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ARTICLE

Genetic Polymorphisms in Immunoresponse Genes *TNFA*, *IL6*, *IL10*, and *TLR4* Are Associated With Recurrent Acute Otitis Media

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ABSTRACT

OBJECTIVE. Cytokines and other inflammatory mediators are involved in the pathogenesis of otitis media. We hypothesized that polymorphisms in inflammatory response genes contribute to the increased susceptibility to acute otitis media in otitis-prone children.

PATIENTS AND METHODS. DNA samples from 348 children with ≥2 acute otitis media episodes, who were participating in a randomized, controlled vaccination trial, and 463 healthy adult controls were included. Polymorphisms in *TNFA*, *IL1B*, *IL4*, *IL6*, *IL10*, *IL8*, *NOS2A*, *C1INH*, *PARP*, *TLR2*, and *TLR4* were genotyped. Genotype distributions in children with recurrent acute otitis media were compared with those in controls. Within the patient group, the number of acute otitis media episodes before vaccination and the clinical and immunologic response to pneumococcal conjugate vaccinations were analyzed.

RESULTS. The *IL6*-174 G/G genotype was overrepresented in children with acute otitis media when compared with controls. In the patient group, *TNFA* promoter genotypes −238 G/G and −376 G/G and the *TLR4* 299 A/A genotype were associated with an otitis-prone condition. Furthermore, lower specific anticapsular antibody production after complete vaccination was observed in patients with the *TNFA*-238 G/G genotype or *TNFA*-863 A allele carriage. Finally, the *IL10*-1082 A/A genotype contributed to protection from the recurrence of acute otitis media after pneumococcal vaccination.

CONCLUSIONS. Variation in innate immunoresponse genes such as *TNFA*-863A, *TNFA*-376G, *TNFA*-238G, *IL10*-1082 A, and IL6-174G alleles in the promoter sequences may result in altered cytokine production that leads to altered inflammatory responses and, hence, contributes to an otitis-prone condition.

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Key Words
genotype-phenotype correlation, human, cytokines, inflammation, otitis media

Abbreviations
AOM—acute otitis media
TNFA—tumor necrosis factor A
IL—interleukin
NOS2A—inducible nitric oxide synthase
C1INH—complement component inhibitor-1
PARP—poly(ADP-ribose) polymerase
TLR—Toll-like receptor
SNP—single-nucleotide polymorphism
OR—odds ratio
rs—reference SNP
IgG—immunoglobulin G

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ACUTE OTITIS MEDIA (AOM) is the most common bacterial infection in children. Overall, 10% to 15% of all children suffer from ≥4 AOM episodes per year, which causes a great disease burden.[1] Genetic polymorphisms in immunoresponse genes are known to influence susceptibility to and severity of infectious diseases. For example, allelic variations in *TNFA* (tumor necrosis factor A), *IL1B* (interleukin 1B), and *IL6* have been associated with meningococcal infection.[2] Although cytokines and other inflammatory mediators are also involved in the pathogenesis of otitis media, the role of polymorphisms in immunoresponse genes in recurrent AOM has been relatively unexplored thus far. Increased expression of TNF-α, IL-1β, IL-6, and IL-10 was observed during experimental otitis media in animals.[3,4] Therefore, in a common disease such as otitis media, genetic variations may lead to altered inflammatory responses and an otitis-prone condition. For instance, bacterial endotoxin is recognized by several Toll-like receptors (TLRs), which in turn stimulate TNF-α production, thus affecting numerous other pathways such as cytokine production, immunoglobulin responses, and mucin production.[5–8] Remarkably, IL-1β, IL-6, and TNF-α levels in nasopharyngeal secretions were found to be lower in children with recurrent otitis media than in healthy children.[9]

The influence of genetically determined variations on otitis media can be illustrated by twin studies, which have shown a heritability of 57% for acute ear infections and 72% for chronic ear infections.[10–14] Correlation for recurrent otitis media is higher in monozygotic twins (65%–71%) compared with dizygotic twins (25%–34%).[15] *Streptococcus pneumoniae* is an important pathogen in otitis media and is involved in at least 20% to 40% of all cases.[16–18] Hence, genetic polymorphisms that influence recurrence of otitis media may also be related to response to pneumococcal antigens.

The effect of polymorphisms may result, for instance, from altered expression levels or altered function caused by amino acid substitutions. Variations in immunoresponse genes such as *IL10*, *IL6*, and *IL4* have been associated with altered cytokine expression levels.[19–21] An altered function caused by amino acid substitutions has been reported for polymorphisms in *TLR4* and poly-(ADP-ribose) polymerase (*PARP*).[22,23] The *PARP* 762A variant was found recently to be associated with reduced activity after H_2O_2 exposure, which is known to be present in inflammation.[23] Other polymorphisms were selected in this study because of previous associations with infectious or inflammatory diseases such as the *IL8* C781T polymorphism, which was reported recently to be associated with bronchiolitis caused by the respiratory syncytial virus.[24]

In this study, we investigated whether polymorphisms in selected immunoresponse genes may contribute to the recurrence of otitis media and to clinical and immunologic response to pneumococcal vaccination.

