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Familial and Microbiological Contribution to the Otitis-Prone Condition

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Abstract

Objective—Our group has an ongoing clinical research project investigating the immunology of the otitis-prone (OP) phenotype. In light of evidence that this condition arises from underlying immunological defects, we examined our sample population of stringently-defined OP (sOP) children suffering 3 episodes of acute otitis media within 6 months or 4 within a year for a familial association with the sOP phenotype.

Methods—We analyzed the frequency of sOP within and between families and the nasopharyngeal (NP) otopathogen colonization patterns within and between families.

Results—The presence of sOP siblings significantly predicted that additional children in the same family would likewise become sOP, with an odds ratio of 3.7 (95% CI 0.77–15.2, 95% lower bound 0.95). We further present evidence for an environmental contribution to this effect by means of prolonged exposure to otopathogens within family units.

Conclusion—sOP children have a significant familial association. The tendency of siblings to share similar patterns of microbial NP colonization contributes to this association. Further research is necessary to determine whether and to what extent genetics are involved.

Keywords

otitis; heritability; microbiome; statistics

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Conflict of interest

The authors have no conflicts of interest to declare.

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1. Introduction

Acute otitis media (AOM) is among the most common causes of visits to pediatric clinics, occurring most frequently in children between the ages of 6 and 24 months [1]. The condition is usually a secondary consequence of a viral upper respiratory infection (URI) which allows normally commensal *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae*, or *Moraxella catarrhalis* to enter the middle ear [2]. A subset of children is especially prone to frequent ear infections. These children are defined as otitis prone (OP) if they suffer 3 cases of AOM in the span of 6 months or 4 cases in a year [3]. We and others have linked the OP condition to immunological defects in the child, including SNPs in genes encoding inflammatory cytokines and deficiencies in immune cell memory and maturation [4–6]. These immunological defects suggest that there may be a genetic component to the OP condition. Our group has been studying a subset of stringently-defined otitis prone (sOP) children based only on AOM episodes which we have confirmed by tympanocentesis and microbiological culture of middle ear fluid [7], thus removing the potential confounding influence of otitis media with effusion (OME), a condition often clinically confused with AOM [1].

We have previously determined that the presence of siblings in a home is a risk factor for the sOP phenotype [8]. Since our sample population contains numerous sets of siblings, we chose to investigate whether there is a familial association with the sOP condition, since this would point to a possible genetic component to recurrent AOM susceptibility.

2. Methods

2.1. Sample population

Our sample population comprised 194 children in 91 different families from whom nasopharyngeal (NP) microbial isolates were obtained. These isolates were identified by culture *in vitro* as previously described [8]. Children were defined as sOP if they suffered at least 3 episodes of AOM confirmed by tympanocentesis and microbial culture within 6 months or 4 within a year, according to previously established criteria. Species of interest in the nasopharynx were the commensal bacteria α -hemolytic *Streptococcus pneumoniae*, *Haemophilus parainfluenzae*, and *Staphylococcus aureus*, and the otopathogens *H. influenzae*, *Moraxella catarrhalis*, and *S. pneumoniae* [3]. Statistical analyses were performed using R (www.r-project.org).

2.2. Analysis of familial association

We define the statistic T ; the total number of sOP subjects in a family beyond the first sOP subject. For example, the contribution of a family with 0, 1, 2, or 3 sOP subjects to T will be 0, 0, 1, or 2, respectively. Thus, a family with only 0 or only 1 sOP subject contributes 0 to the statistic. Under the hypothesis of familial association, the observed value T^* will be larger than under the null hypothesis of no association. We generated a null distribution using a permutation procedure, in which the sOP label was randomly reassigned uniformly among all subjects, independently of family structure. The value of T was captured for each

random permutation. The P-value $P(T \leq T^*)$, was estimated directly from the sample of replicates.

2.3. Statistical comparisons of microbial colonization

The microbes present in the NP of children in the study were identified, and the similarity of colonization patterns over time between siblings, between unrelated children, and within individuals was assessed. Equations are detailed in the Appendix.

