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Genetic Polymorphisms of Functional Candidate Genes and Recurrent Acute Otitis Media With or Without Tympanic Membrane Perforation

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Abstract: Evaluation of the genetic contribution to the development of recurrent acute otitis media (rAOM) remains challenging. This study aimed to evaluate the potential association between single nucleotide polymorphisms (SNPs) in selected genes and rAOM and to analyze whether genetic variations might predispose to the development of complicated recurrent cases, such as those with tympanic membrane perforation (TMP).

A total of 33 candidate genes and 47 SNPs were genotyped in 200 children with rAOM (116 with a history of TMP) and in 200 healthy controls.

INFγ rs 12369470CT was significantly less common in the children with rAOM than in healthy controls (odds ratio [OR] 0.5, 95% confidence interval [CI] 0.25–1, $P = 0.04$). Although not significant, interleukin (IL)-1β rs 1143627G and toll-like receptor (TLR)-4 rs2737191AG were less frequently detected in the children with rAOM than in controls. The opposite was true for IL-8 rs2227306CT, which was found more frequently in the children with rAOM than in healthy controls. The IL-10 rs1800896TC SNP and the IL-1α rs6746923A and AG SNPs were significantly more and less common, respectively, among children without a history of TMP than among those who suffered from this complication (OR 2.17, 95% CI 1.09–4.41, $P = 0.02$, and OR 0.42, 95% CI 0.21–0.84, $P = 0.01$).

This study is the first report suggesting an association between variants in genes encoding for factors of innate or adaptive immunity and the occurrence of rAOM with or without TMP, which confirms the role of genetics in conditioning susceptibility to AOM.

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Abbreviations: AOM = acute otitis media, BFIFA4P = BPI fold containing family A member 3 (BPIFA3) and 4P, CAPN14 = calpain 14, CI = confidence interval, DNAH5 = dynein axonemal heavy chain 5, FAS = Fas cell surface death receptor, FBXO11 = F-box protein 11, IFN = interferon, IL = interleukin, LTA = lymphotoxin α, MBL2 = mannose-binding lectin 2, MUC2 = mucin 2, MUC5AC/AB = mucin 5AC/AB, NOS2 = nitric oxide synthase 2, OME = otitis media with effusion, OR = odds ratio, PARP1 = poly(ADP-Ribose) polymerase-1, rAOM = recurrent acute otitis media, SCN1β = sodium channel voltage-gated type I beta subunit, serpine1 = serine protease inhibitors, SFTPD = surfactant protein D, SLC11A1 = proton-coupled divalent metal ion transporter member 1, SLFN5 = schlafen family member 5), SMAD2 = receptor-regulated SMAD2, SMAD4 = receptor-regulated SMAD4, SNPs = single nucleotide polymorphisms, TGF = transforming growth factor, TLRs = toll-like receptors, TMP = tympanic membrane perforation, TNF = tumor necrosis factor interferon-γ (IFN-γ), TP73 = tumor protein 73, TGFB1 = transforming growth factor β1.

INTRODUCTION

Acute otitis media (AOM) is a very common disease. More than 90% of children suffer from AOM in the first 5 years of life.[1] Moreover, in 20% to 30% of these children, AOM tends to recur, ultimately causing significant medical, social, and economic problems.[1,2] Several factors, including young age, day care attendance, and passive smoke exposure, have been associated with an increased risk of recurrent AOM (rAOM). However, it has been shown that host genetic factors significantly influence the risk of developing AOM.[3] It has been calculated that AOM susceptibility is 40% to 60% heritable,[4] although the exact mechanisms for this heritability have not been precisely described. Because AOM is an infectious disease and because the innate and adaptive immune systems play a fundamental role in the defense from infectious agents, most studies that were specifically designed to evaluate the potential role of genetics in conditioning rAOM have evaluated the association between variants of genes that encode factors of innate and adaptive immunity and susceptibility to AOM. With regard to innate immunity, genes encoding toll-like receptors (TLRs), CD14, mannose-binding lectins, and surfactants have been the most extensively studied.[5] Regarding adaptive immunity, studies have primarily focused on genes encoding various cytokines, such as interleukin-1, (IL-1), IL-6, IL-10, transforming growth factor-β (TGF-β), tumor necrosis factor α (TNF-α), and interferon-γ (IFN-γ).[5] Additionally, some genome-wide association studies of rAOM susceptibility have been performed.[6–8] A set of known genetic polymorphisms that seem

