JARO 14: 791–800 (2013)
DOI: 10.1007/s10162-013-0411-2

JARO
Journal of the Association for Research in Otolaryngology

Research Article

A Genome-Wide Association Study of Chronic Otitis Media with Effusion and Recurrent Otitis Media Identifies a Novel Susceptibility Locus on Chromosome 2

E. Kaitlynn Allen,[1,2] Wei-Min Chen,[1,3] Daniel E. Weeks,[5,6] Fang Chen,[1] Xuanlin Hou,[1] José L. Mattos,[1] Josyf C. Mychaleckyj,[1,3] Fernando Segade,[7] Margaretha L. Casselbrant,[8] Ellen M. Mandel,[8] Robert E. Ferrell,[5] Stephen S. Rich,[1,3,4] Kathleen A. Daly,[9] and Michèle M. Sale[1,2,4]

[1]*Center for Public Health Genomics, University of Virginia, P.O. Box 800717, Charlottesville, VA 22908, USA*

[2]*Department of Biochemistry and Molecular Genetics, Charlottesville, VA 22908, USA*

[3]*Department of Public Health Sciences, Charlottesville, VA 22908, USA*

[4]*Department of Medicine, University of Virginia, Charlottesville, Charlottesville, VA 22908, USA*

[5]*Department of Human Genetics, Pittsburgh, PA 15261, USA*

[6]*Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261, USA*

[7]*Department of Anatomy and Cell Biology, University of Pennsylvania, Philadelphia, PA 19104, USA*

[8]*Department of Otolaryngology, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA 15224, USA*

[9]*Department of Otolaryngology, University of Minnesota, Minneapolis, MN 55455, USA*

Received: 24 October 2012; Accepted: 4 August 2013; Online publication: 23 August 2013

ABSTRACT

Chronic otitis media with effusion (COME) and recurrent otitis media (ROM) have been shown to be heritable, but candidate gene and linkage studies to date have been equivocal. Our aim was to identify genetic susceptibility factors using a genome-wide association study (GWAS). We genotyped 602 subjects from 143 families with 373 COME/ROM subjects using the Illumina Human CNV370-Duo DNA Bead Chip (324,748 SNPs). We carried out the GWAS scan and imputed SNPs at the regions with the most significant associations. Replication genotyping in an independent family-based sample was conducted for 53 SNPs: the 41 most significant SNPs with $P<10^{-4}$ and 12 imputed SNPs with $P<10^{-4}$ on chromosome 15 (near the strongest signal). We replicated the association of rs10497394 (GWAS discovery $P=1.30\times10^{-5}$) on chromosome 2 in the independent otitis media population ($P=4.7\times10^{-5}$; meta-analysis $P=1.52\times10^{-8}$). Three additional SNPs had replication *P*values<0.10. Two were on chromosome 15q26.1 including rs1110060, the strongest association with COME/ROM in the primary GWAS ($P=3.4\times10^{-7}$) in *KIF7* intron 7 ($P=0.072$), and rs10775247, a non-synonymous SNP in *TICRR* exon 2 ($P=0.075$). The third SNP rs386057 was on chromosome 5 in *TPPP* intron 1 ($P=0.045$). We have performed the first GWAS of COME/ROM and have identified a SNP rs10497394 on chromosome 2 is significantly associated with COME/ROM susceptibility. This SNP is within a 537 kb intergenic region, bordered by *CDCA7* and *SP3*. The genomic and functional significance of this newly identified locus in COME/ROM pathogenesis requires additional investigation.

Keywords: otitis media, genetics, genome, susceptibility, locus

INTRODUCTION

Every year, over 10 million children in the United States are treated for otitis media (OM), the inflam-

Correspondence to: Michèle M. Sale · Center for Public Health Genomics · University of Virginia · P.O. Box 800717, Charlottesville, VA 22908, USA. Telephone: +1-434-9820368; fax: +1-434-9821815; email: msale@virginia.edu

mation of the middle ear (Gunasekera et al. 2007; Daly et al. 2010). OM is a leading reason for physician visits during childhood and represents a major component of the pediatric health care burden (Grevers 2010). Antibiotic prescriptions for OM contribute significantly to increasing multi-drug resistance (Grevers 2010), and insertion of ventilation tubes into the tympanic membrane in the middle ear to assist fluid drainage remains one of most common childhood surgeries performed in the U.S. (Schraff 2008).

Acute OM is characterized by middle ear inflammation with signs of middle ear effusion and acute symptoms of infection including middle ear pain and fever (Coker et al. 2010; Toll and Nunez 2012). A minority of children who suffer from acute OM will develop either chronic otitis media with effusion (COME) or recurrent otitis media (ROM). Estimates suggest that by 2 years of age, nearly a third of all children will have had more than three episodes of OM within 6 months or four episodes within 1 year (Poehling et al. 2007). Recurrent and chronic forms of OM are associated with high economic and societal costs such as medical care costs, parental days lost from work, and loss of earnings (Greenberg et al. 2003). Complications of COME/ROM include permanent hearing loss, tympanic membrane abnormalities (e.g., chronic ear drainage), other infections such as meningitis, and other sequelae (Daly et al. 1998; Johnston et al. 2004; Grevers 2010). The annual economic burden of otitis media in the United States is estimated to be at least $5 billion for direct and indirect costs (Gates 1996).

COME/ROM has long been recognized to aggregate in families (Teele et al. 1980; De Melker and Burke 1988; Harsten et al. 1989; Teele et al. 1989). The first family study of COME/ROM (Daly et al. 1996) demonstrated that first-degree relatives (parents and siblings) of probands with COME/ROM had greater than expected rates of OM based on population rates. Subsequent twin and triplet studies confirmed the strong familial aggregation of COME and ROM, with heritability estimates of 0.64–0.74 in monozygotic twins and 0.20–0.53 in dizygotic twins (Kvaerner et al. 1997; Casselbrant et al. 1999; Rovers et al. 2002; Kvestad et al. 2004, 2006). Two linkage studies for COME/ROM have been published to date (Daly et al. 2004; Casselbrant et al. 2009; Chen et al. 2011); however, the loci identified in the two studies did not overlap. Recently, the first GWAS for acute and chronic OM in the first 3 years of life discovered novel associations with OM, including *CAPN14*, *GALNT14*, and the *BPIFA* gene cluster, though the study lacked replication in an independent population of COME/ROM (Rye et al. 2012). In an effort to detect common variants contributing to COME/ROM susceptibility, we have conducted the first genome-wide association study (GWAS) of COME/ROM.

METHODS

This study was conducted with Institutional Review Board approval at the University of Minnesota, Wake Forest University, the University of Virginia, and the University of Pittsburgh, and adhered to the tenets of the Declaration of Helsinki.

