then induced by removing culture media from the inner chamber of the permeable support and maintaining the esophageal epithelial cells for 5–7 days at the ALI in the presence or absence of IL-13 (100 ng/mL).

H3K27Ac Analysis

Ten to 20 million TE-7 cells were fixed with 0.8% formaldehyde by adding 1 ml of 10X fixation buffer (50 mM HEPES-KOH, pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 8% formaldehyde) to 9 ml of growth medium for 8 minutes at room temperature with shaking. The reaction was stopped by adding glycine to a final concentration of 125 mM for an additional 5 minutes. Nuclei were prepared with truChIP™ High Cell Chromatin Shearing Kit with SDS Shearing Buffer (Covaris) according to manufacturer recommendations. Sonication was performed using a Covaris S220 Focused ultrasonicator at 175 pip, 10% output, 200 bursts for 8 minutes. Efficient DNA fragmentation was verified by agarose gel electrophoresis. ChIP was performed with 2 μg of H3K27Ac antibody (ab4729, Abcam) in SX-8G IP-Star® Automated System (Diagenode) in RIPA buffer (TE+0.1% SDS, 1% Triton X-100, 150 mM NaCl, 0.1% Sodium Deoxycholate) following the protocol of the manufacturer. Fastq files from Illumina pipeline were aligned by bowtie (version 1.0.0)[61] and unique reads were identified with no more than one error allowed for alignment. MACS2 (version 2.0.10.20130712)[62] was used to identify islands of enrichment (q-value threshold less than 0.2) and estimated fragment size. For visualization, data were uploaded to the University of California, Santa Cruz genome browser.

Calpain Activity Assay

EPC2 cultures were treated with or without IL-13 (100 ng/mL) for 48 hours and lysed with Mammalian Protein Extraction Reagent lysis buffer (Pierce, #78501) in the presence of 1mM ethylenediaminetetraacetic acid and 1mM dithiothreitol on ice. Calpain activity was measured with the Promega Calpain-Glo protease assay according to the manufacturer's instructions. Briefly, 50 μl of lysate was incubated for 10 minutes with reaction buffer and +/− 100 mM PD150606 (Santa Cruz, s-222133) and luminescence was read using the Biotek Synergy2 Multi-Mode Microplate reader.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Liacouras CA, et al. Eosinophilic esophagitis: updated consensus recommendations for children and adults. J Allergy Clin Immunol. 2011; 128:3–20. e6. quiz 21–2. [PubMed: 21477849]
2. Rothenberg ME. Biology and treatment of eosinophilic esophagitis. Gastroenterology. 2009; 137:1238–49. [PubMed: 19596009]
3. Collins MH, et al. Clinical, pathologic, and molecular characterization of familial eosinophilic esophagitis compared with sporadic cases. Clin Gastroenterol Hepatol. 2008; 6:621–9. [PubMed: 18434257]
4. Putnam PE, Rothenberg ME. Eosinophilic esophagitis: concepts, controversies, and evidence. Curr Gastroenterol Rep. 2009; 11:220–5. [PubMed: 19463222]
5. Abonia JP, Rothenberg ME. Eosinophilic esophagitis: rapidly advancing insights. Annu Rev Med. 2012; 63:421–34. [PubMed: 22034864]
6. Davis BP, Rothenberg ME. Emerging concepts of dietary therapy for pediatric and adult eosinophilic esophagitis. Expert Rev Clin Immunol. 2013; 9:285–7. [PubMed: 23557262]
7. Henderson CJ, et al. Comparative dietary therapy effectiveness in remission of pediatric eosinophilic esophagitis. J Allergy Clin Immunol. 2012; 129:1570–8. [PubMed: 22541246]

