

# *Genetic Influences on Otitis Media*

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The genetics of otitis media are most likely complex and will involve at least several loci that contribute to the overall phenotype. Additionally complexity in analyzing the genetic contribution to otitis media may come from heterogeneity among various populations.

Epidemiologic studies such as population studies, adoption studies, familial aggregation studies and twin and triplet studies had indicated that there must be a genetic component.

A while ago we started a twin and triplet study. Some of our kids that were enrolled in the study are ready to go to college at this time. Three twin and triplet studies that have been performed. The first publish one was by Kvaerner from Norway and she did a retrospective study in 2,750 twin pairs and she found that the estimated heritability was 0.74 in females and 0.45 in males <sup>1</sup>. In our study in Pittsburgh, which was a prospective study in 168 twin pairs and triplets and we've followed them from birth to five years of age <sup>2</sup>. We've found that the estimated heritability was 0.79 for the female and 0.64 for male. The last study was by Rovers in the Netherlands and this was also a prospective study <sup>3</sup>. They enrolled 1.373 twin pairs and they found that the estimated heritability was 0.4 to 0.71 depending on what age you were examining the children.

The strong genetic component that was found in these studies of recurrent and persistent otitis media suggested that it would be feasible to identify one or more genes that contribute to the susceptibility of the disease. **Possible candidate genes that confer the susceptibility may be associated with the anatomical and physiological function and mucosal immunity.**

The **pneumatization of the mastoid** process is more similar in monozygotic than in dizygotic twins. This is a quite old study (Dahlberg, 1945) but it's hard to get approval from the Hospital Ethics Committee (IRB) to do X-Rays on healthy children <sup>4</sup>.

The **racial differences in the eustachian tube (ET)** were studied by Doyle 1997 <sup>5</sup> and Berry 1980 <sup>6</sup>. The differences were: a shorter straighter and more patulous tube in American Indian compared to the Caucasian, which is associated with a higher incidence of chronic middle ear disease.

Also, related to **anatomical mechanisms**, the skeletal and soft tissue dimension of the **nasopharynx** is significantly smaller in children with otitis media compared to controls.

As for **immune response** in the middle ear inflammation, we have **nonspecific immunity** with epithelial barriers and mucins; we have the **innate immunity** with Toll-like receptors, mannose binding lectin, surfactant proteins and cytokines and we have the **adaptive immunity** with the Fc-Gamma receptor and immunoglobulins. The epithelial barriers and mucins allow trapping of foreign material and prevent invasion. Viscosity of mucus is regulated by mucin production and there are 20 mucin genes which have been identified. There are studies that have shown that the MUC5AC, MUC5B and MUC2 genes are up-regulated in acute and chronic otitis media <sup>7</sup>.

As to **innate immunity**, the Toll-like receptors (TLRs) activate reaction related to expression of pro inflammatory genes. Also the TLR4 299A allele is associated with susceptibility to acute otitis media compared to the 299G allele.

The **Mannose Binding Lectin (MBL)** opsonizes pathogens and thereby activate the complement pathway. Also **MBL polymorphism code for low serum levels** and if there is an over representation of MBL2G54D variant on Exon 1, it is associated with recurrent acute otitis media.

**Surfactant Protein**, there are three: A, B and D. They are produced by the epithelial cell in the eustachian tube and middle ear and activate the opsonization and complement pathway. The human SP-A locus is on chromosome 10q22-q23 and there are two functional genes SP-A1 and SP-A2. Studies have shown the frequency of the 6A4-1A5 haplotypes in Surfactant Protein A (SP-A) **is linked to recurrent acute otitis media and early otitis media in children before six months of age** <sup>8</sup>. And **it is also linked to risk for asthma in infants**.

**Cytokines** are may be the studied. Cytokines mediate host response to inflammatory stimuli. There are the **pro-inflammatory cytokines**: the TNF-Alpha, IL-6, and the Interferon Gamma. There are the **anti-inflammatory cytokines**: TGF-Beta and IL-10. Studies have shown that Interferon Gamma at the 874 homozygous A allele is associated with the increased incidence of acute otitis media in infants with respiratory syncycial virus (RSV) infection <sup>9</sup>. The TNF-Alpha-308 and IL-6-174 polymorphic genotypes are associated with increased risk for otitis media and tympanostomy tube insertion. The TNF alpha – 863, TNF alpha 376G, IL-10-1082A and IL-6-174G alleles are also associated with susceptibility to otitis media.

The **adaptive Immunity** on the Fc-Gamma Receptor and Immunoglobulins initiate immune responses. The immune response against *S. pneumoniae* depends on opsonizing antibody, which requires an interaction to the FcγRIIIa -Gamma Receptor. There are two types of receptor, the Histidine and Arginine. If you have a Fc-Gamma Receptor that is homozygous for the Histidine, - this is associated with a significantly decreased risk for recurrent acute otitis media after PCV and PPV immunization compared to homozygous for the Arginine. The G2m(23) genetic variant of the immunoglobulin chain is also related to recurrent acute otitis media.

There are many animal studies but I will mention one and that's a deaf mouse mutant model called Jeff and express a phenotype consisting of chronic proliferative otitis media and mild craniofacial abnormalities <sup>10</sup>. This Jeff mouse

model carries a mutant in the region of a novel gene (Fbxo11) which is located on the distal mouse chromosome 17 (human on chromosome 2) which is expressed in mucin secreting cells of the middle ear. The FBXO11 gene is the human homologue for the gene mutated in the Jeff model. This was evaluated in subjects in the Minnesota Chronic and the Recurrent otitis media family study and they found there was evidence of an association between polymorphism in the gene and increased chronic and recurrent otitis media <sup>13</sup>.