University of Minnesota (UMN) study

Details of recruitment and examination of the study participants have been described previously (Daly et al. 1996, 2004; Segade et al. 2006). Index cases (probands) who had tympanostomy tube surgery for COME/ROM and their family members were recruited for the study. An otolaryngologist performed an ear examination to determine presence of OM sequelae without knowledge of the subject's prior OM history. Tympanometric testing was performed in subjects at three frequencies (226, 630 or 710, and 1,400) to detect abnormal middle ear mechanics, and hearing was screened at 20 dB for speech frequencies. Individuals from 143 families with phenotypic data and DNA available were enrolled in genetic studies. The sample includes 44 families with five to ten members, 55 families with four members, 36 trios, and 8 families with less than three members (Table 1).

University of Pittsburgh (UPitt) study

We carried out a replication analysis in an independent study of OM (Casselbrant et al. 2009) that consisted of 1,584 genotyped individuals from 441 Caucasian families. In order to assure a history of significant ear disease, two or more full siblings who both or all had undergone tympanostomy tube

TABLE 1

Participant characteristics for the University of Minnesota (UMN) and University of Pittsburgh (UPitt) study populations

	UMN study	*UPitt study*
Trait	*Value*	*Value*
Number of families	143	441
Number of genotyped subjects	602	1584
Female (%)	53.3 %	51.4 %
Affected subjects	62.0 % (373/602)	59.1 % (932/1584)
Caucasian	95.7 %	100 %
Non-Hispanic	98.7 %	–

insertion were enrolled. The need for tympanostomy tube insertion established a subject's history of significant middle ear disease. A subject was only considered affected if he/she had undergone tympanostomy tube insertion at least once for recurrent/persistent OM, while a subject was considered unaffected if he/she had never had tympanostomy tubes and had no known history of recurrent/persistent OM. The remaining subjects were considered as having unknown disease status. Otoscopic examinations and tympanometry were conducted at entry when possible, but the condition of the ears at entry did not determine eligibility, and the tubes may have been inserted many years prior to study entry (Casselbrant et al. 2009). The UPitt sample includes 87 families with five to eight members, 330 families with four members, and 12 trios (Table 1).

GWAS genotyping and data cleaning

The Illumina Human CNV370-Duo DNA Bead Chip was used for genotyping DNA family members of the UMN Study. Removal of SNPs was based upon filtering for poor genotype clusters, low minor allele frequency (MAF<0.01), and genotypes inconsistent with Hardy Weinberg proportions ($P<10^{-5}$). Samples and SNPs with excessive Mendelian errors (>0.6 %) and/or low genotype call rates (<95 %) were removed. A total of 324,748 SNPs were genotyped in 602 subjects and available for analyses.

Kinship coefficients were estimated between all pairs of individuals based on the GWAS SNP data using software KING (Manichaikul et al. 2010), and these data were used to correct both within- and between-family pedigree errors. The Minnesota study samples are from a predominantly Caucasian population (95.7 %), with the remaining 4.3 % of the population from Asian, Native American, and Mixed populations. Two families, consisting of two and four individuals, respectively, and had non-Caucasian ethnicity, and 19 individuals self-identified as belonging to more than one race. These individuals did not substantially change the family-based association results and so were retained for analyses.

Imputation

To improve genomic coverage, we used the imputation method implemented in the software package MACH using HapMap3 with the CEU reference population (Li et al. 2009; 2010). This method uses Markov models to identify stretches of shared chromosomes between individuals and then to infer intervening genotypes by contrasting study samples with densely typed HapMap samples. Although imputation is accurate (Li et al. 2010), improvement in accuracy can be achieved by tuning the quality metric threshold. The "most likely" genotypes were used in the QLSw test; therefore, imputed SNPs were treated the same as genotyped SNPs. Imputed SNPs that passed a quality threshold of $r^2>0.3$ and MAF>0.5 were included in analysis. Imputed genotypes that were not consistent with Mendelian inheritance in a family were set to missing. To reduce the multiple comparisons penalty of evaluating all imputed SNPs, our investigation of imputed data was focused only on GWAS regions with prior evidence of association in the primary (genotyped) analysis ($P<10^{-4}$). In our region of interest on chromosome 15, there were 2,818 SNPs available for imputation.

After evidence of replication on chromosome 2, we investigated imputed data in the chromosome 2 region (chr2: 174286992–174307309) using the same imputation methods already described. Eighteen imputed SNPs were available in this region for association analysis.

Statistical analyses

We applied the family-based association test QLS_W to the genome scan data. This method was recently developed (Chen et al. 2010) as an extension of the powerful method MQLS (Thornton and McPeek 2007), and the implementation is publicly available through software package Generalized Disequilibrium Test (GDT) (Chen et al. 2009). The QLS_W method has been shown more powerful than the MQLS method in the presence of population stratification and/or in the presence of family ascertainment such as the affected sibling pairs design. Population substructure does not affect analysis using the QLSw family-based association test as this approach is robust to population stratification. The QLSw family-based association test (January 2011) was used for GWAS analysis and replication analysis.

Association of SNPs on the X chromosome with COME/ROM was analyzed using the GDT (Chen et al. 2009).

The GDT method (Chen et al. 2009) was used for the conditional analysis of the chromosome 2 locus. The genotype at SNP rs10497394 was considered as a covariate and was adjusted in the GDT test statistic.

Replication studies

A total of 53 SNPs were selected for replication genotyping in the UPitt families. The 53 SNPs included the 41 most significant SNPs with $P<10^{-4}$ from our discovery GWAS (n=41), plus 12 imputed SNPs with $P<10^{-4}$ located on chromosome 15 near rs1110060, the only genotyped SNP with marginal significance. Genotyping was conducted on the Sequenom platform using the iPlex assay (Jurinke et al. 2002).

Meta-analysis was carried out on the data from the UMN and UPitt studies. Briefly, a weighted *z* score-based fixed-effects meta-analysis method was used to combine the results of the UMN and UPitt studies using the program METAL (Willer et al. 2010). Weighting was proportional to the sample size of each study (Chen et al. 2008).

In silico eQTL analysis

An eQTL analysis of the chromosomes 2, 5, and 15 regions was conducted using three different databases including "eQTL resources @ the pritchard lab" (http://eqtl.uchicago.edu), Wellcome Trust Sanger Institute's Genevar (Yang et al. 2010), and SCAN: SNP and CNV annotation database (Gamazon et al. 2010). The eQTL analysis using "eQTL resources @ the pritchard lab" was conducted using RNAseq data from a study using total RNA from lymphoblastoid cell lines in 63 HapMap individuals of European ancestry (Montgomery et al. 2010). The eQTL analysis using Genevar was conducted using expression data from the total RNA of 109 lymphoblastoid cell lines of European ancestry (Stranger et al. 2012). The eQTL analysis using SCAN: SNP and CNV annotation database was conducted using expression data from total RNA from lymphoblastoid cell lines of 30 trios of European ancestry and 30 trios of African ancestry (Duan et al. 2008), though based on our population, only significant results from the population of European ancestry are included.

