MAY 2015

INTERNATIONAL SOCIETY FOR OTITIS MEDIA Newsletter #4

Genetics and Otitis Media

The ancient Greeks believed that otitis media was caused by a disruption of four basic bodily fluids: blood, yellow bile, black bile, and phlegm. In the Mid 19th Century aetiology was thought to be due to a mechanical obstruction of the Eustachian tube. By the early 20th Century the role of bacteria was recognised, and by the late 1990s several epidemiological studies showed OM was actually a highly heritable disorder. And so over the last two millennia our view of what cause otitis media has shrunk: from a disruption in four body fluids, to a disruption of the four DNA bases (A, T, C and G) that code our genes.

Our fourth newsletter focuses on genetics and otitis media, an area of my own research interest. We start with Margaretha Casselbrant, who summarises our understanding of OM as a heritable disease. Underlying this heritability will be a complex interaction of thousands of genes, and Lena Hafren and Sarra Jamieson describe two methods to discover some of the genes responsible, through either a "candidate gene" association study looking at a particular gene of interest, or through a "genome-wide" association study that looks across the whole of DNA. Finally, Steve Brown describes how genetic mutant mouse models can be exploited to discover either the identity or function of genes underlying OM.

We have our regular reports from our secretary, our treasurer, and of course our President, in the build up to our conference next month. The presidency will also change at the conference, with Rich Rosenfeld handing over to Tania Sih. Rich's dedication and wisdom have been pivotal in the formation of our society, and he will be a great loss. But I am sure Tania will prove herself a worthy successor.

See you in National Harbor.

Mr Mahmood Bhutta
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As we approach our first International Symposium under the auspices of ISOM there is a palpable sense of vitality and promise in the otitis media universe.

President's report

Prof Richard M. Rosenfeld
President, ISOM
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Let me begin with a quick OM2015 update. Our scientific program committee, chaired by Jian-Dong Li, has assembled an enticing palette of 85 podium presentations, 85 poster presentations, 9 panel sessions, 8 mini-symposia, 4 plenary sessions, and 3 workshops. Our vendor relations committee, chaired by Kenny Chan, has attracted many sponsors and exhibitors, without whom this symposia could not take place. Most importantly, we have plenty of time for networking and fun, including a gala dinner in Old Town Alexandria. Visit **www.om2015.org** to register and to view the full program.

ISOM is thriving, with more than 100 charter members, an active Board of Directors, and preparations underway for our first General Assembly during OM2015. We are identifying new officers, requesting member nominations for new Directors, and soliciting proposals for OM2019. Most importantly, we will emerge from OM2015 with new committees (specified in our Bylaws) that will get busy crafting our society's future. Visit **www.otitismediasociety.org** to become a charter member and qualify for discounted symposium registration.

Last, I defy anyone to find a more engaging and beautiful newsletter, especially in a young society, than the one you are now reading. We are indebted to our editor, Mahmood Bhutta, for terrific content and for sharing the graphic design talents of his wife, Anki. This edition, which focuses on otitis media genetics, makes clear the importance of "choosing your parents carefully" to avoid a childhood chockfull of otitis media.

It has been an honor and privilege to server as founding president of this vibrant society. At the conclusion of OM2015 I will pass the presidential torch to Tania Sih, our energetic president-elect. I very much look forward to personally sharing the excitement with all of you in June at OM2015.

With warm regards,
Richard M. Rosenfeld, MD, MPH

Heritability in Otitis Media

In recent years, several studies have demonstrated a high heritability to otitis media. Heritability is defined as the proportion of variance within a population that is attributable to variation in genetic factors. Twin studies provide a powerful method of determining the contribution of genetics to a disease, because the potentially confounding effects of environmental factors are significantly reduced. Comparison of the concordance rate of a trait among monozygotic (MZ) twins, who share identical genomes, to the concordance in dizygotic (DZ) twins, sharing fifty percent of their genome, can allow estimation of the amount of variation accounted for by genetic factors alone.