MATERIALS AND METHODS

Participants

Patients who initially participated in a randomized, controlled study on prevention of recurrent AOM by pneumococcal vaccinations were included.[25] Children were enrolled in the study after obtaining approval of the medical ethical committee of the participating hospitals and informed consent from the parents or guardians. DNA was available from 348 white Dutch children, 1 to 7 years of age, who suffered from ≥2 AOM episodes in the preceding year. The number of AOM episodes before vaccination was based on both parental report (AOM was defined as having ≥1 of these symptoms: acute earache, new-onset otorrhea, irritability, and fever) and clinical information of the diagnosis by a physician. Children who did not have AOM episodes were not included, because they were not likely to benefit from vaccination. In the present study cohort, 122 children suffered from 2 to 3 otitis media episodes, whereas 226 children suffered from ≥4 episodes (defined as an otitis-prone condition) (Table 1).[26] Children received either pneumococcal vaccinations (n = 168) or control vaccines (ie, hepatitis A [2 years or older] or hepatitis B [younger than 2 years; n = 180 vaccines]). In the pneumococcal vaccine group, 1 dosage of 7-valent conjugate vaccine (Prevnar; Wyeth, Rochester, NY) was administered to children 2 to 7 years of age, whereas 2 dosages were given with a 1-month interval to children of 1 to 2 years of age. In both groups, this procedure was followed after 6 months by 1 dose of 23-valent polysaccharide vaccine (Pneumune, Wyeth). Childrens' progress was followed until 18 months after completion of the vaccine scheme to check for the recurrence of physician-diagnosed AOM.[25]

White Dutch adult controls (n = 463) were derived from the Dutch blood bank Sanquin after informed consent was obtained and represented healthy adult donors. No records of previous history regarding AOM were available for these adult controls; however, in the general population, ≤3.2 AOM episodes is expected in childhood.[26] Children aged 0 to 13 years had an estimated number of 120 episodes of physician-diagnosed AOM per 1000 person-years in the Netherlands in the period 1995–2003.[27] This infers that controls will have experienced, on average, fewer AOM episodes per year than the patients.

Experimental Procedures

Genotyping

Single base extension analysis was used to genotype inducible nitric oxide synthase (*NOS2A*) S608L (reference single-nucleotide polymorphism [rs] 2297518),

TABLE 1 **Characteristics of Patients With ≥2 AOM Episodes**

Characteristics	2–3 Episodes of AOM ($N = 122$)	≥4 Episodes of AOM ($N = 226$)	P[a]
Male gender, *n* (%)	84 (69)	128 (57)	.03
Age, median (minimum–maximum), y	2.42 (1.0–6.3)	2.10 (1.0–7.0)	.40
Age group (12–48 mo), *n* (%)	84 (69)	180 (80)	.03
Age at first AOM, geometric mean (SD), mo	10.1 (2.0)	7.7 (2.1)	.001
Breastfeeding for ≥3 mo, *n* (%)	57 (47)	96 (43)	.50
Tobacco smoke exposure indoors, *n* (%)	41 (34)	70 (31)	.63
Day care			
At age 12–24 mo, *n/N* (%)	22/44 (50)	42/99 (42)	.47
At age 25–48 mo, *n/N* (%)	70/78 (90)	113/127 (89)	1.00
No. of siblings			.02
Median (minimum–maximum)	1.0 (0–3)	1.0 (0–7)	
Mean	0.93	1.17	
Family history of AOM			
Parents (121 of 225), *n* (%)	79 (65)	131 (58)	.21
Siblings, *n/N* (%)	48/86 (56)	98/182 (54)	.79
Atopy, *n* (%)[b]	54 (44)	117 (52)	.22

[a] Fisher's exact test, Mann-Whitney *U* test, and *t* test were performed when appropriate.

[b] Atopy was defined as having eczema, hay fever, or recurrent wheezing or asthma.