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to lead to a predisposition to AOM have been identified, and genome-wide linkage scans have suggested multiple candidate regions of the genome that could be associated with rAOM.[5,9]

However, evaluating the genetic contribution to the development of a disease such as AOM, which is multifactorial in etiology, remains challenging. Most of the data collected to date could be debated. Their relevance varies significantly from study to study and according to the ethnicity and characteristics of the children. Finally, all of these studies have evaluated rAOM as a whole, without considering that in this complex condition, complicated and uncomplicated cases are included and genetics might play a role in conditioning the different clinical pictures. This study was designed to evaluate the potential association between single nucleotide polymorphisms (SNPs) in selected genes and rAOM and to analyze whether genetic variations might predispose to the development of complicated recurrent cases, such as those with tympanic membrane perforation (TMP).

METHODS

Study Population and Recruitment

The study was carried out between November 1, 2014, and January 31, 2015, and it involved children in the age group 1 to 5 years who had a history of rAOM (defined as at least 3 episodes in the preceding 6 months or at least 4 episodes in the preceding 12 months, with the most recent episode in the previous 2–8 weeks) and were regularly followed by the Outpatient Clinic of the Pediatric High Intensive Care Unit at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy. The minimum number of episodes of AOM required to include patients in the otitis-prone group must have been diagnosed by pneumatic otoscopy at the Outpatient Clinic of the Pediatric High Intensive Care Unit and documented by medical records, with at least 2 of these episodes also supported by tympanometric findings. The exclusion criteria were all of the factors that can, per se, favor the development of AOM, including severe atopy, acquired or congenital immunodeficiency, cleft palate, a chronically ruptured eardrum, craniofacial abnormalities or obstructive adenoids, sleep apnea syndrome, or the placement of tympanostomy tubes.

Upon enrollment, the demographic characteristics and medical history of the children were systematically recorded using standardized written questionnaires, paying particular attention to the characteristics of AOM and the occurrence in each episode of TMP. Finally, a 3 mL whole blood sample was obtained for genetic studies.

As the control group, a similar number of age- and gender-matched children without rAOM was enrolled, and a blood sample was drawn for genetic analyses.

The protocol was approved by the Ethics Committee of the Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy, and written informed consent was obtained from the parents or legal guardian of each subject before enrollment.

Genetic Studies

Thirty-three candidate genes and 47 single nucleotide polymorphisms (SNPs) were selected for analysis, including genes that are involved in immune regulation, the pathogenesis of inflammation, and the regulation of cell metabolism and function. Candidate genes included TLR-4, IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IFN-γ, TNF-α1, TNF-α2, lymphotoxin α (LTA), mannose-binding lectin 2 (MBL2), transforming growth factor β1 (TGFB1), nitric oxide synthase 2 (NOS2), poly(ADP-Ribose) polymerase-1 (PARP1), proton-coupled divalent metal ion transporter member 1 (SLC11A1), calpain 14 (CAPN14), mucin 2 (MUC2), mucin 5AC/AB (MUC5AC/AB), surfactant protein D (SFTPD), tumor protein 73 (TP73), serine protease inhibitors (serpine1), receptor-regulated SMAD2 (SMAD2), receptor-regulated SMAD4 (SMAD4), sodium channel voltage-gated type I beta subunit (SCN1β), dynein axonemal heavy chain 5(DNAH5), BPI fold containing family A member 3 (BPIFA3) and 4P (BFIFA4P), Fas cell surface death receptor (FAS), CD14, F-box protein 11 (FBXO11), and schlafen family member 5 (SLFN5). These SNPs are involved in causing or determining the severity or outcome of infectious or chronic immune-mediated diseases in experimental animals or humans or were previously supposed or found to be associated with an increased risk of developing AOM or otitis media with effusion (OME) or an abnormal immune response.[5–8,10–20] The investigated genes and SNPs are listed in Table 1.