In my study from Pittsburgh, Pennsylvania, a total of 168 same-sex twin and 7 triplet sets were studied prospectively with monthly ear examinations to determine the proportion of time with middle ear effusion (MEE). At the 2-year endpoint, the estimated heritability for time with MEE was 73% ($p<0.001$) and estimated discordance for 3 or more episodes of MEE was 0.04 in MZ twins and 0.37 in DZ twins At the five-year follow up, heritability was reported at 72% ($p<0.001$) in 83 twin pairs [1].

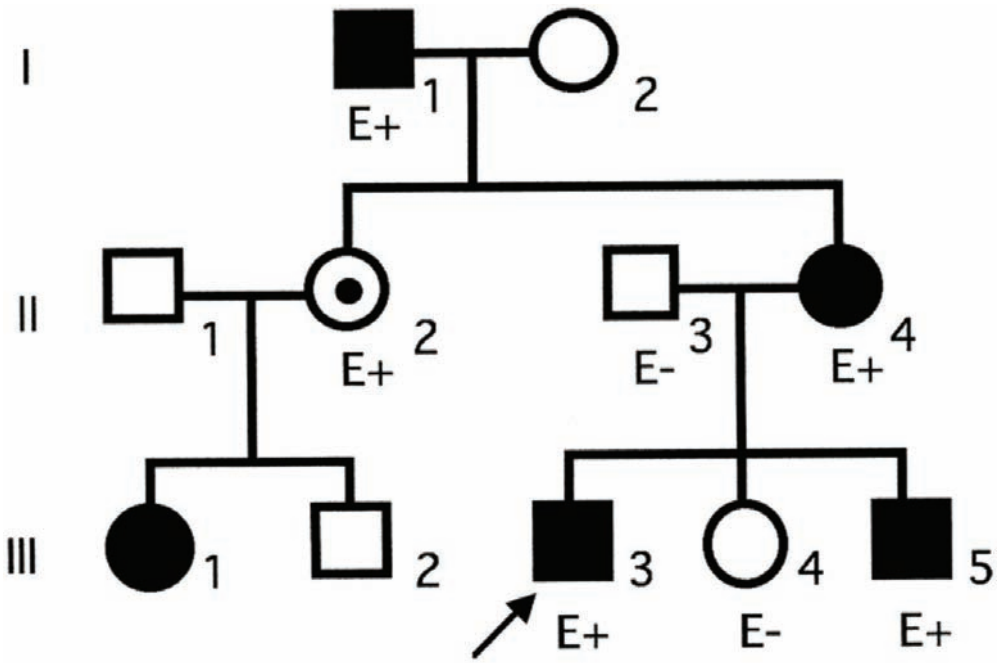
In England and Wales the Twin Early Development Study enrolled same-sex twin pairs born in 1994. This study estimated heritability of otitis media based on parental questionnaires for 715 sets of MZ twins and 658 sets of DZ twins. Heritability at ages 2, 3 and 4 years was reported as 0.49, 0.66 and 0.71, respectively [2].

A retrospective self-reporting study in 2,750 Norwegian twin pairs estimated the heritability for liability to recurrent otitis at 0.74 in females and 0.45 in males [3].

Results from these twin studies evidence a strong genetic component to susceptibility to both recurrent and chronic otitis media. Progress in understanding this susceptibility is being made through candidate gene studies, linkage analyses, and genome-wide association studies, but we have still only touched the surface of this new area of enquiry.

1. Casselbrant, Mandel, Fall, Rockette, Kurs-Lasky, Bluestone, Ferrell – The Heritability of Otitis Media, JAMA, 1999.
2. Rovers, Haggard, Gannon, Koeppen-Schomerus, Plomin – Heritability of Symptom Domains in Otits Media: A Longitudinal Study of 1,373 Twin Pairs, Am J Epidemiol 2002.
3. Kvaerner, Tambs, Harris, Magnus – Distribution and heritability of recurrent ear infections, Ann Otol Rhinol Laryngol, 1997.