3. Results

3.1. Familial association with sOP

The sample population included 194 children in 91 different families. Of these subjects, 26 (13.4%) were sOP. 21 families (23.1%) had at least one sOP child, and among these families 4 (19.0%, 4.4% of total) had more than one sOP child. 13 families had multiple births (14.3%). One such family contained only one sOP child, and one pair of twins were both sOP; all others were non-OP. We have previously determined that the presence of siblings in a home is a risk factor for the OP phenotype [8]. In the sample population of 194 subjects, 26 were sOP. One family had 3 sOP subjects, and 3 families had 2 sOP subjects. By this method we found a significant familial association ($P=0.025$). This hypothesis test is summarized in Table 1.

An odds ratio of 3.7 for sOP given an sOP sibling was estimated directly from the data. A bootstrap procedure (based on family-level resampling, and conditional on observed sOP prevalence) yielded a 95% CI of (0.77, 15.2) and a 95% confidence lower bound of 0.95.

3.2. Comparison of NP isolates

Since AOM is caused by a number of well-characterized bacterial species [9, 10], we investigated whether the observed familial association with sOP corresponded with an increased likelihood of children being colonized with otopathogens. By comparing the microbiological isolates in the NP obtained from the subjects at multiple time points (the commensal bacteria α -hemolytic *Streptococcus pneumoniae*, *Haemophilus parainfluenzae*, and *Staphylococcus aureus*, and the otopathogens *H. influenzae*, *Moraxella catarrhalis*, and *S. pneumoniae*), we established an index of similarity between siblings and between unrelated children, which we tracked over time (Fig. 1). We found that the patterns of bacterial colonization between siblings were substantially more similar than those between unrelated children. The patterns of NP colonization in siblings closely resembled those of a single child for a period of up to 6 months, after which they appear to diverge, falling to the level of unrelated children from separate households.

4. Discussion

We find a significant familial association with the sOP phenotype, where the presence of an sOP child in a household predisposes additional siblings to be sOP themselves with an odds ratio of 3.7. While the 95% confidence interval for the odds ratio (0.77, 15.2) spans 1, we are confident that it represents a significant effect, both because we identified a significant

familial association independent of the odds ratio and because the 95% lower bound of the estimate is 0.95. When we examine the microbes isolated from subjects, we find that siblings, whether sOP or non-OP, tend to resemble each other substantially more than unrelated children. The comparison of colonization patterns within individuals with or without siblings shows that the presence of siblings affects a given individual minimally, if at all, while groups of siblings tend to be colonized like single individuals for months. In other words, the household can be thought of as the basic unit of NP colonization. This suggests that the environment shared by siblings contributes to the familial predisposition to sOP we observe, since the children are in sufficiently close proximity to enable a given episode of bacterial colonization to persist for a longer period of time by passing a population back and forth. Thus, the duration of a period of colonization is extended, and it is this extended exposure to otopathogens which increases the opportunity for middle ear invasion upon a triggering viral URI. In a study of Gambian children who received the 9-valent pneumococcal conjugate vaccine, both recipients and their siblings retained *S. pneumoniae* carriage, though the specific serotypes found in the NP were altered in the recipients [11]. In our sample population, recruited in Rochester, New York, the predominantly isolated bacterial species are the six we have analyzed [12, 13], and a recent study among Indigenous Australian children similarly identified *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* as the most common otopathogens [14], such that we can be confident that our findings are an accurate representation of the nasopharyngeal microbiome. The sharing of bacterial species we observed between siblings followed this pattern, and to our knowledge this is the first study to track the evolution of the NP microbiota among siblings prospectively.

The sample population included numerous multiple births. Children belonging to these sets were generally non-OP, but there were two exceptions. One set of triplets contained only one sOP child, and one pair of twins were both sOP. It is noteworthy that the triplets were fraternal, while the twins were identical. Although this sub-sample is far too small to draw conclusions, it is possible that this points to a polygenic origin of the OP phenotype, such that it is reliably shared only among genetically identical individuals, while siblings are genetically too distinct to possess all of the necessary alleles. A study of 1279 Finnish children and their parents estimated a heritability of 38.5% for recurrent otitis media, where the children of OP parents were more likely to suffer from frequent episodes of AOM [12]. This finding of heritability points to the possibility of a genetic contribution to otitis-proneness, and indeed multiple genes (in particular Toll-like receptor 4, the human leukocyte antigens *HLA-A2* and *HLA-A3*, and the cytokine *IL6*) have been identified as positively associated with recurrent AOM, though many of these studies suffer from small sample sizes [15, 16]. A fifteen-year twin study in the US concluded in 1999 found a close association in time spent suffering from OME between monozygotic twins, while this association was less strong in dizygotic twins [17]. Since OME is preceded by an episode of AOM, this was taken as an indication that susceptibility to otitis is hereditary. However, the fluid recovered from the ear during OME usually does not contain bacteria, and therefore may not be a perfect indicator of the severity or duration of the infection responsible. Our finding of a “horizontal” familial association of the sOP condition between siblings is thus consistent with prior research showing that susceptibility to otitis is hereditary. We further have the

benefit of a rigorous microbiological diagnosis of AOM and a stringent definition of the OP condition.