RESULTS

We performed the first genome-wide association study for COME/ROM by analyzing 324,748 SNPs on the Illumina HumanCNV370-Duo DNA Bead Chip (genomic control lambda value (GC)=0.993) in the UMN family population (Fig. 1). The strongest association with COME/ROM in the UMN families was rs1110060 (P=9.1×10^{-7} (Fig. 1)) located on chromosome 15 within the gene encoding *KIF7*. One region had a "suggestive" P<10^{-5} (Duggal et al. 2008) located on chromosome 6 (rs10499006, P=2.1×10^{-6}), and one region approached the "suggestive" P value threshold on chromosome 3 (rs6438779, P=2.9×10^{-6}) both of which were in intergenic regions. CNV association analyses conducted with PennCNV (Wang et al. 2007) did not reveal any evidence for significant association (data not shown).

Analyses of the X chromosome using the GDT (Chen et al. 2009) did not result in any significant findings. The most significant result on the X chromosome was with rs2215100 (P=3.3×10^{-5}). This was the only SNP that exceeded P=10^{-4}. SNP rs2215100 lies within a large intergenic region with the closest genic feature, the VENT homeobox 1 pseudogene (*VENTXP1*) 115.8 kb distal to the SNP and closest gene Melanoma antigen family B, 6 (*MAGEB6*), 481.3 kb distal to the SNP.

All replication results with P<0.10 are shown in Table 2. All four SNPs listed in Table 2 had genotyping success rates over 99.35 % in the UMN GWAS and over 98.0 % in the UPitt replication study. Five of the 53 SNPs failed quality control assessment (rs3773770, rs11639117, rs17360083, rs6808013, and rs11259905). Three SNPs (rs1361049, rs4701136, and rs11852287) were unable to be successfully genotyped on the Sequenom platform. The significance threshold after performing a conservative Bonferroni correction for multiple testing (*i.e.*, 45 SNPs) was P=1.1×10^{-3}. The locus identified on chromosome 2 (rs10497394, P=2.9×10^{-5}) was successfully replicated in the UPitt Study (P=4.7×10^{-5}) with a meta-analysis P=1.52×10^{-8}. Meta-analysis results are listed in Table 2 and confirmed directionality of these four SNPs in the two studies. Note a naive Bonferroni corrected P value threshold that assumes both datasets are typed at 324,748 SNPs is 0.05/324,748=1.54×10^{-7} and the

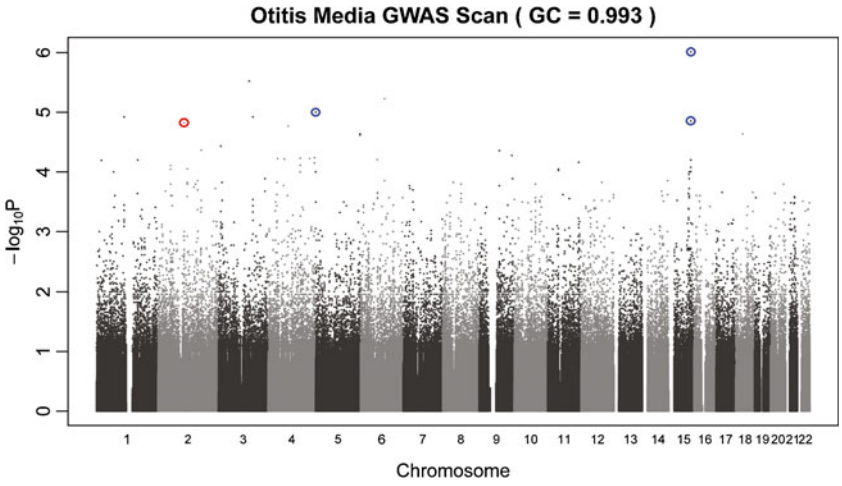

FIG. 1. COME/ROM genome-wide scan association results using the QLS_W method (GC=genomic control value). The *circled* SNPs represent the SNPs in Table 2 (replication P<0.10). The SNP circled in *red* is rs10497394, the significantly replicated SNP. X chromosome analyses were not presented since they were analyzed using a different method (GDT; Chen et al. 2009).

TABLE 2

Replication results with $P<0.10$ and meta-analysis results of those SNPs

				UMN Study				UPitt study				Meta-analysis		
Chr	*SNP*	*Position (hg19)*	*Ref allele*	*Allele freq*	*OR*	*Z*	*Pvalue*	*Allele freq*	*OR*	*Z*	*Pvalue*	*OR (95 % CI)*	*Z*	*Pvalue*
2	rs10497394	174,297,659	G	0.629	1.754	4.18	2.9×10^{-5}	0.637	1.422	4.07	4.7×10^{-5}	1.51 (1.3–1.7)	5.66	1.52×10^{-8}
5	rs386057	685,748	G	0.447	1.733	4.33	1.5×10^{-5}	0.585	1.193	2.01	0.045	1.35 (1.2–1.6)	3.98	6.94×10^{-5}
15	rs10775247	90,126,121	C	0.570	0.573	−4.00	6.3×10^{-5}	0.460	0.861	−1.78	0.075	0.77 (0.67–0.89)	−3.62	3.00×10^{-4}
15	rs1110060	90,190,048	A	0.551	0.510	−4.91	9.1×10^{-7}[a]	0.409	0.856	−1.80	0.072	0.74 (0.64–0.85)	−4.10	4.09×10^{-5}

Information provided in this table relates to the reference allele; *Z* score listed for minor allele

[a]Most significant result from UMN Study GWAS

significance threshold for a more appropriate two-stage joint analysis is 1.80×10^{-6} (Skol et al. 2006).

The replicated SNP on chromosome 2 (rs10497394) maps within a 537 kb intergenic region bordered by two genes (*CDCA7* and *SP3*) coding for nuclear proteins with transcription factor activities. Subsequent imputation analyses of the chromosome 2 region using HapMap3 data found 14 SNPs with $P<10^{-5}$ in this region, with the lowest $P=1.1\times10^{-7}$. *P* values for all 14 of these SNPs exceeded our top genotyped SNP, rs10497394. Haplotype analysis using Haploview (version 3; Release 27; CEU analysis panel) (Barrett 2009) identified two haplotype blocks of high linkage disequilibrium ($r^2>0.9$), one block spanning approximately 16kb (chr2:174286992–174303858) and the other less than 1kb (chr2:174306514–174307309), the total region containing both haplotype blocks spanning chr2:174286992–174307309 (UCSC Genome Browser, build hg19). This region of high LD contains many regulatory and genomic elements that have putative functional effects (Fig. 2). Locus zoom plots using our primary GWAS data with HapMap3 imputation results (Fig. 3) confirmed this region is in high LD and lies between two areas of high recombination (Pruim et al. 2010). HaploReg (Ward and Kellis 2012) identified two SNPs (rs1435769 and rs10185704) in high LD ($r^2=1.0$) with rs10497394 that lie within transcription factor binding sites for *STAT3* and *NFκB*, respectively. In order to investigate whether other SNPs in this region represent independent associations with COME/ROM in this population, we used a conditional analysis using the genotype at SNP rs10497394 as a covariate. There were 14 SNPs in the chromosome 2 region of interest with GDT $P<0.001$ (the smallest $P=8.6\times10^{-5}$) before the conditional analysis, and none of them have $P<0.001$ after the conditional analysis. Note that, although the existing method GDT is slightly less powerful than the method QLSw that we are currently using, it has the ability to adjust for covariates. This analysis suggests that the other SNPs in this region do not represent independent associations with COME/ROM.

