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SHORT REPORT

ABO Genotype and Blood Type Are Associated with Otitis Media

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Aim: To determine if there is an association between *ABO* variants or blood types and otitis media.
Methods: DNA samples from 214 probands from Finnish families with recurrent acute (RAOM) and/or chronic otitis media with effusion (COME) were submitted for exome sequencing. Fisher exact tests were performed when (a) comparing frequencies of ABO genotypes in the Finnish probands with otitis media vs. counts in gnomAD Finnish, and (b) within the Finnish family cohort, comparing occurrence of RAOM vs. COME according to *ABO* genotype/haplotype and predicted blood type.
Results: Female sex is protective against having both RAOM and COME. The wildtype genotype for the *ABO* c.260insG (p.Val87_Thr88fs*) variant resulting in blood type O was protective against RAOM. On the other hand, type A was associated with increased risk for COME. These findings remained significant after adjustment for age and sex.
Conclusions: Within the Finnish family cohort, the wildtype genotype for the *ABO* c.260insG (p.Val87_Thr88fs*) variant and type O are protective against RAOM while type A increases risk for COME. This suggests that the association between the *ABO* locus and otitis media is specific to blood type, otitis media type and cohort.

Keywords: otitis media, ABO, blood type, exome sequencing, Finnish

Introduction

Otitis media is a significant cause of morbidity in the United States and worldwide, and alternative prevention and treatment strategies are required to alleviate burden of the disease. In the United States, it is the most common reason for antibiotic use and office visits in children. Typically, otitis media in young children is acute, lasting for <2 weeks. In acute otitis media (AOM), there is inflammation such as redness of the eardrum and pus in the middle ear, with or without eardrum perforation. The most common causes tend to be bacterial and include *Streptococcus pneumoniae* or *Haemophilus influenzae* (Principi *et al.*, 2017). AOM is often treated with antibiotics and may require surgery if severe enough. In contrast, otitis media with effusion (OME) is defined as the presence of fluid in the middle ear without signs or symptoms of acute infection. Fluid in the middle ear can have few symptoms, especially if it develops slowly. It almost always goes away on its own in a few weeks to a few months, so this type of otitis media usually does not need to be treated with antibiotics (Rosenfeld *et al.*, 2016). In some patients, however, OME may persist and must be treated by surgery. If left untreated, either AOM or OME can lead to complications, the most common of which is hearing loss. Otitis media-related hearing loss can delay language acquisition, alter behavior, and influence quality of life (Bellussi *et al.*, 2005). Therefore, it is paramount to catch this disease sequela early in its progression. Because otitis media may become recurrent or chronic, antibiotics may be repeatedly given for treatment, sometimes inappropriately, and can lead to microbial resistance that is a major public health concern.

Aside from young age, there are many risk factors that contribute to otitis media, such as lack of breastfeeding, allergies, upper respiratory infection, daycare attendance or overcrowding, exposure to tobacco smoke, low socioeconomic status, and family history (Brennan-Jones *et al.*, 2015). Epidemiological and pathologic data suggest that inflammation of the middle ear occurs on a continuum of disease (Bhutta, 2014; Santos-Cortez *et al.*, 2016). Strong evidence exists for genetic susceptibility to otitis media (Casselbrant *et al.*, 1999; Hafrén *et al.*, 2012), but the role of genetic variants in otitis media pathology is not well understood.

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Over the past few years, the availability of new sequencing technologies has sped up the identification of novel genes associated with disease, including infections and immune states. Identification of biomarkers including genetic variants that are common in the population and confer risk of disease holds promise in providing a basis for personalized prevention and treatment. Of the genes studied for otitis media susceptibility, *FUT2* seems to be the most promising for guiding management of otitis media (Santos-Cortez *et al.*, 2018).

FUT2, which encodes alpha-(1,2)-fucosyltransferase, is the human secretor gene at chromosome 19q13.33. The protein encoded by this gene is a Golgi stack membrane protein that is involved in the creation of a precursor of the H antigen, which is required for the final step in the soluble A and B antigen synthesis pathway. In both family and genome-wide association studies (GWASs), *FUT2* variants were implicated in otitis media (Pickrell *et al.*, 2016; Santos-Cortez *et al.*, 2018). *FUT2* is transiently expressed in the middle ear mucosa during AOM (Santos-Cortez *et al.*, 2018). Experimental evidence suggests that the A antigen is used by bacteria for epithelial binding leading to otitis media (Goto *et al.*, 2016). For example, *Lactobacillus* and *Escherichia coli* have affinity for A antigen and may increase in relative abundance in A-antigen-expressing mucosal tissue (Lindstedt *et al.*, 1991; Uchida *et al.*, 2006). However, in Finnish families with otitis media, *FUT2* variants were not associated with otitis media status (Santos-Cortez *et al.*, 2018).