8. Rothenberg ME, et al. Working with the US Food and Drug Administration: progress and timelines in understanding and treating patients with eosinophilic esophagitis. J Allergy Clin Immunol. 2012; 130:617–9. [PubMed: 22935588]
9. Rothenberg ME, et al. Common variants at 5q22 associate with pediatric eosinophilic esophagitis. Nat Genet. 2010; 42:289–91. [PubMed: 20208534]
10. Sherrill JD, et al. Variants of thymic stromal lymphopoietin and its receptor associate with eosinophilic esophagitis. J Allergy Clin Immunol. 2010; 126:160–5. e3. [PubMed: 20620568]
11. Zuo L, et al. IL-13 induces esophageal remodeling and gene expression by an eosinophil-independent, IL-13R alpha 2-inhibited pathway. J Immunol. 2010; 185:660–9. [PubMed: 20543112]
12. Blanchard C, et al. Coordinate interaction between IL-13 and epithelial differentiation cluster genes in eosinophilic esophagitis. J Immunol. 2010; 184:4033–41. [PubMed: 20208004]
13. Blanchard C, et al. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. J Clin Invest. 2006; 116:536–47. [PubMed: 16453027]
14. McAleer MA, Irvine AD. The multifunctional role of filaggrin in allergic skin disease. J Allergy Clin Immunol. 2013; 131:280–91. [PubMed: 23374260]
15. Fallon PG, et al. A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. Nat Genet. 2009; 41:602–8. [PubMed: 19349982]
16. Shin HD, et al. Association of Eotaxin gene family with asthma and serum total IgE. Hum Mol Genet. 2003; 12:1279–85. [PubMed: 12761043]
17. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet. 2007; 39:906–13. [PubMed: 17572673]
18. Altshuler DM, et al. Integrating common and rare genetic variation in diverse human populations. Nature. 2010; 467:52–8. [PubMed: 20811451]
19. Ferreira MA, et al. Identification of IL6R and chromosome 11q13.5 as risk loci for asthma. Lancet. 2011; 378:1006–14. [PubMed: 21907864]
20. Tang HY, et al. Association analysis of single nucleotide polymorphisms at five loci: comparison between atopic dermatitis and asthma in the Chinese Han population. PLoS One. 2012; 7:e35334. [PubMed: 22545103]
21. Esparza-Gordillo J, et al. A common variant on chromosome 11q13 is associated with atopic dermatitis. Nat Genet. 2009; 41:596–601. [PubMed: 19349984]
22. Greisenegger EK, Zimprich F, Zimprich A, Gleiss A, Kopp T. Association of the chromosome 11q13.5 variant with atopic dermatitis in Austrian patients. Eur J Dermatol. 2013
23. Hirota T, et al. Genome-wide association study identifies eight new susceptibility loci for atopic dermatitis in the Japanese population. Nat Genet. 2012; 44:1222–6. [PubMed: 23042114]
24. Paternoster L, et al. Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis. Nat Genet. 2012; 44:187–92. [PubMed: 22197932]
25. Barrett JC, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet. 2008; 40:955–62. [PubMed: 18587394]
26. Ramasamy A, et al. A genome-wide meta-analysis of genetic variants associated with allergic rhinitis and grass sensitization and their interaction with birth order. J Allergy Clin Immunol. 2011; 128:996–1005. [PubMed: 22036096]
27. Tran DQ, et al. GARP (LRRC32) is essential for the surface expression of latent TGF-beta on platelets and activated FOXP3+ regulatory T cells. Proc Natl Acad Sci U S A. 2009; 106:13445–50. [PubMed: 19651619]
28. Fuentebella J, et al. Increased number of regulatory T cells in children with eosinophilic esophagitis. J Pediatr Gastroenterol Nutr. 2010; 51:283–9. [PubMed: 20639775]
29. Stuck MC, Straumann A, Simon HU. Relative lack of T regulatory cells in adult eosinophilic esophagitis - no normalization after corticosteroid therapy. Allergy. 2011; 66:705–7. [PubMed: 21470243]