The strongest locus from our discovery sample (rs1110060) produced a replication $P=0.072$ and a meta-analysis $P=4.09\times10^{-5}$ (Table 2.) The two chromosome 15 SNPs (rs10775247 and rs1110060) lie about 64 kB from each other in adjacent genes *TICRR* and *KIF7* ($r^2=0.47$; $D'=0.77$). SNP rs386057 (replication $P=0.045$; meta-analysis $P=6.94\times10^{-5}$, Table 2) is located on chromosome 5p15.33 within intron 1 of the gene tubulin polymerization promoting protein (*TPPP*).

From our in silico eQTL analysis, the SNPs on chromosomes 2 and 15 were found to regulate genes in the CEU population (Table 3). Analysis of the

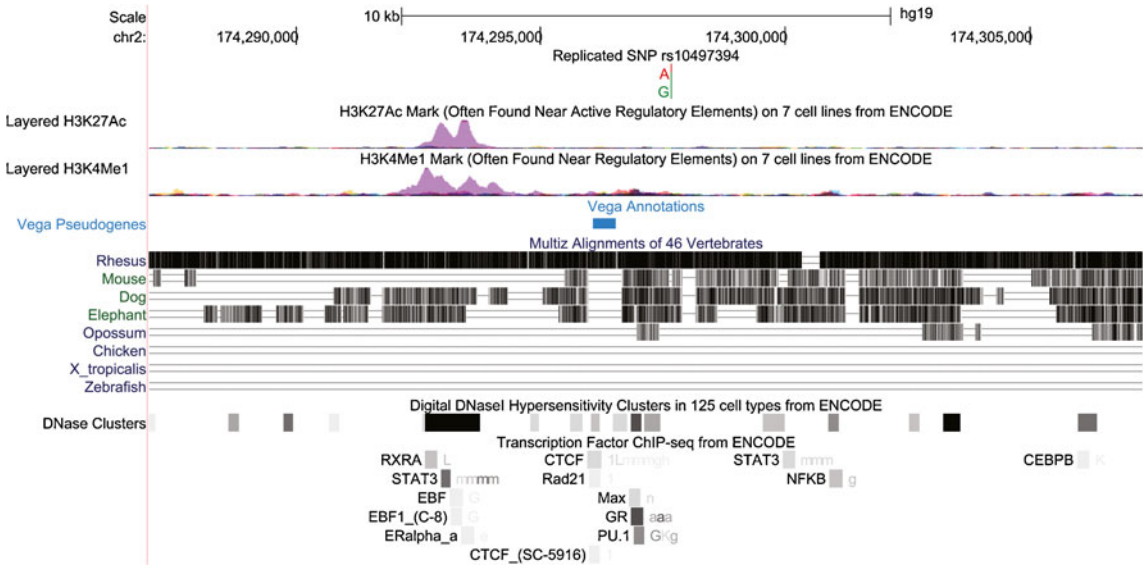

FIG. 2. Genomic characteristics of the chromosome 2 region of high LD (chr2: 174286992–174307309) from UCSC Genome Browser build hg19 which includes a processed pseudogene with 89 % sequence identity to the coding sequence of the hematological and neurological expressed 1 (*HN1*) gene; an H3K27Ac mark and an H3K4Me1 mark from ENCODE; DNaseI hypersensitive regions including those discovered in relevant cell lines (lung, non-pigment ciliary epithelial, and small airway epithelial cell lines); a conserved CTCF insulator binding site; and numerous predicted transcription factor binding sites (The ENCODE Project Consortium 2004; Rosenbloom et al. 2012). Conservation in this region is measured using Multiz Alignments of eight vertebrates.

chromosome 2 region of interest using the eQTL browser of the Pritchard Lab and Genevar produced no significant results. Using the SCAN database, the chromosome 2 SNP (rs10497394) is associated with the expression of the *LDLR* gene on chromosome 19 in the CEU population (P=6×10^{-5}). eQTL analysis of the chromosome 15 region of interest with the "eQLT resources @ the pritchard lab" found our most significantly associated SNP in the discovery population (rs1110060) acts in *cis* as an exon-QTL for *IQGAP1* (Montgomery et al. 2010). Using the SCAN database, SNP rs10775247 regulates expression of Resistin (*RETN*) in the CEU population (P=4×10^{-5}). Due to differences in sample sets and methods for measuring and analyzing expression data, results varied between the databases.

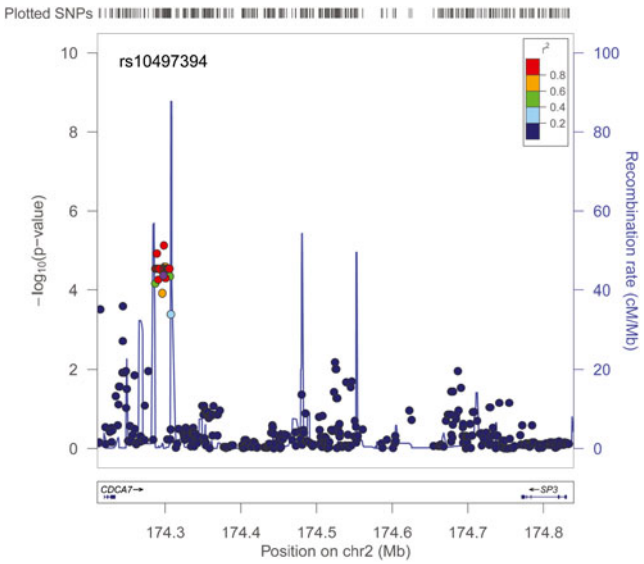

FIG. 3. Locus Zoom plots of the chromosome 2 region using UMN GWAS data and HapMap3 imputation. SNP shown in *purple* is our replicated SNP rs10497394, and linkage disequilibrium (LD) is in relation to this SNP.

DISCUSSION

We have conducted the first GWAS of COME/ROM and identified and investigated replication in an independent collection of families with otitis media. In our discovery GWAS, one SNP on chromosome 15q26.1 (rs1110060) approached significance (P=9.1×10^{-7}). A panel of 53 SNPs with Pvalues less than 10^{-4} from our GWAS was tested for replication in an independent otitis media family population at the University of Pittsburgh. We successfully replicated a novel locus on chromosome 2q31.1 (rs10497394), with replication P=4.7×10^{-5} and meta-analysis P=1.52×10^{-8}.