PARP V762A (rs1136410), complement component inhibitor-1 (*C1INH*), V480M (rs4926), *IL4* C-524T (rs2243250), *IL10* G-1082A (rs1800896), *IL10* C-819T (rs3021097), *IL1B* C-31T (rs1143627), *TNFA* A-863C (rs1800630), *TNFA* T-857C (rs1799724), *TNFA* G-376A (rs3093659), *TNFA* G-308A (rs1800629), *TNFA* G-238A (rs361525), *IL6* G-174C (rs1800795), *IL8* C781T (rs2227306), *TLR4* D299G (rs4986790), and *TLR4* T399I (rs4986791) (www.ncbi.nlm.nih.gov/SNP). In short, the genomic region of interest was amplified by using polymerase chain reaction. After purification, a single base extension was performed by using a primer ending 1 nucleotide before the single-nucleotide polymorphism (SNP) location. Up to 7 SNPs were analyzed in 1 multiplex assay. A poly-T tail attached to the primer combined with the use of a Liz size marker served to distinguish SNPs in the multiplex analysis (Tables 2 and 3). The *TLR2* R753Q polymorphism (rs5743708) was determined by using Taqman analysis with primers TLR2-753F CCATTCCCCAGCGCTTCT and TLR2-753R CCAGGTAGGTCTTGGTGTTCATT and probes TLR2-753V1 VIC-AAGCTGCAGAAGAT and TLR2-753M1 FAM-AAGCTGCGGAAGAT. A subset of polymerase chain reaction samples was sequenced to confirm genotypes. All genotypes were annotated independently by 2 investigators who were blinded to the clinical data.

Antibody Measurements

In children with ≥2 AOM episodes, a blood sample was taken for immunologic assessment before and 1 month

TABLE 2 **Polymerase Chain Reaction Primer Sequences**

Pool No.	Upper 5′→3′	Lower 5′→3′	rs No.	Change
Pool 1				
NOS2A	GCAGGGCTAGGAGTAGGA	AGCCCCATATGTAAACCAA	2297518	608 S/L
PARP	CTGCCCCTGTTCTACCA	ACTGTAGGCCACCTCGAT	1136410	762V/A
Pool 2				
C1INH	CCTCCGCCATCTCTGT	GCTCGCCCTAACCTGA	4926	480 V/M
IL4	CTTGCCAAGGGCTTCCTTAT	TGGAAACTGTCCTGTCATGG	243250	C-524T
Pool 3				
IL10	TCCCCTTACCTTCTACACAC	GACCCCTACCGTCTCTATTT	1800896	G-1082A
IL1B	CTTGCCCTTCCATGAAC	TGCCTCGAAGAGGTTTG	1143627	C-31T
TNFA.1	CCCCTCCCAGTTCTAGTT	GGGACACACAAGCATCA	361525	G-238A
			1800629	G-308A
			3093659	G-376A
TNFA.2	GGAGAATGTCCAGGGCTATG	AAAATCAGGGACCCCAGAGT	1800630	A-863C
			1799724	T-857C
Pool 4				
TLR4	ATGCCCCTACTCAATCTCTCT	GCCAGCCATTTTCAAGACT	4986790	299 D/G
			4986791	399 T/I
IL6	TTGTCAAGACATGCCAAGTGCT	GCCTCAGAGACATCTCCAGTCC	1800795	G-174C
IL8	AGCTTGCCTACTATAAATAACA	CTAGCCCTTGACCTCAG	2227306	C781T
Pool 5				
IL10	TCCCCTTACCTTCTACACAC	GACCCCTACCGTCTCTATTT	3021097	C-819T

TABLE 3 **Primer Sequences for Single Base Extension Reactions**

Pool No.	Primer Sequence 5′→3′	Primer Length, Bases
Pool 1		
PrNOS2A_U	TTTTGCTCTTTCAGCATGAAGAGC	24
PrPARP_L	TTTTTTTTTTGCAGGTTGTCAAGCATTTCC	30
Pool 2		
PrIL4_U	TAAACTTGGGAGAACATTGT	20
PrC1INH_U	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCAGCAGCCCTTCCTCTTC	66
Pool 3		
PrTNFA_2863U	AGTCGAGTATGTGGACCCCC	20
PrIL10_1082L	TTTTTTTTTTTTACCTATCCCTACTTCCCC	30
PrTNFA1238U	TTTTTTTTTTTTTTTTTTTTTTAGAAGACCCCCCTCGGAATC	42
PrIL1B_L	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTCCCTCGCTGTTTTTAT	48
PrTNFA_1308L	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTAGAGGCTGAACCCCGTCC	54
PrTNFA_1376U	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCCTGCATCCTGTCTGGAA	66
PrTNFA_2857L	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTCTACATGGCCCTGTCTTC	72
Pool 4		
PrIL6_L	AATGTGACGTCCTTTAGCAT	24
PrIL8_L	TTTTTTTTTTCATAACTGACAACATTGAAC	30
PrTLR4_299U	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTTAGACTACTACCTCGATG	60
PrTLR4_399L	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGATCTAAATACTTTAGGCTG	72
Pool 5		
PrIL10_819U	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCCTTGTACAGGTGATGTAA	82

after complete vaccination. Prevaccination and postvaccination immunoglobulin G (IgG) levels to the 7 pneumococcal serotypes included in the conjugate vaccine were measured in serum by enzyme-linked immunosorbent assay as described previously.[28,29]