DNA was extracted using the Masterpure DNA Purification kit (Epicentre, Madison, FL) in accordance with the manufacturer's instructions, and a 50 μL final elution volume was obtained after purification. Single nucleotide polymorphisms in the 33 genes were genotyped using the Custom TaqMan Array Microfluidic Cards genotyping system on an ABI 7900HT (Applied Biosystems, Foster City, CA). After PCR amplification, the alleles were detected by means of end-point analysis using SDS and the TaqMan Genotyper software (Applied Biosystems, Foster City, CA). The data were entered into a Progeny database (Progeny Software, LLC, South Bend, IN) to generate datasets for analysis.

Statistical Analysis

The categorical data were compared between the groups using contingency table analysis with Fisher's exact test. The continuous data were analyzed using a 2-sided Wilcoxon rank-sum test after ensuring that they were not normally distributed (by means of the Shapiro–Wilk statistic).

Genotype frequencies were determined by direct counting. To investigate Hardy–Weinberg equilibrium (HWE), the expected number of each genotype was compared with the observed number, and potential deviations were assessed using Fisher's exact test. Univariate odds ratios (OR), their 95% confidence intervals (CI), and pertinent *P* values obtained by Fisher's exact test were calculated to measure the associations between selected SNPs and (1) susceptibility to AOM by comparing all the children with rAOM and controls and (2) susceptibility to rAOM without TMP with respect to rAOM and at least 1 episode with TMP. The data were controlled for multiple testing using the false discovery rate method (with the Benjamini–Hochberg procedure). All of the statistical analyses were made using the R software package, version 3.1.1, with library genetics and epitools added.

RESULTS

A total of 200 children with rAOM (129 men; median age, 31 months) and 200 healthy controls (129 men; median age, 31 months) were enrolled. Among the children with rAOM, 84 (42.0%) had never experienced TMP, and 116 had a history of rAOM with TMP. Their demographic and clinical characteristics are reported in Table 2. The 2 groups were similar for all of the studied variables. None of the healthy controls had a previous history of rAOM.

TABLE 1. Gene and Single Nucleotide Polymorphisms (SNPs)