Previously in a GWAS using DNA samples from >120,000 European-descent individuals, an intronic variant rs781822643 within the *ABO* locus at 9q34.2 was identified as a protective factor against otitis media (Pickrell *et al.*, 2016). Earlier studies in smaller cohorts of children from Denmark and Greece demonstrated an association between blood type and otitis media, for example, type O is protective whereas type A increased risk for secretory or effusive otitis media (Mortensen *et al.*, 1983; Apostolopoulos *et al.*, 2002). This is consistent with a role for A antigen in otitis media pathology. In this study, our goal was to determine the association between *ABO* variants or blood type and otitis media in Finnish probands from families with otitis media.

Methods

DNA samples from 214 probands from Finnish families with recurrent (R) AOM and/or chronic (C) OME were subjected to exome sequencing at the Northwest Genomics Center. Using 1 μg DNA, a shotgun library was constructed through an automated pipeline and underwent exome capture using the Roche/Nimblegen SeqCap EZ Exome v2.0 (~ 36.5 MB target). Exome sequencing was performed on an Illumina HiSeq 4000 instrument at 40–60 × mean coverage. The generated base calls were aligned to the hg19 human reference genome sequence using Burrows-Wheeler Aligner v0.7.10 (Li and Durbin, 2009) to generate binary alignment map files for each sample. Variants were called using the Genome Analysis Toolkit v3.2–2 (McKenna *et al.*, 2010) and annotated using SeattleSeq Annotation 151.

Of the 214 Finnish probands, 40 had blood types available from clinical records. Using exome data and available blood types, ABO type was predicted for the rest of the Finnish probands (Table 1): (1) carriage of a haplotype consisting of four *ABO* variants [c.526C>G (p.Arg176Gly); c.703G>A (p.Gly235Ser); c.796C>A (p.Leu266Met); c.803G>C (p.Gly268Ala)] conferred a B phenotype. (2) For *ABO* c.260insG (p.Val87_Thr88fs*), the wild type genotype resulted in type O whereas homozygous genotype conferred the A phenotype. (3) Type AB was assigned if carriage of the four-variant haplotype and homozygosity for the c.260insG variant co-occurred. (4) All other genotype combinations were assigned as type A. Fisher exact tests were performed when (a) comparing ABO genotype frequencies in the Finnish probands with otitis media versus Finnish genotypes in the gnomAD database as representation of the general population and (b) among Finnish probands, comparing occurrence of recurrent acute otitis media (RAOM) versus chronic otitis media with effusion (COME) according to *ABO* genotype/haplotype and predicted blood type. Age and sex were included as covariates in logistic regression analysis. Statistical analyses were performed using R.

Results

From the exome data, 14 coding *ABO* variants were identified (Table 1). Of these variants, the [c.526C>G (p.Arg176-Gly); c.703G>A (p.Gly235Ser); c.796C>A (p.Leu266Met); c.803G>C (p.Gly268Ala)] and c.260insG variants were the most significant expression quantitative trait loci (eQTL) for airway or mucosal tissue in the Genotype-Tissue Expression database (GTEx) (Table 1). However, no association between OM and homozygous genotype for *ABO* c.260insG or the four-variant haplotype was identified. In contrast, wild type genotype for c.260insG conferring blood type O was protective against RAOM (odds ratio [OR] = 0.33; 95% confidence interval [CI]: 0.11–1.04; $p = 0.04$).

The proportions of *ABO* genotypes and predicted blood types among Finnish probands were comparable with the general Finnish population according to gnomAD and national data (Nevanlinna, 1973), except for Type O (Table 2). In addition to type O as being protective against RAOM, type A was associated with increased risk for COME (OR = 2.14; 95% CI: 1.04–4.50; $p = 0.03$).

Female sex was protective against having both RAOM and COME (OR = 0.51; 95% CI: 0.28–0.93; $p = 0.02$). However, the association between blood type A and COME and blood type O and RAOM remained significant after adjustment for age and sex (Table 2).