30. Tantibhaedhyangkul U, Tatevian N, Gilger MA, Major AM, Davis CM. Increased esophageal regulatory T cells and eosinophil characteristics in children with eosinophilic esophagitis and gastroesophageal reflux disease. Ann Clin Lab Sci. 2009; 39:99–107. [PubMed: 19429794]
31. Zhu X, Wang M, Crump CH, Mishra A. An imbalance of esophageal effector and regulatory T cell subsets in experimental eosinophilic esophagitis in mice. Am J Physiol Gastrointest Liver Physiol. 2009; 297:G550–8. [PubMed: 19571233]
32. Frischmeyer-Guerrerio PA, et al. TGFbeta receptor mutations impose a strong predisposition for human allergic disease. Sci Transl Med. 2013; 5:195ra94.
33. Maher B. ENCODE: The human encyclopaedia. Nature. 2012; 489:46–8. [PubMed: 22962707]
34. Ecker JR, et al. Genomics: ENCODE explained. Nature. 2012; 489:52–5. [PubMed: 22955614]
35. Skipper M, Dhand R, Campbell P. Presenting ENCODE. Nature. 2012; 489:45. [PubMed: 22955612]
36. Ueta M, Mizushima K, Yokoi N, Naito Y, Kinoshita S. Expression of the interleukin-4 receptor alpha in human conjunctival epithelial cells. Br J Ophthalmol. 2010; 94:1239–43. [PubMed: 20610477]
37. Ueta M, Sotozono C, Kinoshita S. Expression of interleukin-4 receptor alpha in human corneal epithelial cells. Jpn J Ophthalmol. 2011; 55:405–10. [PubMed: 21617960]
38. Sherrill JD, et al. Analysis and expansion of the eosinophilic esophagitis transcriptome by RNA sequencing. Genes and Immunity. 2014 In Press.
39. Bonnelykke K, et al. Meta-analysis of genome-wide association studies identifies ten loci influencing allergic sensitization. Nat Genet. 2013; 45:902–6. [PubMed: 23817571]
40. Hinds DA, et al. A genome-wide association meta-analysis of self-reported allergy identifies shared and allergy-specific susceptibility loci. Nat Genet. 2013; 45:907–11. [PubMed: 23817569]
41. Krahn M, et al. Eosinophilic infiltration related to CAPN3 mutations: a pathophysiological component of primary calpainopathy? Clin Genet. 2011; 80:398–402. [PubMed: 21204801]
42. Krahn M, et al. CAPN3 mutations in patients with idiopathic eosinophilic myositis. Ann Neurol. 2006; 59:905–11. [PubMed: 16607617]
43. Bartoli M, et al. Validation of comparative genomic hybridization arrays for the detection of genomic rearrangements of the calpain-3 and dysferlin genes. Clin Genet. 2012; 81:99–101. [PubMed: 22150418]
44. Brown RH Jr, Amato A. Calpainopathy and eosinophilic myositis. Ann Neurol. 2006; 59:875–7. [PubMed: 16718709]
45. Amato AA. Adults with eosinophilic myositis and calpain-3 mutations. Neurology. 2008; 70:730–1. [PubMed: 18299526]
46. Oflazer PS, Gundesli H, Zorludemir S, Sabuncu T, Dincer P. Eosinophilic myositis in calpainopathy: could immunosuppression of the eosinophilic myositis alter the early natural course of the dystrophic disease? Neuromuscul Disord. 2009; 19:261–3. [PubMed: 19285864]
47. Sorimachi H, Hata S, Ono Y. Calpain chronicle--an enzyme family under multidisciplinary characterization. Proc Jpn Acad Ser B Phys Biol Sci. 2011; 87:287–327.
48. Arnandis T, et al. Differential functions of calpain 1 during epithelial cell death and adipocyte differentiation in mammary gland involution. Biochem J. 2014
49. Wang Y, Zhang Y. Regulation of TET Protein Stability by Calpains. Cell Rep. 2014; 6:278–84. [PubMed: 24412366]
50. Meephansan J, Tsuda H, Komine M, Tominaga S, Ohtsuki M. Regulation of IL-33 expression by IFN-gamma and tumor necrosis factor-alpha in normal human epidermal keratinocytes. J Invest Dermatol. 2012; 132:2593–600. [PubMed: 22673732]
51. Hayakawa M, et al. Mature interleukin-33 is produced by calpain-mediated cleavage in vivo. Biochem Biophys Res Commun. 2009; 387:218–22. [PubMed: 19596270]
52. Wu C, et al. BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. Genome Biol. 2009; 10:R130. [PubMed: 19919682]
53. McCall MN, Uppal K, Jaffee HA, Zilliox MJ, Irizarry RA. The Gene Expression Barcode: leveraging public data repositories to begin cataloging the human and murine transcriptomes. Nucleic Acids Res. 2011; 39:D1011–5. [PubMed: 21177656]

54. Prahalad S, et al. Juvenile rheumatoid arthritis: linkage to HLA demonstrated by allele sharing in affected sibpairs. Arthritis Rheum. 2000; 43:2335–8. [PubMed: 11037894]
55. Purcell S, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–75. [PubMed: 17701901]
56. Pruim RJ, et al. LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics. 2010; 26:2336–7. [PubMed: 20634204]
57. Zimmermann N, et al. Dissection of experimental asthma with DNA microarray analysis identifies arginase in asthma pathogenesis. J Clin Invest. 2003; 111:1863–74. [PubMed: 12813022]
58. Flicek P, et al. Ensembl 2013. Nucleic Acids Res. 2013; 41:D48–55. [PubMed: 23203987]
59. Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics. 2009; 25:1105–11. [PubMed: 19289445]
60. Trapnell C, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol. 2010; 28:511–5. [PubMed: 20436464]
61. Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 2009; 10:R25. [PubMed: 19261174]
62. Zhang Y, et al. Model-based analysis of ChIP-Seq (MACS). Genome Biol. 2008; 9:R137. [PubMed: 18798982]