### **Genome-Wide Linkage Scans**

There are two studies that have been performed and the first one was by Daly in Minnesota, "Chronic and Recurrent Otitis Media: A Genome Scan for Susceptibility Loci" <sup>12</sup>, and the other one was done in Pittsburgh, "A genome-wide linkage scan with evidence on **susceptibility loci within 17q12 and 10q22.3 regions**" <sup>13</sup>. The **objective was to map possible otitis media susceptibility genes using linkage analysis.**

Daly's study included 133 families with 692 subjects and the proband had a history of tympanostomy tube insertion. Their method was a single-point non-parametric linkage analysis. Their goal was a genome scan for susceptibility loci in chronic and recurrent otitis media patients. They **found linkage to chromosome 10q 26.3 and 19q13.43.**

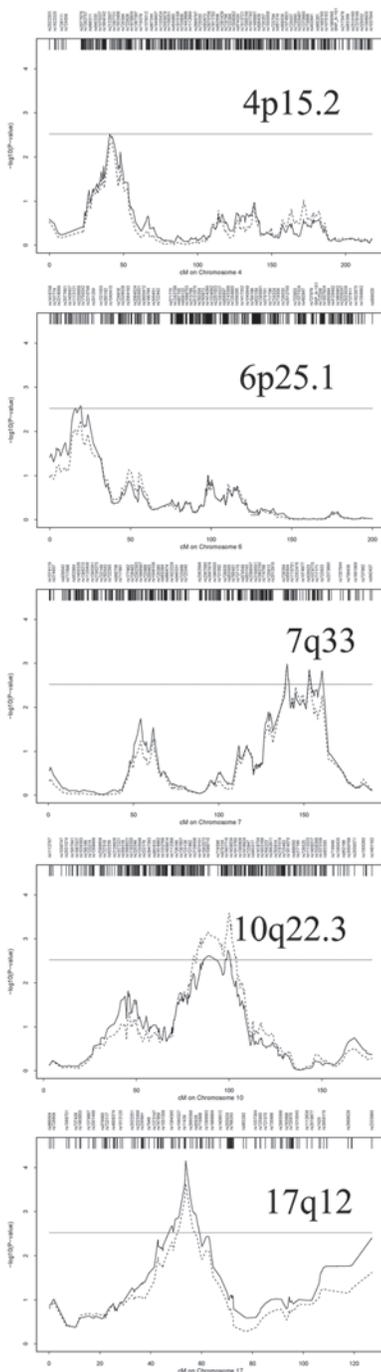
Their conclusion was that **genetic susceptibility to otitis media is determined, in part, by the contribution of genes in distinct chromosomal regions: 10q and 19q.** These regions contain genes that are involved in the defense response or in the modulation of the inflammatory response. However "true" positional candidates remain unresolved as the regions remain broad and not precisely defined.

In our study we enrolled healthy full siblings, two or more, that had had tympanostomy tubes insertion due to a significant history of otitis media <sup>2</sup>. We also enrolled their parents and full sibling(s) with no history of tympanostomy tube insertion.

Our purpose was to scan a genome-wide linkage for genes influencing otitis media. Our methods included isolation of high molecular weight DNA and the genome-wide linkage scan was done using the 10K Affymetrix SNP panel.

We had 403 Caucasian families with 1,431 genotyped individuals with **377 genotyped affected siblings' pairs.** We also had 26 African-American families with 75 genotyped individuals with **27 affected siblings' pairs.**

Using the Mendel-Based Linkage Analysis we found five peaks, but we focused on **peak 10 and peak 17 because we know that have been indication that there are candidate genes on those chromosomes (Figure 1).**

**Figure 1.** The Mendel-based linkage analysis.

The horizontal reference line indicates a nominal  $P$ -value of 0.003

If you look at the 17q12 linkage peak, the very tip occurred in the adaptor-related protein complex, which plays a role in the Nef-mediated CD8 down-regulation. And **we know that children with recurrent otitis media have low numbers of CD8 producing Interferon-Gamma cells in the adenoids.** And this peak also contains a cluster of CCL genes. **And the CCL5 gene is known as a RANTES gene which has been associated with otitis media** and is an eosinophil chemo attractant.

As a genome-wide linkage scan for **genes influencing otitis media, the linkage peak is on 10q22.3**<sup>15</sup>. You remember I told you to remember that number, it's close to the human surfactant protein A (SFTPA2), which is expressed in the Eustachian tube and plays a role in innate host defense by up-regulating phagocytosis of many otitis media pathogens.

As a genome-wide linkage scan for **genes influencing otitis media**, two strong linkage peaks **have been identified on chromosomes 17 and 10** which **both contain** previously implicated candidate **genes that influence the risk for recurrent and persistent otitis media**. Further fine-mapping, replication and functional studies are needed.

### Conclusions

Once otitis media susceptibility genes have been identified, molecular diagnostic assays could be developed to aid the clinician in identifying the child at risk for otitis media. Also, the identification of susceptibility genes may enable us to better understand the pathogenesis of otitis media, leading to the development of better and more innovative methods for prevention and treatment.

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