Gene	dbSNP	HGVS Description	Functional Consequence	Position (bp)	Chr	Gene Location
SLC11A1	rs17221959	NG_012128.1:g.10879C>T	Synonymous codon	218387907	2	Exon
SLC11A1	rs17235409	NG_012128.1:g.17981G>A	Missense	218395009	2	Exon
SLC11A1	rs2276631	NG_012128.1:g.7262C>T	Synonymous codon	218384290	2	Exon
SLC11A1	rs2279014	NG_012128.1:g.19425C>T	3′UTR variant	218396453	2	Intergenic
SLC11A1	rs2279015	NG_012128.1:g.17519G>A	Intron variant	218394547	2	Intron
SLC11A1	rs2695343	NG_012128.1:g.13672A>G	Intron variant	218390700	2	Intron
SLC11A1	rs3731863	NG_012128.1:g.10457C>T	Intron variant	218387485	2	Intron
SLC11A1	rs3731865	NG_012128.1:g.8252G>C	Intron variant	218385280	2	Intron
SLC11A1	rs7576974	NG_012128.1:g.6039C>T	Synonymous codon	218383067	2	Exon
BPIFA3	rs17305657	NC_000020.11:g.33218782T>C	Intron variant	33218782	20	Intron
DNAH5	rs17265607	NG_013081.1:g.135237T>G	Intron variant	13814244	5	Intron
BPIFA4P	rs17396317	NC_000020.11:g.33202571G>A	Nc transcript variant	33202571	20	Exon
SERPING1 (C1INH)	rs4926	NG_009625.1:g.21963G>A	Missense	57614516	11	Exon
CAPN14	rs13408922	NC_000002.12:g.31221960C>A	Intron variant	31221960	2	Intron
CAPN14	rs13386745	NC_000002.12:g.31222749A>G	Intron variant	31222749	2	Intron
CAPN14	rs13386850	NC_000002.12:g.31222825A>C	Intron variant	31222825	2	Intron
NDUFA2 (CD14)	rs778591	NG_021417.1:g.6887C>T	Intron variant	140645899	5	Intron
FAS	rs12765241	NG_011541.1:g.15647C>T	Intron variant	88980744	10	Intron
FBXO11	rs330787	NG_008397.1:g.96438T>C	Intron variant	47814238	2	Intron
IFNγ	rs12369470	NC_000012.12:g.68151116T>C	Downstream variant	68151116	12	Intergenic
IL10	rs1800896	NG_012088.1:g.3943A>G	Upstream variant 2KB	206773552	1	Intergenic
IL10	rs3021094	NG_012088.1:g.5888A>C	Intron variant	206771607	1	Intron
IL1α	rs6746923	NC_000002.12:g.112795849A>G	Upstream variant	112795849	2	Intergenic
IL1β	rs1143627	NG_008851.1:g.4970C>T	Upstream variant 2KB	112836810	2	Intergenic
IL1β	rs3917368	NC_000002.12:g.112825205C>T	Downstream variant	112825205	2	Intergenic
IL4	rs243250	NC_000022.11:g.33990089C>T	Upstream variant	33990089	22	Intergenic
IL6	rs10499563	NC_000007.14:g.22720869T>C	Upstream variant	22720869	7	Intergenic
IL8	rs2227306	NG_029889.1:g.5833C>T	Intron variant	73741338	4	Intron
MBL2	rs1800450	NG_008196.1:g.5226G>A	Missense	52771475	10	Exon
MUC2	rs7934606	NC_000011.10:g.1100037C>T	Intron variant	1100037	11	Intron
MUC5AC/ MUC5B	rs6421966	NC_000011.10:g.1133071G>T	Upstream variant	1133071	11	Intergenic
NOS2	rs2297518	NG_011470.1:g.35959C>T	Missense	27769571	17	Exon
PARP1	rs1136410	NC_000001.11:g.226367601A>G	Missense	226367601	1	Exon
SCN1B	rs2278996	NG_013359.1:g.14190A>C	3′ UTR variant	35039877	19	Intergenic
SERPINE1	rs2227631	NG_013213.1:g.4160A>G	Upstream variant 2KB	101126257	7	Intergenic
SFTPD	rs1923539	NC_000010.11:g.79935194G>A	Downstream variant	79935194	10	Intergenic
SLFN5	rs1564580	NC_000017.11:g.35236775T>C	Upstream variant	35236775	17	Intergenic
SMAD2	rs4940086	NG_029946.1:g.16209A>G	Intron variant	47919936	18	Intron
SMAD4	rs12958604	NG_013013.2:g.70916A>G	Intron variant	51033955	18	Intron
TGFβ1	rs10417924	NC_000019.10:g.41327262T>C	Downstream variant	41327262	19	Intergenic
TLR4	rs4986790	NG_011475.1:g.13843A>G	Missense	117713024	9	Exon
TLR4	rs4986791	NG_011475.1:g.14143C>T	Missense	117713324	9	Exon
TLR4	rs2737191	NC_000009.12:g.117700437A>G	Upstream variant	117700437	9	Intergenic
LTA	rs2229092	NG_012010.1:g.5882A>C	Missense	31572980	6	Exon
TNFα.1	rs361525	NG_007462.1:g.4752G>A	Upstream variant 2KB	31575324	6	Intergenic
TNFα.1	rs1800629	NG_007462.1:g.4682G>A	Upstream variant 2KB	31575254	6	Intergenic
TNFα.2	rs1799724	NG_007462.1:g.4133C>T	Upstream variant 2KB	31574705	6	Intergenic
TP73	rs3765766	NG_017035.2:g.70532T>C	Intron variant	3718096	1	Intron