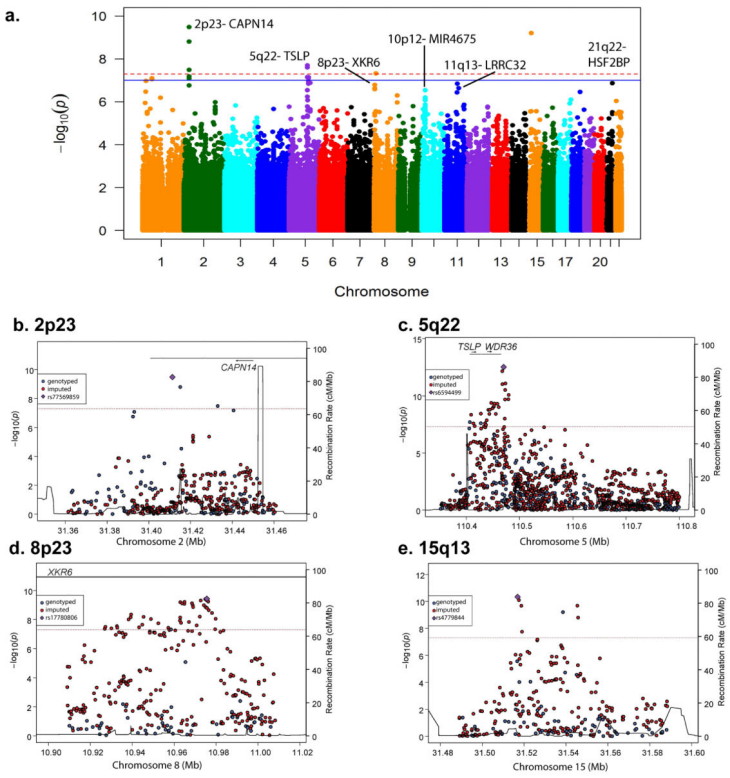

Figure 1. Manhattan plot of the p values obtained from the genome-wide association analysis

a. Data are from 736 subjects with EoE and 9,246 controls having 1,468,075 genetic variants, with minor allele frequencies greater than 1% in the subjects with EoE. The −log of the probability is shown as a function of the genomic position of the autosomes. Genome-wide significance (red dotted line, $p \leq 5\times10^{-8}$) and suggestive significance (solid blue line, $p \leq 10^{-7}$) are indicated. b–e. Genetic association of variants at the 2p23, 5q22, 8p23, and 15q13 loci with EoE risk. P values ($-\log_{10}$) of the genetic association analysis of genotyped and imputed variants are plotted against the genomic positions of each genotyped (blue) and imputed (red) SNPs on the *x* axis on chromosomes 2, 5, 8, and 15. Genes in the region are shown above. The black lines indicate the recombination rates in cM per Mb using subjects of European Ancestry from the 1,000 genomes project.

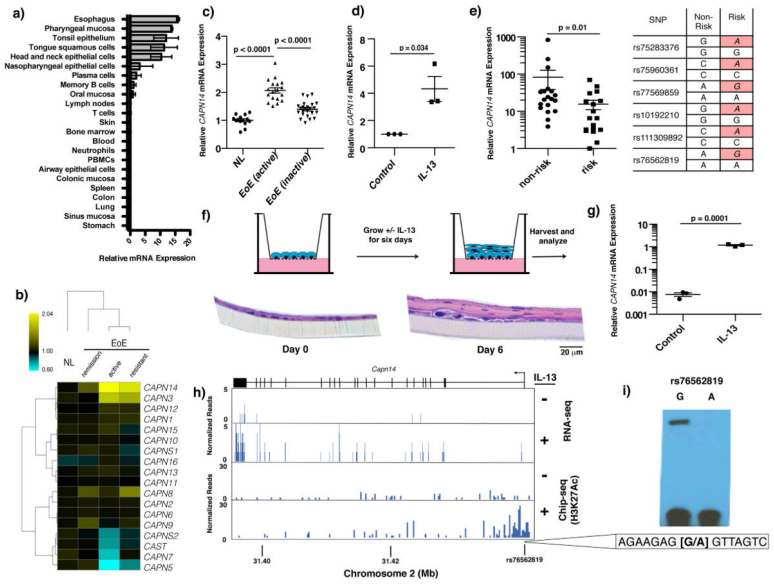

Figure 2. *CAPN14* is specifically expressed in esophageal epithelium, dynamically upregulated as a function of disease activity and genetic haplotype and after exposure of epithelial cells to IL-13

