Genome-wide association analysis of diverse immune-related phenotypes highlights complex overlapping pathways of immune response

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Introduction

The immune system's defense of the body against assault requires a complex and highly interconnected interplay of cell-mediated and humoral immunities including a multitude of cell types, antigen processing and presentation systems, cytokines, and inflammatory factors. As a result, teasing out which components underlie each individual's susceptibility to immunological conditions is a daunting task. Genome-wide analyses studying these conditions can shed light on which pathways play the biggest roles for different types of conditions. Here we compare the top associations for a diverse set of inflammatory phenotypes including poison oak contact dermatitis, mosquito bite itchiness, and tonsillectomy (as a proxy for chronic tonsillitis) with each other, uncovering substantial differences and a few similarities in immune functions involved.

Table 1. Sex and age distribution of cohorts

Phenotype	Levels	Male	Female	0-30 years	30-45 years	45-60 years	60+ years
Tonsillectomy	Case	19862	19149	2388	5997	11375	19251
	Control	44383	34842	16046	28830	19723	14626
Poison Oak Rash	None	11108	11971	4553	7763	5864	4899
	Mild	9424	6308	2464	4391	4208	4669
	Severe	4622	5247	834	2291	3291	3453
Mosquito Bite Itchiness	Very itchy	2455	6836	1396	2954	2600	2341
	Itchy	12653	12705	4715	8267	6383	5993
	Mildly itchy	14364	7378	3114	6307	5900	6421
	Not itchy	1570	788	362	694	677	625

Results

Table 2. Top associations from GWAS on poison oak rash, mosquito bite itchiness, and tonsillectomy.

Band	SNP	P-value	Effect size	95% CI	Proposed Gene	Function
POISON OAK						
1q23.1	rs59038586	3.3e-12	0.056	[0.040,0.071]	CD1A, CD1C, CD1B	Antigen presentation
MOSQUITO BITE ITCHINESS						
4q27	rs309407	2.0e-33	0.057	[0.047,0.066]	IL2, IL21	Interleukin signaling
5q31.1	rs17516457	9.3e-26	-0.049	[-0.059,-0.040]	IRF1	Interferon signaling
6p21.33	rs3093977	5.6e-18	0.054	[0.042,0.067]	HLA-B, HLA-C	Antigen presentation
22q12.3	rs5756391	4.2e-17	-0.039	[-0.048,-0.030]	CSF2RB	Interleukin signaling
12q15	rs4141135	1.7e-13	-0.034	[-0.043,-0.025]	IFNG	Interferon signaling
19p13.2	rs2967677	2.0e-10	0.049	[0.034,0.064]	HNRNPM	Interleukin signaling
12q13.3	rs3024971	2.5e-10	-0.046	[-0.061,-0.032]	STAT6	Interleukin signaling
6p21.1	rs11751172	1.2e-08	0.030	[0.020,0.041]	RUNX2	Osteoblast differentiation
19p13.3	rs778798	1.2e-08	-0.029	[-0.039,-0.019]	FUT6	Bacterial infection defense
TONSILLECTOMY						
22q12.2	rs9620943	7.40E-21	0.898	[0.877,0.918]	LIF	Interleukin signaling
12p13.31	rs10849448	6.10E-20	0.895	[0.874,0.917]	TNFRSF1A	Lymphoid development
6p21.33	rs115846244	6.70E-14	1.208	[1.150,1.269]	HLA-B, HLA-C	Antigen presentation
7p12.3	rs80077929	2.90E-12	1.133	[1.094,1.173]	IGFBP3	IGF signaling
4q21.1	rs4859854	6.90E-12	0.923	[0.902,0.944]	CXCL13	Lymphoid development
7p12.2	rs11773763	1.10E-11	0.927	[0.906,0.947]	IKZF1	Lymphoid development
14q21.1	rs762083	1.80E-09	1.064	[1.043,1.086]	FOXA1	Liver development
7p15.2	rs6668	1.90E-09	1.067	[1.044,1.089]	HOXA2	Facial development
1q41	rs55728445	1.20E-08	1.105	[1.068,1.144]	DUSP10	Stress signaling
20q13.12	rs1883832	1.20E-08	1.067	[1.044,1.091]	CD40	TNF signaling
22q13.31	rs55651132	1.50E-08	1.079	[1.051,1.108]	UPK3A	Bacterial infection defense
4q24	rs72696109	1.70E-08	1.063	[1.041,1.086]	NFKB1	NFKB1 signaling
3q21.2	rs1980080	2.90E-08	0.943	[0.923,0.962]	SLC12A8	?
9q34.2	rs532436	4.50E-08	0.934	[0.911,0.957]	ABO	Blood group

Band = chromosomal band; SNP = SNP identifier; P-value = p-value of association; Effect size = odds ratio (for tonsillectomy) or beta (for poison oak or mosquito bite itchiness); 95% CI = 95% confidence interval; proposed gene = gene most likely to be involved; pathway = functional pathway most likely to be involved

Discussion

Mosquito bite itchiness, poison oak rash, and infection of the tonsils (using tonsillectomy as a proxy for tonsillitis) represent a set of diverse phenotypes rarely studied together, but related as they represent reactions of the immune system to different foreign substances (mosquito saliva proteins, urushiol, and bacteria or viruses, respectively). Determining which parts of the immune system are key to each reaction can help us identify which pathways are common to many reactions and which are specific to certain reactions and therefore might be more specific targets for intervention. See Figure 1. Hits near genes that are important for antigen presentation were found in all three phenotypes, while genes involved in defense against bacterial infection and interleukin signaling were found for both tonsillectomy and mosquito bite rash. The differences between phenotypes were also interesting. The development of lymphoid tissue (of which tonsils are one example), facial morphology, TNFα signaling, NFκB1 signaling, and blood group were all implicated in the tonsillectomy GWAS. By contrast, genes related to interferon signaling, which might be more related to the inflammation reaction to a mosquito bite, were seen in the mosquito bite itchiness GWAS. This analysis demonstrates that analyzing a diverse set of phenotypes united by a set of pathways can provide insight into how different parts of a system are utilized for specific biological processes.