Bp = base pairs; chr = chromosome; HGVS = Human Genome Variation Society–Genome; SNPs = single nucleotide polymorphisms. Build 37.1 (www.ncbi.nlm.nih.gov); the position reflects the distance from the short-arm telomere.

TABLE 2. Demographic, Clinical, and Familial Characteristics of Subjects With Recurrent Acute Otitis Media (rAOM) by Disease Characteristics

Characteristics	Children With rAOM (n = 200)	rAOM Without TMP (n = 84)	rAOM With TMP (n = 116)	*P* Value
Median age (range), months	31 (10–121)	30.5 (11–73)	31 (10–121)	0.649
Males, n (%)	129 (64.5)	54 (64.3)	75 (64.7)	1
Caucasian ethnicity, n. (%)	191 (95.5)	79 (94.0)	112 (96.6)	0.96
Older siblings, n (%)	120 (60)	50 (59.5)	70 (60.3)	1
Day-care attendance, n (%)	159 (79.5)	71 (84.5)	88 (75.9)	0.157
Pacifier use, n (%)	83 (41.2)	36 (42.9)	47 (40.5)	0.772
Exposed to passive smoking, n (%)	41 (20.5)	16 (19)	25 (21.6)	0.725
History of allergy, n (%)	30 (15)	12 (14.3)	18 (15.5)	0.844
Vaccinated with 13-valent pneumococcal vaccine, n (%)	153 (76.5)	62 (73.8)	91 (78.4)	0.5
Vaccinated with influenza vaccine, n (%)	129 (64.5)	48 (57.1)	81 (69.8)	0.073
Median AOM episodes since birth (range)	6 (3–21)	6 (3–20)	6 (3–21)	0.624
Median AOM episodes in the last 6 months (range)	3 (0–8)	2.5 (0–8)	3 (0–8)	0.43
Median AOM episodes in the last 12 months (range)	5 (3–14)	5 (3–12)	5 (3–14)	0.297

AOM = acute otitis media; rAOM = recurrent acute otitis media; TMP = tympanic membrane perforation.

Table 3 lists the genotype frequencies with differences in the selected SNPs between the children with rAOM and otherwise healthy controls. As shown, INFγ rs 12369470CT was significantly less common in the children with rAOM than in healthy controls (OR 0.5, 95% CI 0.25–1, $P = 0.04$). Similarly, although not significant at the conventional 5% level, IL-1β rs 1143627G and TLR-4 rs2737191AG were less frequently detected in the children with rAOM than in controls (OR 0.57, 95% CI 0.31–1.05, $P = 0.06$; OR 0.68, 95% CI 0.43–1.05, $P = 0.07$, respectively). The opposite was observed for IL-8 rs2227306CT, which was found more frequently among the children with rAOM than in the healthy controls, although the difference was not statistically significant in this case (OR 1.52, 95% CI 0.97–2.04, $P = 0.06$). No other relevant genetic variation association was found between the children with rAOM and those without.

Table 4 summarizes the genotype frequencies with differences in the selected SNPs between the children with rAOM with TMP and those who did not experience TMP. The SNP IL-10 rs1800896TC and the IL-1α rs6746923A and AG SNPs were significantly more and less common, respectively, among children without a history of TMP than among those who suffered from this complication (OR 2.17, 95% CI 1.09–4.41, $P = 0.02$, and OR 0.42, 95% CI 0.21–0.84, $P = 0.01$, respectively). The other SNPs were similar in both groups of children with rAOM, regardless of the history of TMP.