The replicated SNP rs10497394 is in a large intergenic region on chromosome 2q31.1 between the genes *CDCA7* and *SP3*. Although predicting functional consequences of non-protein coding SNPs proves challenging, loci have been identified in a GWAS in gene deserts and have been found to have

TABLE 3

Significant results from the three eQTL browsers used for the in silico eQTL analysis for the four SNPs of interest

Chromosome	*SNP*	*Database*	*Gene*	*Population*	*P value*	*Cis/trans*
2	rs10497394	eQTL browser @ pritchard lab	*LDLR*	CEU	6.00E-05	Trans (Chr19)
5	rs386057	–	–	–	–	–
15	rs10775247	SNP and CNV annotation database	*RETN*	CEU	4.00E-05	Trans (Chr19)
15	rs1110060	eQTL browser @ pritchard lab	*IQGAP1*	CEU	3.07E-05	*Cis* (exonQTL)

functional significance in complex diseases (Jia et al. 2009). SNP rs10497394 was found to regulate expression of *LDLR* on chromosome 19 using SCAN: SNP and CNV annotation database. Low-density lipoprotein receptor (*LDLR*) is expressed on ciliated airway epithelial cells, and *LDLR* has been found to have a role in the pathogenesis of asthma in mice (Yao et al. 2011). Additionally, *LDLR* is a binding site for human rhinovirus species C which have been implicated in upper and lower respiratory infections in children and adults with chronic respiratory disease (Bochkov et al. 2011). Replication and significant meta-analysis results for rs10497394, when combined with evidence for potential functional consequences, support a role in COME/ROM pathogenesis that warrants further investigation.

Association results on chromosomes 5 and 15 did not meet Bonferroni-corrected significance for replication but are of interest for further evaluation in additional populations. SNP rs1110060, with strongest evidence for association in the primary GWAS, is located on 15q26.1 in the proximity of the 5′ end of intron 7 of *KIF7*, a location that may conceivably affect the splicing of the primary *KIF7* transcript. The likelihood of this SNP affecting splicing was confirmed by Spliceman Web Server, an online tool used to predict how likely mutations around annotated splice sites are to disrupt splicing (Lim and Fairbrother 2012). *KIF7* is involved in regulating mammalian *Sonic Hedgehog* (*Shh*) (Ingham and McMahon 2009) and Indian hedgehog (*IHH*) (Hsu et al. 2011) via protein trafficking within the primary cilium (Ingham and McMahon 2009), resulting in skeletal abnormalities, possibly providing a link between craniofacial development such as Eustachian tube formation and COME/ROM susceptibility. Hedgehog interacting protein (*HHIP*), a regulator of the Hedgehog (Hh) pathway, and Patched (*PTCH1*), a Shh protein upstream of *KIF7*, have been associated with abnormal lung function and chronic obstructive pulmonary disease in two large meta-analyses of GWAS studies of lung function (Li et al. 2011). Polymorphisms in a related kinesin gene, *KIF3A*, were recently shown to be associated with aspirin-intolerance in asthma and the decline of forced expiratory volume at 1 s (FEV_1)% by aspirin provocation (Kim et al. 2010), suggesting a possible role in immune response.

The second SNP on chromosome 15 in Table 2, rs10775247, is a missense mutation (Arg287Cys) in *C15orf42* (*TICRR*). *TICRR* encodes the protein Treslin which has been shown to play a role in initiation of DNA replication (Kumagai et al. 2010). Although the link with COME/ROM is unclear, Treslin interacts with TopBP1, a member of a complex impacted by inactivation of host DNA replication by certain viruses, including adenovirus and parainfluenza virus type 2 (Lilley et al. 2007) which are both commonly coincident with otitis media (Massa et al. 2009). This suggests that *TICRR* may be worth evaluating in a larger sample to determine a role in COME/ROM susceptibility. A SNP in *TICRR* (rs8032553, not genotyped in our study) was found to be associated with cholesterol/LDL in the Framingham Heart Study (Kathiresan et al. 2007). As far as we are aware, none of the other regions reported in our study have been previously associated in any GWAS.

The eQTL analysis of the chromosome 15 region using the "eQTL resources @ the pritchard lab" showed regulation of *IQGAP1* by rs1110060 as an exon-QTL. *IQGAP1* is located about 741 kB distal to this SNP (chr15:90931473–91045475; hg19). *IQGAP1* is a widely conserved and ubiquitously expressed scaffold protein with numerous protein-interaction domains allowing for binding to diverse proteins (Brown and Sacks 2006). *IQGAP1* has been implicated as a target of several bacteria as a player in their mechanisms of infection. *Salmonella typhimurium* has been shown to bind to *IQGAP1* which allows *S. typhimurium* to escape the host immune response allowing for the establishment of chronic infection (Kim et al. 2011). Additionally, *IQGAP1* was found to be needed to organize ROS-dependent *VEGF* signaling via *VEGFR2* in endothelial cells which may contribute to the repair and maintenance of blood vessels and angiogenesis (Yamaoka-Tojo et al. 2004). *VEGF* signaling is known to induce angiogenesis, increase vascular permeability, and influence inflammation in several ways, all which could increase risk of chronic otitis media by effusion build-up and in-

creased inflammation (Angelo and Kurzrock 2007). Inhibitors of *VEGF* signaling have been found to reduce hearing loss and fluid buildup in the middle ear of *Junbo* mice, a spontaneous mouse model of OM (Cheeseman et al. 2011). These roles of *IQGAP1* indicate that *IQGAP1* may be involved in COME/ROM pathogenesis though further investigation is necessary.

SNP rs386057 is located on chromosome 5p15.33 in the intronic region of the gene tubulin polymerization promoting protein (*TPPP*). *TPPP* is important in microtubule (MT) assembly, MT bundling, and the stabilization of existing MTs, and may also play a role in mitotic spindle assembly and nuclear envelope breakdown (Ovádi and Orosz 2009). It has been identified as a brain specific protein, but its orthologs are ciliary proteins, suggesting a role of *TPPP* in the basic function of cilia (Orosz and Ovádi 2008). This potential role ciliary function is interesting due to our findings of the association of rs1110060 in *KIF7*, an important intraflagellar transport protein.

Many epidemiological studies have shown that male children are more likely to be affected with COME/ROM than females (Auinger et al. 2003; Damoiseaux et al. 2006; Daly et al. 2010). Despite evidence of a gender bias, analysis of the X chromosome from our GWAS data did not result in any significant loci associated with COME/ROM.