a. Barcode Z-score relative microarray expression of *CAPN14* in various human tissue samples. Barcode analysis of 18,656 publically available microarrays as displayed by biogps.org[52,53]. Representative data from multiple cellular subtypes. Error bars represent the median absolute deviation. (Refer to Supplemental Figure 2 for an exhaustive list). b. Microarray expression heat map of calpain family in esophageal biopsies from normal controls (NL, n = 14), therapy-responsive EoE patients (EoE remission, n = 18), active EoE patients (EoE active, n = 18), and therapy-non-responsive EoE patients (EoE resistant, n = 19). c. Microarray expression analysis of *CAPN14* expression from esophageal biopsies (NL, n = 14; EoE active, n = 18; EoE inactive, n = 18) (b. and c.) and primary esophageal epithelial cells with or without IL-13 stimulation for 48 hours (d). Error bars represent standard error of the mean (s.e.m.), n=3. e. Real-time PCR analysis of *CAPN14* expression in biopsies from EoE patients with the non-risk haplotype (n = 19) or with at least one copy of the risk haplotype at the 2p23 loci (n = 17). The risk haplotype is defined as having the EoE risk allele (highlighted in red) at each of the six most highly associated variant locations. Error bars represent s.e.m. f. Schematic of esophageal epithelial air-liquid interface (ALI) transwell culture system and H&E staining demonstrating stratification. g. RNA-seq expression analysis of *CAPN14* expression from ALI cultures with or without IL-13 stimulation for 6 days (n = 3 for each group). Error bars represent s.e.m. h. Chip-seq on TE-7 cells shows increased H3K27Ac marks with IL-13 stimulation over the transcriptional start site of *CAPN14*, which correlates with an increase in transcriptional activity by RNA-seq. One significantly associated SNP (rs76562819) is in this acetylation region. i. EMSA was used to probe nuclear lysates from an esophageal epithelial cell line using oligonucleotides with the risk [G] or non-risk [A] allele of rs7656219.

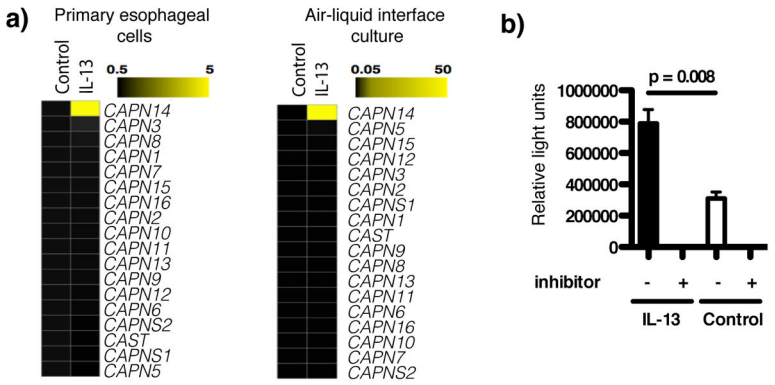

Figure 3. IL-13 induces *CAPN14* expression and calpain activity

a. Heat map of microarray expression of the calpain family in primary esophageal epithelial cell culture (left) and in EPC2 air-liquid interface cultures (right). b. Calpain activity assay in EPC2 cells with or without IL-13 stimulation for 48 hours in the presence or absence of the calpain inhibitor PD150606. Error bars represent s.e.m. Error bars represent s.e.m. (data representative of 3 independent experiments)

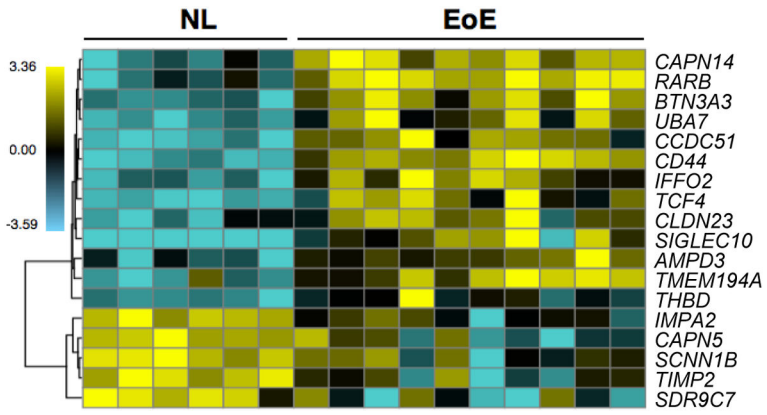

Figure 4. Genes at EoE risk loci with differential expression in biopsies from EoE patients compared to controls