Statistical Analysis

Statistical analysis was performed by using SPSS 11.0 (SPSS Inc, Chicago, IL) and Stata 8 (Stata Corp, College Station, TX). Verification of Hardy-Weinberg equilibrium of genotypes was performed by using the χ^2 test (1 degree of freedom). Binomial variables were analyzed by using Pearson's χ^2 test (2 degrees of freedom) or Fisher's exact test when appropriate. Continuous variables were compared for the different genotypes by using the Mann-Whitney *U* test. When necessary, variables were log-transformed to obtain an approximately normal distribution.

A comparison of genotype frequencies was made between patients and the reference control group. In addition, genotype frequencies in children who suffered from 2 to 3 episodes per year were compared with those of patients who had ≥4 episodes after correction for gender, number of siblings, age, log-transformed age at the time of the first AOM episode, and the interaction term between the latter 2 by using binary logistic regression. Log-transformed age at the first AOM episode was included in the analysis, because an early first infection predisposes to a second AOM episode. A child who has had a first AOM episode at a younger age has had a longer period of time to develop multiple AOM episodes than a child of the same age who suffered from the first infection at a later age. Because the interaction between the age at the first AOM episode and the age of inclusion was significant, it was accounted for in the analyses. Log-transformed antibody levels were compared between individuals with different genotypes. Age, number of AOM episodes (2–3 vs ≥4), and the number of conjugate vaccinations (1 or 2) were included in the analyses that assessed the effect of the different genotypes. Only when genotypes were consistently associated with different serotype-specific antibodies was this association considered relevant.

To determine the involvement of SNPs on the occurrence of AOM after complete vaccination, negative binomial regression was used, because it allows for extra variation (overdispersion). The time of follow-up was measured from 1 month after complete vaccination to the end of the study. Effects were corrected for treatment (antipneumococcal vaccinations or hepatitis vaccinations) and the number of AOM episodes in the year preceding vaccinations (2–3 vs ≥4). *P* values of ≤.05 were considered to be statistically significant. No correction was made for multiple testing.

RESULTS

We investigated the association between variations in the genes listed in Table 2 and the occurrence of ≥2 episodes of AOM, the number of AOM episodes before vaccination, specific antipneumococcal IgG levels after vaccination, and AOM after complete vaccination, respectively. Only significant associations will be discussed. SNPs not mentioned here showed no significant associations.

Genotype distribution for all SNPs except for the *IL10* G-819A polymorphism reached Hardy-Weinberg equi-

librium in controls. To rule out technical problems, 14 individuals were typed by using the reverse and forward primer in the single base extension reaction. Results for both strands were identical. Sequencing of 13 random controls also showed identical genotypes, excluding technical errors (data not shown). Genotype distributions in controls are listed in Table 4.

IL6-174 G/G Genotype Is Associated With Susceptibility to AOM

The number of children with the *IL6*-174 C/C, *IL6*-174 C/G, and *IL6*-174 G/G genotypes in the total cohort of patients with AOM was 49 (14.1%), 156 (45%), and 142 (40.9%), respectively. The corresponding frequencies in controls were 82 (17.8%), 232 (50.4%), and 146 (31.7%), respectively. The *IL6*-174 G/G genotype was found more frequently in patients with AOM (1014) compared with controls than the C/G genotype (odds ratio [OR]: 1.45; $P = .02$) or the C/C genotype (OR: 1.64; $P = .02$).

TNFA Promoter Genotypes −238 G/G and −376 G/G and TLR4 299 D/D Genotype Are Associated With an Otitis-Prone Condition

Similar to other otitis studies, in our study, population risk factors for AOM such as a high number of siblings and low age at first AOM are related to an otitis-prone condition (Table 1).[13,30,31] Girls were overrepresented in the group of children who suffered from ≥4 AOM episodes when compared with the children who had 2 to 3 AOM episodes. Children under the age of 4 were more likely to have had ≥4 AOM episodes in the previous year than older children. The interaction of age and age at the first AOM episode was significantly associated with the AOM recurrence rate ($P < .01$). Therefore, the effect of the polymorphisms on the recurrence rate of AOM was corrected for these factors (Table 5).

The *TNFA*-238 G/G genotype was overrepresented in the otitis-prone children compared with the children with 2 to 3 AOM episodes (crude OR: 2.13; $P = .03$; adjusted OR: 2.29; $P = .03$). Because a difference was observed in the recurrence-rate distribution in children over and under the age of 4 years, these data were also analyzed separately. The association was mainly attributable to children under the age of 4 years.