The primary strength of our study is the family-based study design for both the GWAS and replication phases, permitting an analytical approach robust to population stratification and the application of a powerful new test of association. Though the QLSw family-based association test is powerful and robust, it is unable to model covariates. The main limitations to this study include SNP density and modest population size. We carried out a power analysis for a two-stage genome-wide association study using the software tool CaTS (Skol et al. 2006). In a study with 1,309 cases and 877 controls, with 27.5 % of the samples genotyped at all 324,748 SNPs in the first stage, and 0.015 % of SNPs followed up by genotyping the remaining samples in Stage 2, assuming the prevalence of OM is 0.15, and the genotype relative risk is 1.5 at a causal SNP with the disease allele frequency 0.5, we have 59 % power to identify a variant at significance level 1.5×10^{-7}. In common diseases, statistical power has been shown to be slightly lower in family-based studies than in case–control studies (Laird and Lange 2006); therefore, the statistical power calculated is slightly inflated. Another limitation is that the UMN Study included only a small sample of non-European families, and the UPitt Study excluded all non-European families. Consequently, we are unable to draw conclusions about whether the observed associations are also present in populations of non-European ancestry. Due to the nominal significance of the chromosome 5 and 15 SNPs in the replication study, we cannot exclude the possibility of Type I error in the discovery GWAS or Type II error in the replication study.

The RAINE cohort GWAS of OM discovered novel associations with combined acute/chronic OM, including *CAPN14*, *GALTN14*, and the *BPIFA* gene cluster, which are all found within or connected to the TGFβ pathway (Rye et al. 2012). The TGFβ pathway has recently been identified as important in COME/ROM in multiple studies (Tateossian et al. 2009; Rye et al. 2011; Tateossian et al. 2013). These loci were not able to be replicated in the Western Australia Family Study of Otitis Media. In our family-based population, we also found no evidence of association with the reported SNPs at the loci that had genotype data in our study (N=21). The lack of replication could be due to the differences in inclusion criteria for case classification (acute/chronic OM in first 3 years versus COME/ROM) or due to lack of power in the UMN population to detect associations.

This investigation has demonstrated that a family-based GWAS approach can successfully identify susceptibility loci for COME/ROM, despite the high prevalence of acute OM in the general population and modest sample size of our study. Larger genome-scale investigations of COME/ROM would be anticipated to lead to identification of additional susceptibility loci. Functional investigation of the chromosome 2 susceptibility locus identified by our study is warranted and may provide insights into COME/ROM initiation and pathophysiology.

ACKNOWLEDGMENTS

We thank the families for their participation. The study would not have been possible without the UMN Study recruitment team: Donna Brauer, Karen Savonna, Ruth Selvius, Ann Brauti, Cynthia Roh, Michele Bochert, Jennifer Palmer, Kendra Herrell, Judy Monroe, Norrita Rech, Kim Canfield, Heather Nelson, and JoAnn Knox. Thanks also to Kathleen Day and Pamela Hicks for technical assistance. This work was supported by National Institutes of Health grants DC003166 and DC003166-S1 (KAD). The replication work was supported primarily by the National Institutes of Health grant R01DC005630 (MLC), as well as by the National Institutes of Health General Clinical Research Center grant M01RR000084, National Institutes of Health Clinical and Translational Science Award UL1RR024153, Children's Hospital of Pittsburgh of UPMC, and the University of Pittsburgh. Ms. Allen received support from the National Institute of General Medical Sciences of the National Institutes of Health under Award Number T32GM008715.

REFERENCES

ANGELO LS, KURZROCK R (2007) Vascular endothelial growth factor and its relationship to inflammatory mediators. Clin Cancer Res 13:2825–2830

AUINGER P, LANPHEAR BP, KALKWARF HJ, MANSOUR ME (2003) Trends in otitis media among children in the United States. Pediatrics 112:514–520

BARRETT JC (2009) Haploview: visualization and analysis of SNP genotype data. Cold Spring Harbor protocols 2009: pdb.ip71, CSHL Press, Cold Spring Harbor

BOCHKOV YA, PALMENBERG AC, LEE W-M, RATHE JA, AMINEVA SP, SUN X, PASIC TR, JARJOUR NN, LIGGETT SB, GERN JE (2011) Molecular modeling, organ culture and reverse genetics for a newly identified human rhinovirus C. Nat Med 17:627–632

BROWN MD, SACKS DB (2006) IQGAP1 in cellular signaling: bridging the GAP. Trends Cell Biol 16:242–249

CASSELBRANT ML, MANDEL EM, FALL PA, ROCKETTE HE, KURS-LASKY M, BLUESTONE CD, FERRELL RE (1999) The heritability of otitis media: a twin and triplet study. JAMA 282:2125–2130

CASSELBRANT ML, MANDEL EM, JUNG J, FERRELL RE, TEKELY K, SZATKIEWICZ JP, RAY A, WEEKS DE (2009) Otitis media: a genome-wide linkage scan with evidence of susceptibility loci within the 17q12 and 10q22.3 regions. BMC Med Genet 10:85

CHEESEMAN MT, TYRER HE, WILLIAMS D, HOUGH TA, PATHAK P, ROMERO MR, HILTON H, BALI S, PARKER A, VIZOR L, PURNELL T, VOWELL K, WELLS S, BHUTTA MF, POTTER PK, BROWN SDM (2011) HIF–VEGF pathways are critical for chronic otitis media in *Junbo* and *Jeff* mouse mutants. PLoS Genet 7:e1002336

CHEN W-M ET AL (2008) Variations in the G6PC2/ABCB11 genomic region are associated with fasting glucose levels. J Clin Investig 118:2620–2628

CHEN WM, MANICHAIKUL A, RICH SS (2009) A generalized family-based association test for dichotomous traits. Am J Human Genet 85:364–376

CHEN WM, MANICHAIKUL A, RICH SS (2010) Testing and estimating within-family association for dichotomous traits. Genetic Epidemiol 34:959–959

CHEN WM, ALLEN EK, MYCHALECKYJ JC, CHEN F, HOU X, RICH SS, DALY KA, SALE MM, CHEN WM, ALLEN EK, MYCHALECKYJ JC, CHEN F, HOU X, RICH SS, DALY KA, SALE MM (2011) Significant linkage at chromosome 19q for otitis media with effusion and/or recurrent otitis media (COME/ROM). BMC Med Genet 12:124

COKER TR CLSNSJ ET AL (2010) Diagnosis, microbial epidemiology, and antibiotic treatment of acute otitis media in children: A systematic review. JAMA 304:2161–2169

DALY KA, HUNTER LL, LEVINE SC, LINDGREN BR, GIEBINK GS (1998) Relationships between otitis media sequelae and age. Laryngoscope 108:1306–1310

DALY KA, RICH SS, LEVINE S, MARGOLIS RH, LE CT, LINDGREN B, GIEBINK GS (1996) The family study of otitis media: design and disease and risk factor profiles. Genet Epidemiol 13:451–468

DALY KA, HOFFMAN HJ, KVAERNER KJ, KVESTAD E, CASSELBRANT ML, HOMOE P, ROVERS MM (2010) Epidemiology, natural history, and risk factors: panel report from the Ninth International Research Conference on Otitis Media. Int J Pediatr Otorhinolaryngol 74:231–240