DISCUSSION

The results of this study contribute to the knowledge about the relationship between genetics and rAOM. Together with data regarding the potential role of SNPs in conditioning rAOM, this study suggests for the first time that genetics might be implicated in the determination of AOM with TMP. Concerning rAOM, this study shows that the SNP rs 12369470CT of the IFN-γ gene is less common among children with rAOM than in healthy controls, which suggests that this genetic variant might protect children from repeated AOM episodes. Although they are not supported by a statistically significant difference at the conventional 5% level, the same conclusions could be drawn for the SNP rs 1143627G of the IL-1β gene and the SNP rs2737191AG of the TLR4 gene. Conversely, a negative effect with an increased risk of rAOM seems to be associated with the IL-8 rs2227306CT SNP, which was more common among children with rAOM than in healthy controls, although the difference between the groups did not reach statistical significance in this case. For the further study, it is suggested that increasing the number of patients with rAOM might increase the statistical power of a similar study.

The impact of polymorphisms of the IFNγ, IL-1β, and TLR4 genes has been well studied in several clinical conditions, including AOM, with conflicting results. The protective effect of the IFNγ rs 12369470CT SNP found in this study is new information that might be useful for differentiating otitis-prone children from those for whom rAOM is less likely. Gentile et al found that genetic variations of the IFNγ gene were associated with an increased frequency of AOM.[21] Ilia et al reported that the same SNP could be considered a predictor of progress to AOM following upper respiratory infection (URI).[22] By contrast, results consistent with our study were published by Alper et al, who did not find any association between this SNP and the development of AOM in children with URIs.[23]

The data describing the IL-1β rs 1143627G and TLR4 rs2737191AG SNPs merit further evaluation because the protective effect associated with these genetic variations is not substantiated by the statistical analysis and was not found in

TABLE 3. Genotype Frequencies With Differences in the Selected SNPs Between Controls and Children With Recurrent Acute Otitis Media (rAOM)

Gene and Polymorphic Alleles	Control Group (n = 200)		Children With rAOM (n = 200)		HWE, χ^2 Controls	HWE, χ^2 rAOM	Outcome		
	N	%	N	%	*P* Value	*P* Value	OR	95% CI	*P* Value*
IFNγ.rs12369470									
C	3	1.5	0	0	0.18	1	0	0–2.24	0.11
C/T	29	14.5	16	8			0.5	0.25–0.99	0.04
T	168	84	184	92			1	(reference)	
IL1β.rs1143627									
C	77	38.5	94	47	0.06	0.11	1	(reference)	
C/T	83	41.5	79	39.5			0.78	0.5–1.23	0.27
T	39	19.5	27	13.5			0.57	0.31–1.05	0.06
NA	1	0.5	0	0					
IL8.rs2227306									
C	90	45	75	37.5	0.01	0.88	1	(reference)	
C/T	74	37	94	47			1.52	0.97–2.4	0.06
T	36	18	31	15.5			1.03	0.56–1.9	1
TLR4.rs2737191									
A	88	44	104	52	0.87	0.71	1	(reference)	
A/G	89	44.5	71	35.5			0.68	0.43–1.05	0.07
G	20	10	14	7			0.59	0.26–1.32	0.19
NA	3	1.5	11	5.5					

CI = confidence intervals; HWE = Hardy–Weinberg equilibrium; NA = not available; OR = odds ratio.
* *P* values from univariate analysis, not adjusted for multiple testing. None of the *P* values was significant after adjusting for multiple testing.

previously published studies in which the same or other IL-1β SNPs were examined. Nokso-Koivisto et al studied the occurrence of AOM following URI and concluded that the presence of the IL-1β rs1143627G SNP did not increase susceptibility to AOM.[24] By contrast, the IL-1β rs1143634 SNP was associated with a higher risk of severe inflammation after AOM.[25] Similarly, conflicting data were reported for the TLR4 SNPs. The TLR4 rs4986790 and TLR4 rs49867912 SNPs, 2 of the SNPs evaluated in this study for which no association with rAOM was found, were reported to be more common in otitis-prone children by Emonts et al[13] but were considered independent from AOM by Carroll et al.[26]