Discussion

In our cohort of Finnish families, wild type genotype for c.260insG and blood type O were protective against RAOM, whereas type A increased risk for COME, in agreement with previous findings (Mortensen *et al.*, 1983; Apostolopoulos *et al.*, 2002). Similarly in the Pickrell *et al.* (2016) GWAS, an intronic *ABO* variant c.28 + 1482delT was identified as protective (OR = 0.94) against recurrent ear infections in childhood. Taken together, our findings replicate the novel association between the *ABO* locus and otitis media that was identified in previous GWASs. In addition our findings suggest that the association between the *ABO* locus and otitis media is specific to genotype, blood type, and otitis media type.

It is interesting that even though the *FUT2* variants in these Finnish families were not associated with otitis media

Table 1. Coding *ABO* Variants Identified in Finnish Probands with Otitis Media

chr:pos_ref/alt (hg19)	*rsID*	*Variant*[a]	*GTEx eQTL tissue*[b]	*GTEx eQTL* p-*value*	*gnomAD Finnish allele count*	*gnomAD Finnish allele number*	*gnomAD Finnish MAF*	n *Finnish proband carriers (%)*[c]	*Blood type:* n *(%)*[d]
9:136131056_CG/C	56392308	c.1061delC(p.Pro354Argfs*)	NA	NA	2381	22672	0.105	11 (28)	A: 10 (91)
9:136131289_C/T	8176748	c.829G>A(p.Val277Met)	Esophagus-mucosa	3.8×10^{-21}	4427	24872	0.178	13 (32)	A: 7 (54)
9:136131315_C/G	**8176747**	**c.803G>C(p.Gly268Ala)**	Lung	1.5×10^{-43}	3375	24602	0.137	10 (25)	B: 8 (80)/AB: 2 (20)
9:136131322_G/T	**8176746**	**c.796C>A(p.Leu266Met)**	Lung	1.5×10^{-43}	3361	24576	0.137	10 (25)	B: 8 (80)/AB: 2 (20)
9:136131415_C/T	**8176743**	**c.703G>A(p.Gly235Ser)**	Lung	1.5×10^{-43}	2935	21424	0.137	10 (25)	B: 8 (80)/AB: 2 (20)
9:136131472_A/T	8176740	c.646T>A(p.Phe216Ile)	Esophagus-mucosa	6.7×10^{-21}	3804	21946	0.198	13 (32)	A: 7 (54)
9:136131523_G/A	8176739	c.595C>T(p.Arg199Cys)	NA	NA	255	23488	0.011	2 (5)	A: 2 (100)
9:136131592_G/C	**7853989**	**c.526C>G(p.Arg176Gly)**	Lung	1.3×10^{-36}	3884	24828	0.156	10 (25)	B: 8 (80)/AB: 2 (20)
9:136131651_G/A	1053878	c.467C>T(p.Pro156Leu)	Lung	1.6×10^{-07}	2748	24962	0.110	11 (28)	A: 10 (91)
9:136132908_T/TC	**8176719**	**c.260insG(p.Val87_Thr88fs*)**	Esophagus-mucosa	4.8×10^{-45}	11730	24958	0.470	36 (90)[e]	Wt 7, O: 7 (100); Het 22, A: 14 (64); Hom 11, A: 9 (82)/AB: 2 (18)[f]
9:136133506_A/G	512770	c.220C>T(p.Pro74Ser)	Esophagus-mucosa	1.0×10^{-12}	21136	24894	0.849	40 (100)	A: 23 (58)
9:136135238_T/C	549446	c.188G>A(p.Arg63His)	Esophagus-mucosa	3.0×10^{-20}	20513	24972	0.821	13 (32)	A: 7 (54)
9:136136770_A/C	688976	c.106G>T(p.Val36Phe)	Esophagus-mucosa	1.4×10^{-19}	19220	23386	0.822	40 (100)	A: 23 (58)
9:136136773_C/T	8176696	c.103G>A(p.Gly35Arg)	NA	NA	473	23194	0.020	4 (10)	A: 2 (50)

[a]Variants that are primary determinants of blood type are in bold.
[b]Top epithelial tissue in eQTL list for variant according to smallest *p*-value.
[c]Counts and proportions out of 40 Finnish probands with blood types and exome data. Variants with similar counts are in strong linkage disequilibrium, for example, the 4-variant haplotype comprising variants [c.526C>G (p.Arg176Gly); c.703G>A (p.Gly235Ser); c.796C>A (p.Leu266Met); c.803G>C (p.Gly268Ala)] is carried by the same 10 probands who all express B antigen.
[d]Counts and proportions out of variant carriers in previous column. The blood types shown are based on majority counts for each variant. If carriers of the four-variant haplotype that confers type B and individuals who are wild type for the c.260insG (p.Val87_Thr88fs*) with blood type O are excluded, all remaining probands with available blood types are type A. Two probands who are type AB are both heterozygous for the four-variant haplotype and homozygous for the c.260insG variant.
[e]Blood type O or A antigen expression depends on genotype for the c.260insG variant: wild type individuals are type O, whereas homozygous individuals have A antigen.
[f]Proportions reported are based on genotype for the c.260insG variant.
eQTL, expression quantitative trait loci; GTEx, Genotype-Tissue Expression database.