DALY KA, BROWN WM, SEGADE F, BOWDEN DW, KEATS BJ, LINDGREN BR, LEVINE SC, RICH SS (2004) Chronic and recurrent otitis media: a genome scan for susceptibility loci. Am J Human Genet 75:988–997

DAMOISEAUX RA, ROVERS MM, VAN BALEN FA, HOES AW, DE MELKER RA (2006) Long-term prognosis of acute otitis media in infancy: determinants of recurrent acute otitis media and persistent middle ear effusion. Fam Pract 23:40–45

DE MELKER RA, BURKE PD (1988) Epidemiology of otitis media and the role of the general practitioner in management. Fam Pract 5:307–313

DUAN S, HUANG RS, ZHANG W, BLEIBEL WK, ROE CA, CLARK TA, CHEN TX, SCHWEITZER AC, BLUME JE, COX NJ, DOLAN ME (2008) Genetic architecture of transcript-level variation in humans. Am J Hum Genet 82:1101–1113

DUGGAL P, GILLANDERS EM, HOLMES TN, BAILEY-WILSON JE (2008) Establishing an adjusted *p*-value threshold to control the family-wide type 1 error in genome wide association studies. BMC Genomics 9:516

GAMAZON ER, ZHANG W, KONKASHBAEV A, DUAN S, KISTNER EO, NICOLAE DL, DOLAN ME, COX NJ (2010) SCAN: SNP and copy number annotation. Bioinformatics 26:259–262

GATES GA (1996) Cost-effectiveness considerations in otitis media treatment. Otolaryngol - Head and Neck Surg 114:525–530

GREENBERG D, BILENKO N, LISS Z, SHAGAN T, ZAMIR O, DAGAN R (2003) The burden of acute otitis media on the patient and the family. Eur J Pediatr 162:576–581

GREVERS G (2010) Challenges in reducing the burden of otitis media disease: an ENT perspective on improving management and prospects for prevention. Int J Pediatr Otorhinolaryngol 74:572–577

GUNASEKERA H, HAYSON L, MORRIS P, CRAIG J (2007) The global burden of childhood otitis media and hearing impairment: a systematic review. In: 9th International Symposium on Recent Advances in Otitis. St. Petersburg, FL

HARSTEN G, PRELLNER K, HELDRUP J, KALM O, KORNFALT R (1989) Recurrent acute otitis media. A prospective study of children during the first three years of life. Acta Otolaryngol 107:111–119

HSU SH, ZHANG X, YU C, LI ZJ, WUNDER JS, HUI CC, ALMAN BA (2011) Kif7 promotes hedgehog signaling in growth plate chondrocytes by restricting the inhibitory function of Sufu. Development 138:3791–3801

INGHAM PW, MCMAHON AP (2009) Hedgehog signalling: Kif7 is not that fishy after all. Curr Biol 19:R729–731

JIA L, LANDAN G, POMERANTZ M, JASCHEK R, HERMAN P, REICH D, YAN C, KHALID O, KANTOFF P, OH W, MANAK JR, BERMAN BP, HENDERSON BE, FRENKEL B, HAIMAN CA, FREEDMAN M, TANAY A, COETZEE GA (2009) Functional enhancers at the gene-poor 8q24 cancer-linked locus. PLoS Genet 5:e1000597

JOHNSTON LC, FELDMAN HM, PARADISE JL, BERNARD BS, COLBORN DK, CASSELBRANT ML, JANOSKY JE (2004) Tympanic membrane abnormalities and hearing levels at the ages of 5 and 6 years in relation to persistent otitis media and tympanostomy tube insertion in the first 3 years of life: a prospective study incorporating a randomized clinical trial. Pediatrics 114:e58–67

JURINKE C, VAN DEN BOOM D, CANTOR CR, KOSTER H (2002) Automated genotyping using the DNA MassArray technology. Methods Mol Biol 187:179–192

KATHIRESAN S, MANNING AK, DEMISSIE S, D'AGOSTINO RB, SURTI A, GUIDUCCI C, GIANNINY L, BURTT NP, MELANDER O, ORHO-MELANDER M, ARNETT DK, PELOSO GM, ORDOVAS JM, CUPPLES LA (2007) A genome-wide association study for blood lipid phenotypes in the Framingham Heart Study. BMC Med Genet 8(Suppl 1):S17

KIM H, WHITE CD, SACKS DB (2011) IQGAP1 in microbial pathogenesis: targeting the actin cytoskeleton. FEBS Lett 585:723–729

KIM JH, CHA JY, CHEONG HS, PARK JS, JANG AS, UH ST, KIM MK, CHOI IS, CHO SH, PARK BL, BAE JS, PARK CS, SHIN HD (2010) KIF3A, a cilia structural gene on chromosome 5q31, and its polymorphisms show an association with aspirin hypersensitivity in asthma. J Clin Immunol. 31:112-121

KUMAGAI A, SHEVCHENKO A, DUNPHY WG (2010) Treslin collaborates with TopBP1 in triggering the initiation of DNA replication. Cell 140:349–359

KVAERNER KJ, TAMBS K, HARRIS JR, MAGNUS P (1997) Distribution and heritability of recurrent ear infections. Ann Otol Rhinol Laryngol 106:624–632

KVESTAD E, KVAERNER KJ, ROYSAMB E, TAMBS K, HARRIS JR, MAGNUS P (2004) Otitis media: genetic factors and sex differences. Twin Res 7:239–244

KVESTAD E, KVAERNER KJ, ROYSAMB E, TAMBS K, HARRIS JR, MAGNUS P (2006) Recurrent otitis media and tonsillitis: common disease predisposition. Int J Pediatr Otorhinolaryngol 70:1561–1568

LAIRD NM, LANGE C (2006) Family-based designs in the age of large-scale gene-association studies. Nature reviews Genetics 7:385–394

LI X, HOWARD TD, MOORE WC, AMPLEFORD EJ, LI H, BUSSE WW, CALHOUN WJ, CASTRO M, CHUNG KF, ERZURUM SC, FITZPATRICK AM, GASTON B, ISRAEL E, JARJOUR NN, TEAGUE WG, WENZEL SE, PETERS SP, HAWKINS GA, BLEECKER ER, MEYERS DA (2011) IMPORTANCE OF HEDGEHOG INTERACTING PROTEIN AND OTHER LUNG FUNCTION GENES IN ASTHMA. J ALLERGY CLIN IMMUNOL 127(6):1457-65

LI Y, WILLER C, SANNA S, ABECASIS G (2009) Genotype imputation. Annu Rev Genomics Hum Genet 10:387–406

LI Y, WILLER CJ, DING J, SCHEET P, ABECASIS GR (2010) MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol 34:816-834

LILLEY CE, SCHWARTZ RA, WEITZMAN MD (2007) Using or abusing: viruses and the cellular DNA damage response. Trends Microbiol 15:119–126