The IL-8 rs2227306CT SNP seems to be associated with an increased risk of rAOM because it is more common among children with this condition than controls. Although the difference between groups did not reach statistical significance, this finding merits attention and further studies to confirm the data because IL-8 has been repeatedly reported as a factor that increases susceptibility to ear disease and chronic ear inflammation both in vitro and in vivo.[27,28]

TABLE 4. Genotype Frequencies With Differences in the Selected SNPs Between Children With Recurrent Acute Otitis Media (rAOM) With Tympanic Membrane Perforation (TMP) or rAOM Without TMP

Gene and Polymorphic Alleles	Children With rAOM and TMP (n = 116)		Children With rAOM Only (n = 84)		HWE, χ^2 Controls	HWE, χ^2 rAOM	Outcome		
	N	%	N	%	*P* Value	*P* Value	OR	95% IC	*P* Value*
IL10.rs1800896									
C	19	16.4	12	14.3	0.44	0.03	1.45	0.53–3.86	0.49
T/C	51	44	52	61.9			2.33	1.17–4.77	0.01
T	46	39.7	20	23.8			1	(reference)	
IL1α.rs6746923									
A	24	20.7	13	15.5	0.46	0.35	0.44	0.17–1.08	0.06
A/G	63	54.3	35	41.7			0.45	0.22–0.89	0.02
G	29	25	36	42.9			1	(reference)	

CI = confidence intervals; HWE = Hardy–Weinberg equilibrium; NA = not available; OR = odds ratio.
* *P* values from univariate analysis, not adjusted for multiple testing. None of the *P* values was significant after adjusting for multiple testing.

The IL-10 rs1800896TC SNP and the IL-1α rs6746923A and AG SNPs were associated with a reduced or an increased risk, respectively, of rAOM with TMP. Differences in genetic characteristics between subjects with and without TMP are not surprising because AOM complicated by TMP significantly differs from AOM without this complication in several factors. AOM with TMP is frequently caused by *Streptococcus pyogenes*, a bacterial pathogen that is not common in AOM without TMP, and it frequently has a complicated course.[29] Moreover, the administration of vitamin D [30] or influenza vaccine,[31] which can reduce the incidence of new episodes of AOM in children who have never had TMP, is not effective in patients with rAOM that is occasionally complicated by TMP. The importance of SNPs in IL-10 and IL-1α in conditioning susceptibility to respiratory infections has been reported by others. Nokso-Koivisto et al showed that the IL-10 rs1800896TC SNP was more common in subjects with a reduced risk of URI and an occurrence of AOM during URI episodes,[24] whereas Joki-Erkkila et al found that SNPs in the IL-1α gene were associated with an increased risk of rAOM.[32] Conversely, the role of IL-1α in predisposing to ear diseases is supported by the demonstration that the expression of IL-1α is higher in cases of chronic otitis media, with a strong positive correlation between the cytokine level and the degree of bone destruction.[33] However, the findings of this study extend previous knowledge and seem to indicate that genetic variants of the IL-1α genes might be associated with complicated AOM at risk of negative evolution.

In this study, several associations between variants in genes encoding for factors of innate or adaptive immunity and the occurrence of rAOM were identified, which confirmed the role of genetics in conditioning susceptibility to AOM. Moreover, for the first time, an association between genetic variants of IL-10 and IL-1α and the risk of development of rAOM complicated by TMP was indicated. However, before these data can be used in clinical practice, linkage studies and genome-wide association studies might be useful to definitively solve the problem of the real role of genetic variants in conditioning susceptibility to rAOM and in the development of complicated cases.

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