Prof Margaretha Casselbrant
University of Pittsburgh



A word from the Secretary

Mahmood has set the agenda for this Newsletter to focus on genetics. Although he had meant for this to be particularly related to OM I have been thinking a lot about genetic duplication of your Secretary given the need for more time as we have a flurry of activity leading up to our annual meeting. It is truly the mark of a growing and thriving Society when there is such an increase in emails and plans and conference calls.

The leadership of ISOM has met frequently as we plan for our meeting in National Harbor, Maryland, just outside DC and it has been a pleasure to work to keep many items aligned. One particular piece of mention has been the review of the Bylaws and the setting forward of many of the structures of your organization described therein. First I would like to mention the extremely important work that Bob Ruben and Rich Rosenfeld put into the initial crafting of these Bylaws. They have truly set us up for success and have guided us well in our early moments as a society. Much like that first chromosomal duplication (work with me on the genetic references) in which the single cell works to grow and perfectly split into two new cells we as a Society are growing in an extremely healthy fashion. Those Bylaws have allowed for us to continue to develop committees, plan for leadership transitions and the election of new members to the Board. They have outlined ample ways in which the Membership can participate in the Society and I hope a growing number of you will be willing to give of your time to help our growth.

One final comment on genetics and OM is that we have now sequenced the model organism for the study of OM, the chinchilla, and if you would like to learn more about this you may visit the chinchilla genome website or attend the workshop session at the upcoming meeting. I look forward to seeing you all there!

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A word from the Treasurer

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It is hard to believe that there are not even three months left until the 18th International Symposium on Recent Advances in Otitis Media in National Harbor, Maryland June 7-11, 2015. Everyone has worked extremely hard to make this the best symposium ever!

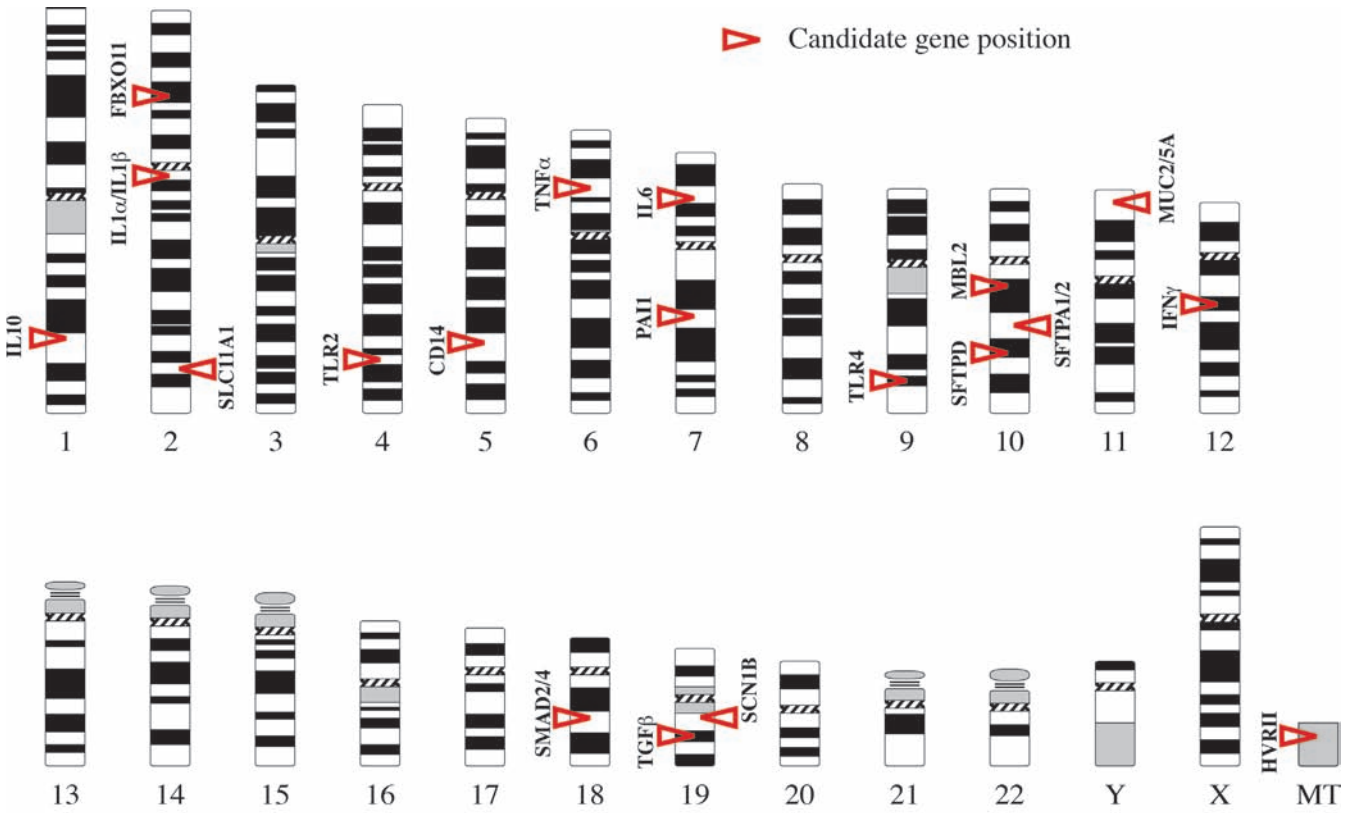
The ISOM membership has continued to grow to a total of 101 active members, 6 Honorary members and 6 Students members, which is really exiting. For those of you who have not yet become an ISOM member I strongly encourage you to join. One of the benefits of membership is the discounted symposium registration fee, in addition to the privilege of being part of the development of this very young Society. I also want to remind you that you must join ISOM prior to June 30, 2015 in order to become a charter member.