LIM KH, FAIRBROTHER WG (2012) Spliceman–a computational web server that predicts sequence variations in pre-mRNA splicing. Bioinformatics 28:1031–1032

MANICHAIKUL A, MYCHALECKYJ JC, RICH SS, DALY K, SALE M, CHEN WM (2010) Robust relationship inference in genome-wide association studies. Bioinformatics 26:2867–2873

MASSA HM, CRIPPS AW, LEHMANN D (2009) Otitis media: viruses, bacteria, biofilms and vaccines. The Med J Aust 191:S44–49

MONTGOMERY SB, SAMMETH M, GUTIERREZ-ARCELUS M, LACH RP, INGLE C, NISBETT J, GUIGO R, DERMITZAKIS ET (2010) Transcriptome genetics using second generation sequencing in a Caucasian population. Nature 464:773–777

OROSZ F, OVÁDI J (2008) TPPP orthologs are ciliary proteins. FEBS Lett 582:3757–3764

OVÁDI J, OROSZ F (2009) An unstructured protein with destructive potential: TPPP/p25 in neurodegeneration. BioEssays 31:676–686

POEHLING KA, SZILAGYI PG, GRIJALVA CG, MARTIN SW, LAFLEUR B, MITCHEL E, BARTH RD, NUORTI JP, GRIFFIN MR (2007) Reduction of frequent otitis media and pressure-equalizing tube insertions in children after introduction of pneumococcal conjugate vaccine. Pediatrics 119:707–715

PRUIM RJ, WELCH RP, SANNA S, TESLOVICH TM, CHINES PS, GLIEDT TP, BOEHNKE M, ABECASIS GR, WILLER CJ (2010) LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics 26:2336–2337

ROSENBLOOM KR ET AL (2012) ENCODE whole-genome data in the UCSC Genome Browser: update 2012. Nucleic Acids Res 40:D912–D917

ROVERS M, HAGGARD M, GANNON M, KOEPPEN-SCHOMERUS G, PLOMIN R (2002) Heritability of symptom domains in otitis media: a longitudinal study of 1,373 twin pairs. Am J Epidemiol 155:958–964

RYE MS, WARRINGTON NM, SCAMAN ESH, VIJAYASEKARAN S, COATES HL, ANDERSON D, PENNELL CE, BLACKWELL JM, JAMIESON SE (2012) Genome-wide association study to identify the genetic determinants of otitis media susceptibility in childhood. PloS one 7:e48215

RYE MS, WIERTSEMA SP, SCAMAN ES, OOMMEN J, SUN W, FRANCIS RW, ANG W, PENNELL CE, BURGNER D, RICHMOND P, VIJAYASEKARAN S, COATES HL, BROWN SD, BLACKWELL JM, JAMIESON SE (2011) FBXO11, a regulator of the TGFbeta pathway, is associated with severe otitis media in Western Australian children. Genes and Immunity 12:352–359

SCHRAFF SA (2008) Contemporary indications for ventilation tube placement. Curr Opin Otolaryngol Head Neck Surg 16:406–411

SEGADE F, DALY KA, ALLRED D, HICKS PJ, COX M, BROWN M, HARDISTY-HUGHES RE, BROWN SD, RICH SS, BOWDEN DW (2006) Association of the FBXO11 gene with chronic otitis media with effusion and recurrent otitis media: the Minnesota COME/ROM Family Study. Arch of Otolaryngol-Head Neck Surg 132:729–733

SKOL AD, SCOTT LJ, ABECASIS GR, BOEHNKE M (2006) Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat Genet 38:209–213

STRANGER BE, MONTGOMERY SB, DIMAS AS, PARTS L, STEGLE O, INGLE CE, SEKOWSKA M, SMITH GD, EVANS D, GUTIERREZ-ARCELUS M, PRICE A, RAJ T, NISBETT J, NICA AC, BEAZLEY C, DURBIN R, DELOUKAS P, DERMITZAKIS ET (2012) Patterns of cis regulatory variation in diverse human populations. PLoS Genet 8:e1002639

TATEOSSIAN H, HARDISTY-HUGHES RE, MORSE S, ROMERO MR, HILTON H, DEAN C, BROWN SD (2009) Regulation of TGF-beta signalling by Fbxo11, the gene mutated in the Jeff otitis media mouse mutant. PathoGenet 2:5

TATEOSSIAN H, MORSE S, PARKER A, MBURU P, WARR N, ACEVEDO-AROZENA A, CHEESEMAN M, WELLS S, BROWN SD (2013) Otitis media in the Tgif knockout mouse implicates TGFbeta signalling in chronic middle ear inflammatory disease. Hum Mol Genet 22:2553-2565

TEELE DW, KLEIN JO, ROSNER BA (1980) Epidemiology of otitis media in children. Ann Otol Rhinol Laryngol Suppl 89:5–6

TEELE DW, KLEIN JO, ROSNER B (1989) Epidemiology of otitis media during the first seven years of life in children in greater Boston: a prospective, cohort study. J Infect Dis 160:83–94

THE ENCODE PROJECT CONSORTIUM (2004) The ENCODE (ENCyclopedia Of DNA Elements) Project. Science 306:636–640

THORNTON T, MCPEEK MS (2007) Case–control association testing with related individuals: a more powerful quasi-likelihood score test. Am J Human Genet 81:321–337

TOLL EC, NUNEZ DA (2012) Diagnosis and treatment of acute otitis media: review. Laryngol Otol 126:976–983

WANG K, LI M, HADLEY D, LIU R, GLESSNER J, GRANT SF, HAKONARSON H, BUCAN M (2007) PennCNV: an integrated hidden Markov model designed for high-resolution copy number variation detection in whole-genome SNP genotyping data. Genome Res 17:1665–1674

WARD LD, KELLIS M (2012) HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res 40:D930–934

WILLER CJ, LI Y, ABECASIS GR (2010) METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics 26:2190–2191

YAMAOKA-TOJO M, USHIO-FUKAI M, HILENSKI L, DIKALOV SI, CHEN YE, TOJO T, FUKAI T, FUJIMOTO M, PATRUSHEV NA, WANG N, KONTOS CD, BLOOM GS, ALEXANDER RW (2004) IQGAP1, a novel vascular endothelial growth factor receptor binding protein, is involved in reactive oxygen species-dependent endothelial migration and proliferation. Circ Res 95:276–283

YANG T-P, BEAZLEY C, MONTGOMERY SB, DIMAS AS, GUTIERREZ-ARCELUS M, STRANGER BE, DELOUKAS P, DERMITZAKIS ET (2010) Genevar: a database and Java application for the analysis and visualization of SNP-gene associations in eQTL studies. Bioinformatics 26:2474–2476

YAO X, REMALEY AT, LEVINE SJ (2011) New kids on the block apolipoprotein mimetics for asthma treatment the emerging role of apolipoproteins in the pathogenesis and treatment of asthma. Chest 140:1048–1054