The *TNFA*-376 G/G genotype was associated with the otitis-prone condition in children (crude OR: 3.10; $P = .05$; adjusted OR: 3.06; $P = .07$). No significant association was found when older and younger children were analyzed separately.

In addition, carriage of the *TLR4* 299 G allele was associated with a lower number of AOM episodes (OR: 0.5; $P = .04$). This finding, however, remained significant only after correction for confounding factors when the data for the children under the age of 4 years were

TABLE 4 **Genotype Distribution of Polymorphisms in White Dutch Controls**

SNP	Genotype Frequency, *n* (%)
NOS2a 608	
G/G	302 (65.2)
G/A	140 (30.2)
A/A	21 (4.5)
PARP 762	
T/T	300 (66.7)
T/C	140 (31.1)
C/C	10 (2.2)
C1INH 480	
G/G	256 (55.5)
G/A	176 (38.2)
A/A	29 (6.3)
TNFα-863	
C/C	330 (71.4)
C/A	117 (25.3)
A/A	15 (3.2)
TNFα-857	
C/C	387 (84.5)
C/T	66 (14.4)
T/T	5 (1.1)
TNFα-376	
G/G	447 (96.8)
G/A	15 (3.2)
A/A	—
TNFα-308	
G/G	301 (65.2)
G/A	147 (31.8)
A/A	14 (3.0)
TNFα-238	
G/G	415 (89.8)
G/A	47 (10.2)
A/A	—
IL4–524	
C/C	340 (73.8)
C/T	112 (24.3)
T/T	9 (2.0)
IL1B-31	
C/C	57 (12.5)
C/T	215 (47)
T/T	185 (40.5)
Il10–1082	
G/G	123 (26.7)
G/A	219 (47.6)
A/A	118 (25.7)
IL10–819	
C/C	284 (61.6)
C/T	145 (31.5)
T/T	32 (6.9)
IL6–174	
C/C	82 (17.8)
C/G	232 (50.4)
G/G	146 (31.7)
IL8 781	
C/C	152 (32.8)
C/T	235 (50.8)
T/T	76 (16.4)
TLR4 299	
A/A	374 (86.4)
A/G	58 (13.4)
G/G	1 (0.2)
TLR4 399	
C/C	379 (87.1)
C/T	54 (12.4)
T/T	2 (0.5)

TABLE 5 **Genotype Distribution of *TNFA* and *TLR4* Polymorphisms in Children With AOM**

SNP	Controls, *n* (%)	2–3 Episodes of AOM, *n* (%)	≥4 Episodes of AOM, *n* (%)	2–3 vs ≥4	
				Crude OR (*P*)	Adjusted OR (*P*)[a]
All patients				G/G vs G/A	
TNFA G-238A	462	120	222		
G/G	415 (89.8)	101 (84.2)	204 (91.9)	2.13 (.03)	2.29 (.03)
G/A	47 (10.2)	19 (15.8)	18 (8.1)	—	—
A/A	0 (0.0)	0 (0.0)	0 (0.0)	—	—
Patients <4 y					
G/G	—	68 (82.9)	162 (92.0)	2.38 (.03)	3.06 (.01)
G/A	—	14 (17.1)	14 (8.0)	—	—
A/A	—	0 (0.0)	0 (0.0)	—	—
Patients >4 y					
G/G	—	33 (86.8)	42 (92.3)	1.59 (.51)	1.41 (.66)
G/A	—	5 (13.2)	4 (8.7)	—	—
A/A	—	0 (0.0)	0 (0.0)	—	—
All patients				G/G vs G/A and A/A	
TNFA G-376A	462	120	222		
G/G	447 (96.8)	112 (93.3)	217 (97.7)	3.10 (.05)	3.06 (.07)
G/A	15 (3.2)	8 (6.7)	4 (1.8)	—	—
A/A	0 (0.0)	0 (0.0)	1 (0.5)	—	—
Patients <4 y					
G/G	—	77 (93.9)	172 (97.7)	2.79 (.13)	3.05 (.12)
G/A	—	5 (6.1)	3 (1.7)	—	—
A/A	—	0 (0.0)	1 (0.6)	—	—
Patients >4 y					
G/G	—	35 (92.1)	45 (97.8)	3.85 (.25)	2.74 (.44)
G/A	—	3 (7.9)	1 (2.2)	—	—
A/A	—	0 (0.0)	0 (0.0)	—	—
All patients				D/D vs D/G and G/G	
TLR4 299, D/G	433	121	216		
D/D	374 (86.4)	99 (81.8)	194 (89.8)	1.96 (.04)	1.66 (.14)
D/G	58 (13.4)	20 (16.5)	22 (10.2)	—	—
G/G	1 (0.2)	2 (1.7)	0 (0.0)	—	—
Patients <4 y					
D/D	—	67 (80.7)	157 (91.3)	2.50 (.02)	2.49 (.03)
D/G	—	14 (16.9)	15 (8.7)	—	—
G/G	—	2 (2.4)	0 (0.0)	—	—
Patients >4 y					
D/D	—	32 (84.2)	37 (84.1)	0.99 (.99)	0.65 (.54)
D/G	—	6 (15.8)	7 (15.9)	—	—
G/G	—	0 (0.0)	0 (0.0)	—	—

Data are from the crude and adjusted logistic regression analysis.