Table 2. Variables Studied in 214 Finnish Probands with Otitis Media[a]

Variable	n *(%)*
Female sex	88 (41)
ABO genotypes[b]	
Carriers of four-variant haplotype	57 (27)
Wild type for c.260insG variant	50 (23)
Homozygous for c.260insG variant	48 (22)
All other	81 (38)
Blood type[c]	
A	107 (50)
O[d]	50 (23)
B	35 (16)
AB	22 (10)
Otitis media type[e]	
RAOM[f]	191 (89)
COME[g]	148 (69)
Both RAOM and COME[h]	125 (58)

[a]Average age was 12.9 ± 3.3 years.
[b]A total of 22 (10%) probands are both carriers of the 4-variant haplotype and homozygous for c.260insG.
[c]Known proportions for each blood type in the general Finnish population using a sample of $n = 5536$ (ref. 4): A (44%); O (31%); B (17%); AB (8%).
[d]The 95% CI for type O does not overlap in the two groups using the binomial test, with lower frequency in OM probands—OM 95% CI: 17.9–29.6; general population 95% CI: 29.8–32.2.
[e]Probands were ascertained upon referral for ventilation tube insertion for OM. COME was diagnosed for middle ear effusion >2 months, whereas RAOM was diagnosed for those with >3 AOM episodes in 6 months or >4 AOM episodes in 12 months.
[f]For model RAOM ~ Age+Sex+TypeO, $\beta_{TypeO} = -0.098 \pm 0.046$, $p = 0.03$.
[g]For model COME ~ Age+Sex+TypeA, $\beta_{TypeA} = 0.134 \pm 0.061$, $p = 0.03$.
[h]For model BothRAOM/COME ~ Age+Sex+TypeA, $\beta_{TypeA} = 0.131 \pm 0.066$, $p = 0.0495$ and $\beta_{Sex} = -0.160 \pm 0.067$, $p = 0.02$. This suggests that increased susceptibility to having both RAOM and COME due to blood type A is more predominant in males.
AOM, acute otitis media; CI, confidence interval; COME, chronic otitis media with effusion; RAOM, recurrent acute otitis media.

(Santos-Cortez *et al.*, 2018), an ABO genotype and blood types A and O were associated with specific otitis media types. *FUT2* variants decrease presentation of A antigen that is used by bacteria to gain access to the middle ear lining (Santos-Cortez *et al.*, 2018). Although *FUT2* variants are not significant in these Finnish families, that the *ABO* locus is involved in otitis media in these families also supports the role of epithelial expression of A antigen in otitis media pathology.

The probands studied here are a subset of the familial cohort in which an intronic SNP rs16974263 on 19q13.2 was also identified as genome-wide significant for COME (Einarsdottir *et al.*, 2016). From GTEx data, the rs16974263 variant is an eQTL affecting expression of *SERTAD3* in skin and blood. *SERTAD3* is involved in the late response to TLR-induced immune effector expression, thereby supporting a potential role in middle ear immunity (Lin *et al.*, 2017). These findings indicate that multiple genes contribute to otitis media susceptibility within these Finnish families.

By using new genomic technologies and analytic techniques, genes for otitis media susceptibility and how they function in influencing bacterial abundance within the middle ear should continue to be identified. Genetic studies can identify pathways involved in host–bacterial interactions that may be targeted for prevention and treatment of otitis media.

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Web Resources

Burrows-Wheeler Aligner, http://bio-bwa.sourceforge.net
dbSNP, www.ncbi.nlm.nih.gov/snp
Genome Aggregation Database, gnomad.broadinstitute.org
Genome Analysis Toolkit, software.broadinstitute.org/gatk/
Genotype-Tissue Expression Portal, www.gtexportal.org/
R, R v3.5.1, www.r-project.org
SeattleSeq, snp.gs.washington.edu/SeattleSeqAnnotation151/
UCSC Genome Browser, genome.ucsc.edu

Author Disclosure Statement

No competing financial interests exist.

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