5. Conclusions

Susceptibility to otitis and the sOP condition are similar but distinct terms. Time spent with middle ear effusion may reflect a delay in inflammatory resolution inside the ear, rather than the severity or duration of the initiating infection, whereas to be defined as sOP in our study a child must have abnormally frequent episodes of active bacterial infection of the middle ear confirmed by laboratory culture. In our study population, we find that the sOP condition clusters within families, and identify an environmental contribution to this effect. While this study does not present strong, direct evidence for a genetic component, the possibility of inheritance among identical twins is intriguing, and bears further investigation.

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Appendix

Statistical comparisons of microbial colonization

For each visit i we are given a binary vector $x_i = (x_{i1}, \dots, x_{i3})$, with $x_{ik} = 1$ for positive isolate k at visit i . Set $N_i = \sum_k x_{ik}$. We define a *similarity index* between x_i and x_j as follows:

$$A(x_i, x_j) = \begin{cases} \frac{2 \sum_k x_{ik} x_{jk}}{N_i + N_j} & ; \quad N_i + N_j > 0 \\ 0 & ; \quad N_i + N_j = 0 \end{cases}$$

which is interpretable as the probability that one positive isolate chosen at random from the pooled isolates of both subjects is positive in both subjects. We have

$$0 \leq A(x_i, x_j) \leq 1,$$

with $A(x_i, x_j) = 1$ if and only if $x_{ik} = x_{jk}$ for all k . For each visit i the following variables are available:

$$\begin{aligned} sub_i &= \text{subjectID} \\ fam_i &= \text{familyID} \\ x_i &= \text{binary vector of isolate presence} \\ day_i &= \text{day of visit} (day_i = 1 \text{ on } 01/01/2006) \end{aligned}$$

For each pair of visits i, j the following variables are calculated:

$$\begin{aligned} si_{ij} &= A(x_i, x_j) \\ \delta_{ij} &= day_i - day_j \\ \mathfrak{R}_{ij} &= \begin{cases} 0 & ; \quad fam_i \neq fam_j \\ 1 & ; \quad fam_i = fam_j, sub_i \neq sub_j \\ 2 & ; \quad sub_i = sub_j \end{cases} \end{aligned}$$

The variable $\mathfrak{R}_{ij}$ represents relationship categories, identifying subjects for visit i, j as unrelated ($=0$), siblings ($=1$), or the same individual ($=2$). For $\mathfrak{R}_{ij} = 2$, we necessarily have $score_{ij} = 1$ when $\delta_{ij} = 0$, so these observation pairs are not included in the analysis.

For each relation category $\mathfrak{R}_{ij}$, the functional relationships between si_{ij} and δ_{ij} were estimated using generalized additive models (GAMs) [18, 19]. This is a family of models which incorporates the structure of generalized linear models (GLMs), but permits the use of splines in place of linear terms. This offers considerably more flexibility in modeling nonlinear functional forms, while permitting the type of formal statistical inference available for GLMs, including confidence bands and formal statistical hypothesis testing. The package `gam()` was used within the R statistical computing environment.

The analysis performed for data from families with multiple siblings ($\Re I_{ij} = 0,1,2$) and with no siblings ($\Re I_{ij} = 0,2$).

Figure 1.

Comparison of similarity of microbiological isolates between siblings and between unrelated children. Similarity index represents the fraction of bacterial species shared between children according to the number of days between clinic visits. Figure also compares duration of colonization in individual subjects with and without siblings. Solid lines depict means, dashed lines show 95% confidence intervals.

Table 1

Identification of a familial association with the OP phenotype. NF , the number of families with at least 1 OP child; T^* , number of families with multiple OP children accounting for families with 3 or more; null mean T , expected T under null hypothesis of no association.

Phenotype	NF	T^*	null mean T	P
OP	21	5	1.98	0.025