[a] Adjusted OR indicates the effect of SNP adjusted for gender, number of siblings, age, log (age at first AOM episode), and the interaction of the latter 2.

analyzed separately (crude OR: 2.50; $P = .02$; adjusted OR: 2.49; $P = .03$).

TNFA-863 C/C Genotype and Carriage of the *TNFA*-238 A Allele Are Associated With Higher Specific Antipneumococcal IgG Levels After Complete Vaccination

IgG antipneumococcal antibody levels were evaluated in 80 children who were in the pneumococcal vaccine group: 34 were aged 12 to 24 months and 46 were aged 25 to 84 months. Four or more AOM episodes in the year preceding inclusion were observed in 36 children, whereas 44 had 2 to 3 episodes of AOM. Before vaccination, all IgG antipneumococcal antibody levels were low for patients with 2 to 3 and ≥4 AOM episodes (Table 6). Serum IgG antipneumococcal antibody levels against all serotypes were lower in the children with an AOM recurrence rate ≥4 compared with those with only 2 to 3 AOM episodes after complete vaccination. This was significant for all except the anti–serotype 6B and 23F antibody levels. Age, independent from the number of AOM episodes (2–3 or ≥4), had a significant effect on specific IgG levels against serotype 14 at baseline, and on anti–serotype 4 antibody levels after vaccination. By using a logistic regression model adjusting for age, number of AOM episodes, and number of conjugate vaccinations, a significantly lower antipneumococcal serotype 23F antibody level was observed for the carriers of the *TNFA*-863 A allele compared with children homozygous for the C allele ($P < .001$). Furthermore, a trend for lower specific IgG levels was observed against 5 of 7

vaccine serotypes (P = .05–.10) in the carriers of the 863A allele. The *TNFA*-238 A allele carriers had higher geometric mean specific IgG levels against all serotypes (Table 7). This finding, however, was only significant for antibodies against pneumococcal serotypes 18C and 19F without correction for confounding factors (P = .04 and .04, respectively).

No significant differences in specific IgG levels were observed for the *IL6*-174 G/C and the *IL10*-1082 G/A and *IL10*-819 C/T genotypes (data not shown).

IL10-1082 A/A Genotype Is Associated With Protection From AOM Recurrence After Vaccination

Previously, we have reported that the number of AOM episodes in the per-protocol analysis was higher in the pneumococcal vaccine group than in the control vaccine group, particularly among children who suffered ≥4 AOM episodes in the year preceding inclusion.[25] We corrected for this feature to assess whether the polymorphisms were correlated to the number of AOM episodes after complete vaccination. Patient characteristics did not differ between the pneumococcal and control vaccine group.[25] We observed that the *IL10*-1082 A/A genotype protects patients from AOM recurrence during follow-up after vaccination (incidence rate ratio: 0.63; P = .01).

No significant differences were observed for polymorphisms in *TNFA* or *IL6* (data not shown).

DISCUSSION

In this study, we found an association between *TNFA* promoter polymorphisms and otitis-prone condition and specific IgG production after pneumococcal vaccination. The *TNFA*-238 G/G genotype was associated with the otitis-prone condition and a trend for lower specific antipneumococcal antibody levels after vaccinations compared with carriers of the A allele. The *TNFA*-863 A allele was also associated with a trend for lower specific antipneumococcal IgG levels compared with children with the *TNFA*-863 C/C genotype. The *TNFA*-376 G/G genotype was also associated with an otitis-prone condition. Similar to Joki-Ekkila et al[32], we found no association between the *TNFA*-308 promoter polymorphism and recurrent AOM. In a recent study, an association was reported between carriage of the rare *TNFA*-308 allele and susceptibility to AOM. The allele frequencies of the *TNFA*-308 polymorphism, however, differed from what is usually reported for this polymorphism.[33] Differences in the observed associations between the *TNFA* promoter polymorphisms may, indeed, result from differences in allele frequency in the population and the effect of haplotypes. This finding may be specifically true for rare polymorphisms such as the *TNFA*-376 G/A polymorphism. For our study, however, the limited number of patients prohibited haplotype analysis.