Acknowledgments

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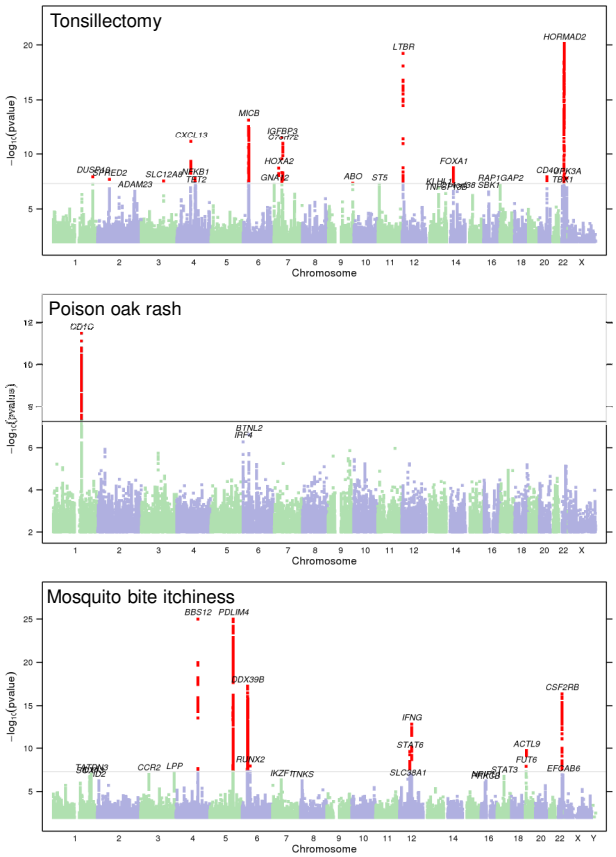

Figure 1. Manhattan plots of association test statistics for tonsillectomy, poison oak rash, and mosquito bite itchiness.

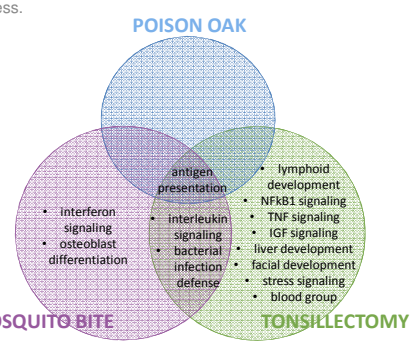

Figure 2. Overlapping immune-response functions amongst tonsillectomy, mosquito bite itchiness, and poison oak rash.

Methods

To identify variants associated with tonsillectomy, poison oak, and mosquito bite reaction, we conducted a genome-wide association analysis (GWAS) on a cohort of unrelated 23andMe customers (Table 1) genotyped on custom Illumina arrays. We restricted participants to a set of individuals who have >97% European ancestry, as determined through an analysis of local ancestry via comparison to the three HapMap 2 populations. A maximal set of unrelated individuals was chosen for the analysis using a segmental identity-by-descent (IBD) estimation algorithm. Individuals were defined as related if they shared more than 700 cM IBD, including regions where the two individuals share either one or both genomic segments identical-by-descent. Samples were genotyped on at least one of three genotyping platforms based on either the Illumina HumanHap550+ BeadChip or the Illumina Human OmniExpress+ BeadChip. The platforms included assays for 586,916, 584,942, and 1,008,948 SNPs, respectively, and had to meet quality control criteria of minor allele frequency > 0.001, Hardy-Weinberg equilibrium $P>10^{-20}$, call rate > 95%, and without large allele frequency discrepancies compared to the 1000 Genomes reference data. SNP genotypes were imputed against the August 2010 release of the 1000 genomes data using Beagle and Minimac. For each phenotype, approximately 7.4 million SNPs met our quality control criteria of avg.rsq>0.5 and min.rsq>0.3 in any imputation batch, no strong evidence of an imputation batch effect. We also removed logistic regression results that did not converge due to complete separation, identified by abs(effect)>10 or stderr>10 on the log odds scale.

For tonsillectomy, we computed association test results by logistic regression assuming additive allelic effects, including covariates for age, gender, and the top five principal components. For tests using imputed data, we use the imputed dosages rather than best-guess genotypes. Cases were defined as those who answered yes to "Have you ever had any of the following surgeries? (Tonsillectomy)" or "Have you had your tonsils removed?" Controls answered no. For poison oak and mosquito bite reaction, association tests were performed by linear regression. Poison oak rash was assessed by "Have you ever had a skin rash because of poison oak, poison ivy, or poison sumac? – No, – Yes, a mild reaction (itching, redness), – Yes, a severe reaction (blisters, swelling, oozing), – I'm not sure". Mosquito bite itchiness was assessed by "When you are bitten by mosquitoes, how much do the bites typically itch? – Very badly (impossible to ignore), – Somewhat badly (definitely noticeable, at times hard to ignore), – Only mildly (noticeably itchy, but easy to ignore), – Not at all (no noticeable itching), – I'm not sure". See Table 1 for how responses were grouped into levels for analysis.