Genes within 25 kb of the 768 genetic variants associated with EoE (combined $p < 10^{-4}$) were used in this analysis of RNA-seq data. The expression of 208 genes was assessed. Normalized fold-change is shown for all genes with 2-fold average change and corrected $p < 0.05$. NL, normal controls.

Table 1

Most highly associated EoE risk variants

Chr band	SNP	BP	Minor allele	MAF cases	MAF Controls	Major allele	p value	OR	Nearest Gene	Genomic context	p value CCHMC	OR CCHMC	p value CoFAR	OR CoFAR
1p13	rs2000260	108673405	G	0.37	0.43	A	6.56×10^{-7}	0.757	*SLC25A24*		1.10×10^{-4}	0.773	1.7×10^{-3}	0.725
2p23	**rs77569859**	**31411287**	**G**	**0.09**	**0.05**	**A**	$\mathbf{3.30\times10^{-10}}$	**1.98**	***CAPN14***	**intronic**	$\mathbf{1.41\times10^{-7}}$	**1.93**	$\mathbf{1.2}\times10^{-3}$	**2.06**
5q22	**rs3806933**	**110406742**	**A**	**0.37**	**0.44**	**G**	$\mathbf{2.00\times10^{-8}}$	**0.731**	***TSLP***	**non-coding**	$\mathbf{1.32\times10^{-6}}$	**0.724**	$\mathbf{3.1}\times10^{-3}$	**0.740**
5q23	rs2055376	116181428	A	0.04	0.02	C	7.12×10^{-8}	2.30	near *SEMA6a*		1.89×10^{-6}	2.29	7.7×10^{-3}	2.40
8p23	**rs2898261**	**10958539**	**A**	**0.35**	**0.42**	**C**	$\mathbf{4.84\times10^{-8}}$	**0.735**	***XKR6***	**intronic**	$\mathbf{1.73\times10^{-6}}$	**0.724**	$\mathbf{6.2}\times10^{-3}$	**0.755**
10p12	rs11819199	20865157	G	0.09	0.06	A	2.89×10^{-7}	1.62	*MIR4675*		1.64×10^{-5}	1.62	2.00×10^{-3}	1.70
11q13	rs2155219	76299194	C	0.413	0.491	A	3.65×10^{-7}	0.729	between *C11orf30* and *LRRC32*		2.64×10^{-5}	0.743	5.8×10^{-3}	0.699
11q14	rs118086209	86104495	C	0.03	0.02	A	2.35×10^{-7}	2.19	CCDC81	intronic	1.93×10^{-4}	1.99	6.61×10^{-5}	2.83
15q13	**rs8041227**	**31538542**	**A**	**0.2**	**0.28**	**G**	$\mathbf{6.34\times10^{-10}}$	**0.657**	**between *LOC283710* and *KLF13***		$\mathbf{3.97\times10^{-6}}$	**0.693**	$\mathbf{3.38\times10^{-5}}$	**0.581**
21q22	rs17004598	45078556	C	0.03	0.01	A	1.37×10^{-7}	2.57	*HSF2BP*	intronic	4.48×10^{-6}	2.59	3.3×10^{-3}	2.75

Loci included in this table were significant or suggestive ($p<5\times10^{-6}$) and consistently associated in the CCHMC cohort ($p<0.0005$), local analysis ($p<0.05$), and CoFAR cohort ($p<0.05$). Those loci that failed one of these consistency checks can be found in Supplementary Table 2. The most highly associated variant is shown for each loci. BP, build 37 map position of the SNP; CCHMC, Cincinnati Children's Hospital Medical Center; CoFAR, NIH Consortium of Food Allergy Research); Chr and band, chromosome and cytogenetic band; MAF. minor allele frequency across cases or controls; Nearest gene, spanning or flanking (<1 Mb away from) the index SNP; OR, odds ratio for the minor allele; p value, the weighted Z-score method implemented in METAL was used to combine the p values for the CCHMC and CoFAR cohorts; SNP, rs ID of variant. Bold font indicates genome-wide significant loci.