I am looking forward to a great scientific meeting and reconnecting with old friends as well as making new friends at the 18th International Symposium on Recent Advances in Otitis Media.

See you there.

Please do not hesitate to contact me if you have any question about ISOM membership or the 18th Symposium.

Candidate gene association studies



The Candidate gene approach for identifying susceptibility genes
Candidate gene association studies can be used to identify genes predisposing to risk of disease. The genes or genetic markers are hypothesised based on previous findings, for example from animal studies or through educated guesswork. The drawback of the candidate gene approach is that it restricts analysis to only a few specified genes, and so will not find entirely novel risk genes responsible for otitis media.

The approach is to compare the frequencies of these genetic markers among subjects affected by RAOM/COME with those in healthy or unaffected subjects. The control individuals can be siblings and other near relatives (family study), or healthy controls from the same population. If a candidate genetic polymorphism (Single nucleotide polymorphism) in a gene is identified, the analysis of larger sample sets and more detailed analysis or the genetic region of interest is likely to yield more robust and replicable data.

Most candidate gene studies in OM have focused on genes involved in innate or acquired immunity. A compilation of positive association findings is presented below (adapted from Hafren et al 2012). Only a few have been replicated in multiple cohorts, perhaps due to lack of power, differences in populations, in phenotype definition, or environmental factors. Our voyage of discovery continues!

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Sweden

Dr Lena Hafrén
Helsinki University Hospital
Finland

Genetic mouse models

Similarities between the auditory and immune systems in mice and humans, along with the close evolutionary relationship between the two genomes, make the mouse a key model system for otitis media research. Moreover, the extensive genetic toolkit in the mouse, that allows us to alter genes at will and investigate their function, has provided a number of profound insights into molecular pathways involved in OM and new targets for therapeutic intervention.