TNF-α levels in nasopharyngeal secretions are decreased in children with recurrent otitis media compared with healthy children.[9] TNF-α stimulates Ig and mucin production, and low TNF-α concentrations may compromise these defense mechanisms.[7] The association between various *TNFA* polymorphisms and otitis media parameters found in our study may indicate that, indeed, there is a role for these polymorphisms in TNF-α production in vivo. In the recent past, numerous studies have been performed to investigate the association of *TNFA* promoter polymorphisms and TNF-α levels in different inflammatory and infectious diseases, and contradictory results have been reported[34]. TNF-α expression is probably not determined by one polymorphism but, rather, by a combination of polymorphisms in *TNFA* and *TNFA*-associated genes. Different pathogens may induce a variety of cytokine responses and, because numerous pathogens, both bacterial and viral, are known to cause otitis media, unraveling the role of polymorphisms on TNF-α production in the human setting is very difficult. Because TNF-α levels are expected to change during the course of infection, the timing of sampling is likely to be a very important determinant of the results.

In addition, the *IL6*-174 G/G promoter genotype was found more frequently in patients with AOM than in healthy adult controls in our study. Our findings support the findings of others (eg, the study by Nieters et al[35])

TABLE 6 **Geometric Mean (95% Confidence Interval) of Specific Antipneumococcal IgG in Children With 2 to 3 AOM Episodes and Children With ≥4 AOM Episodes Before Vaccination and 1 Month After Complete Vaccination**

Total IgG Against Capsular Type	Before Vaccination		P[a]	1 mo After Complete Vaccination		P[a]
	2–3 AOM Episodes (n = 36)	≥4 AOM Episodes (n = 44)		2–3 AOM Episodes (n = 36)	≥4 AOM Episodes (n = 44)	
4	0.05 (0.04–0.07)	0.05 (0.04–0.06)	.61	5.91 (4.6–7.6)	3.55 (2.6–4.8)	<.01
6B	0.04 (0.03–0.05)	0.04 (0.04–0.05)	.74	1.39 (0.7–2.7)	0.75 (0.4–1.4)	.20
9V	0.22 (0.16–0.31)	0.18 (0.13–0.25)	.37	43.48 (31–61)	20.18 (14–30)	<.01
14	2.20 (1.27–3.82)	1.17 (0.84–1.61)	.05	117.84 (85–164)	59.94 (47–77)	.001
18C	0.27 (0.18–2.50)	0.20 (0,14–0.28)	.29	12.80 (9.9–17)	7.79 (6.0–10)	<.01
19F	0.43 (0.26–0.71)	0.23 (0.18–0.30)	.02	25.81 (18–36)	8.16 (5–12)	<.001
23F	0.53 (0.40–0.71)	0.57 (0.43–0.75)	.65	4.91 (3.6–6.7)	3.28 (2.3–4.8)	.12

[a] P values were corrected for age by using regression analysis.

TABLE 7 **Geometric Mean (95% Confidence Interval) of Specific Antipneumococcal Antibodies 1 Month After Complete Vaccination for Different *TNFA*-863 and *TNFA*-238 Genotypes**

SNP	IgG Against Pneumococcal Capsular Type						
	4	6B	9V	14	18C	19F	23F
TNFA-863							
C/C ($n = 54$)	5.00 (3.9–6.4)	1.27 (0.8–2.1)	33.29 (24–45)	90.11 (69–118)	10.12 (8.0–13)	16.00 (11–23)	5.22 (4.0–6.8)
C/A and A/A ($n = 26$)	3.52 (2.3–5.3)	0.60 (0.3–1.4)	20.66 (12–36)	65.52 (47–92)	8.99 (6.4–13)	9.93 (6.0–17)	2.19 (1.4–3.5)
P (crude)	.12	.10	.10	.16	.80	.13	.001
P (adjusted)[a]	.05	.10	.06	.10	.42	.05	<.001
TNFA-238							
G/G ($n = 66$)	4.53 (3.7–5.5)	0.93 (0.6–1.5)	27.36 (21–36)	76.22 (61–95)	8.87 (7.4–11)	11.86 (8.6–16)	3.74 (2.9–4.9)
G/A ($n = 12$)	4.17 (1.6–11)	1.89 (0.5–6.7)	34.88 (9.7–125)	125.04 (61–257)	15.54 (6.9–35)	27.83 (12–67)	5.03 (2.1–12)
P (crude)	.78	.23	.54	.10	.04	.04	.41
P (adjusted)[a]	.70	.46	.77	.21	.06	.07	.39

Data are from the crude and adjusted logistic regression analysis.