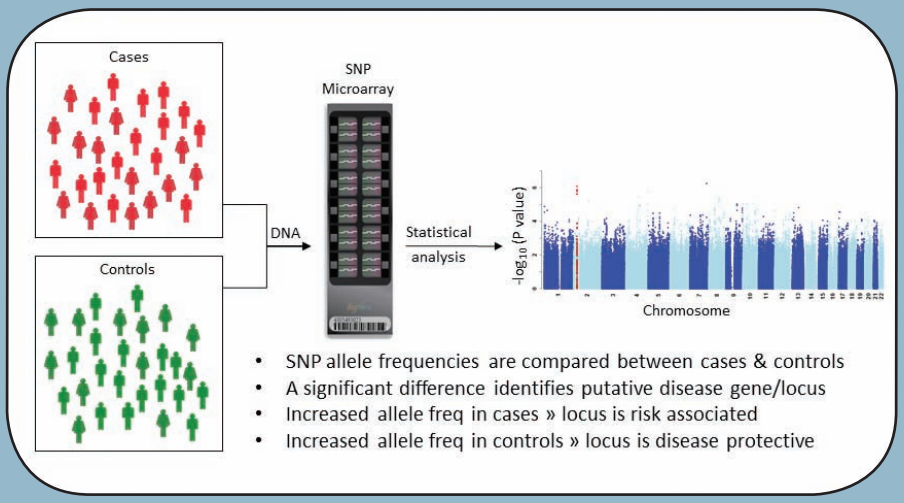
At Harwell we have generated a number of mouse models of chronic OM, including the Junbo, Jeff and Tgif1 knockout mouse. Interestingly these mice carry mutations in the genes Fbxo11, Evi1 and Tgif1, and all of these genes impact upon the molecule TGFb, suggesting that the Tgfb pathway is a critical regulator of chronic inflammation in the ear. We know that TGFb signalling interacts with hypoxia signalling, and indeed we have shown that the chronically inflamed middle ear in all of these models in also hypoxic. The hypoxia responsive genes HIF-1a and VEGF are upregulated in the middle ear of these mice, and systemic therapy with VEGF inhibitors leads to reduced inflammation in these models, suggesting that hypoxia signalling is a potential molecular target in chronic OM.

Are these findings relevant to human disease? We think so. Variants in two of the mouse OM genes (Fbxo11 and Tgif1) have been significantly associated with the development of chronic OM in several human populations. Analysis of gene expression in children with chronic OM shows that hypoxia pathways are indeed upregulated in the human middle ear.

In conclusion, work on the humble mouse is playing an important role in deciphering the mechanisms of OM, and presenting exciting new targets for novel therapies.

Prof Steve Brown
Director MRC Harwell, Oxfordshire UK

The genome wide association study



Dr Sarra Jamieson
Telethon Kids Institute
The University of Western Australia

Over the last decade the genome-wide association study (GWAS) has been used to delineate the genetic risk factors underlying many common diseases, including otitis media. In a GWAS the frequencies of (usually) several million single nucleotide polymorphisms (SNPs) across the genome are analysed on a SNP microarray. The frequencies of each SNP's alleles are then compared in hundreds/thousands of unrelated cases versus hundreds/thousands of ethnically matched controls. Statistical evidence that specific SNP alleles differ in frequency between cases and controls suggests that the SNP either directly influences risk of the disease (i.e. it is a causal genetic variant) or that it is linked with the true genetic variant.

To date two GWASs of OM have been reported. The first identified associations at several genes not previously implicated in OM (CAPN14, GALNT14, BPIFA3 and BPIFA1) but which are known to play a role in the invasion, adherence or recognition of respiratory bacterial pathogens, suggesting a functional role[1].

The second GWAS I identified associations at additional novel genes/regions (intergenic 2q31.1 SNP, KIF7, TICRR and TPPP), several of which are known to play a role in ciliary function or are expressed on ciliated airway epithelial cells.[2]

These studies demonstrate that the GWAS is a powerful approach to identifying genetic risk factors in OM and the recent formation of OTIGEN, an international consortium of researchers focused on delineating the genetic basis of OM, will greatly facilitate ongoing GWAS investigations. Knowledge of the genes that contribute to OM susceptibility provides important insights into the biological complexity of this disease, and could ultimately contribute to improved preventative and therapeutic strategies to reduce incidence.

1. Rye MS, Warrington NM, et al. Genome-wide association study to identify the genetic determinants of otitis media susceptibility in childhood. PloS one. 2012;7(10):e48215.
2. Allen EK, Chen WM, et al. A genome-wide association study of chronic otitis media with effusion and recurrent otitis media identifies a novel susceptibility locus on chromosome 2. JARO. 2013 Dec;14(6): 791-800.

Save the dates!