[a] *P* (adjusted) indicates the effect of SNP adjusted for age, AOM rate (2–3 vs ≥4), and number of conjugate vaccinations (1 or 2).

who found homozygosity of the *IL6*-174C allele to be associated with a lower frequency of reported common colds. Common colds are known to predispose for recurrent otitis media. In addition, Patel et al[33] reported *IL6*-174 G allele carriage to be increased in otitis-media-susceptible children. The *IL6* G/G genotype was shown previously to be associated with high IL-6 levels compared with the C/C genotype[21]. This association, however, is not consistent, and a more complex regulation of IL-6 production that depends on multiple polymorphisms in the *IL6* promoter region seems to play a role.[36,37] Furthermore, IL-6 expression is influenced by TNF-α, and the interaction of polymorphisms in these and other genes may codetermine the phenotype.

Carriage of the *TLR4* 299 G allele was associated with a lower number of AOM episodes but was significant only in children younger than 4 years after corrections for confounding factors. Possibly, a low G allele frequency in our population (10% in the group with 2–3 AOM episodes versus 5% in the otitis-prone group) hampered identification of an association. TLRs recognize microbial patterns in a specific way. It is thought that, depending on the microorganism involved, a combination of TLRs is triggered, which determines the direction of the immune response.[38] Detailed information on the causative pathogens in each disease episode is needed to elucidate the precise role of TLRs and their genetic variation in AOM and other diseases. Unfortunately, these data are not available in our study.

The *IL10*-1082 A/A genotype was associated with protection from AOM after vaccination. The *IL10* promoter haplotype, which includes the *IL10*-1082 A/A genotype, is associated with low IL-10 production.[19,39,40] For the *IL10*-819 C/C genotype, which is in the same haplotype, a similar trend was observed (data not shown). In IL-10-deficient mice immunized with nonvirulent unencapsulated *Streptococcus pneumoniae* (strain R36A), elevated induction of proinflammatory cytokines was observed, which supports the hypothesis that low IL-10 producers confer better response after vaccination. Antibody titers against pneumococcal proteins were increased compared with those in wild-type mice.[41] Although no association was observed between the *IL10* polymorphism and specific antibody levels in our population, one might expect a similar effect. Possibly, the concentration of IL-10 in low producers is sufficient to preclude finding differences in antibody titers.

Because otitis media is a multifactorial disease, the effect of each polymorphism on its own is expected to be limited. In addition, we are aware that most of these associations are expected to lose significance after correction for multiple testing. However, because no consensus has been reached on what method to use to correct for multiple testing in genetic-association studies, we felt it most appropriate to provide *P* values as they are.

The associations in our study were attributable mainly to children younger than 4 years. Several factors may explain this finding. First, a selection for children suffering from ≥2 episodes of otitis media for the vaccination trial may have resulted in a biased group in the older children, including those children with the highest recurrence rates or ongoing infection. Second, the immune system and anatomy, like the Eustachian tube, of young children are both still developing and differ from older children and adults, which likely results in a more prominent role for innate immunity at younger age. In contrast to previous studies, our study only included patients with recurrent AOM and no age-matched controls without otitis were included.[30,42]

CONCLUSIONS

We have shown that several polymorphic variants in immunoresponse genes (ie, IL6-174 G/C, TNFA-863

A/C, TNF-376 G/A, TNFA-238 G/A, TLR4 D/G, and IL10-1082 G/A) are suggested to have a potential influence on middle-ear infections. Because the various genotypes are expected to interact with each other and numerous environmental and host factors, additional functional and genetic studies are warranted to elucidate their individual contributions to the recurrence of AOM.

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REFERENCES

1. Alho OP, Koivu M, Sorri M. What is an "otitis-prone" child? *Int J Pediatr Otorhinolaryngol.* 1991;21:201–209
2. Emonts M, Hazelzet JA, de Groot R, Hermans PW. Host genetic determinants of *Neisseria meningitidis* infections. *Lancet Infect Dis.* 2003;3:565–577
3. Long JP, Tong HH, Shannon PA, DeMaria TF. Differential expression of cytokine genes and inducible nitric oxide synthase induced by opacity phenotype variants of *Streptococcus pneumoniae* during acute otitis media in the rat. *Infect Immun.* 2003;71:5531–5540
4. Melhus A, Ryan AF. Expression of cytokine genes during pneumococcal and nontypeable *Haemophilus influenzae* acute otitis media in the rat. *Infect Immun.* 2000;68:4024–4031
5. Kohase M, Henriksen-DeStefano D, May LT, Vilcek J, Sehgal PB. Induction of beta 2-interferon by tumor necrosis factor: a homeostatic mechanism in the control of cell proliferation. *Cell.* 1986;45:659–666
6. Dinarello CA, Cannon JG, Wolff SM, et al. Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J Exp Med.* 1986;163:1433–1450
7. Lin J, Kim Y, Juhn SK. Increase of mucous glycoprotein secretion by tumor necrosis factor alpha via a protein kinase C-dependent mechanism in cultured chinchilla middle ear epithelial cells. *Ann Otol Rhinol Laryngol.* 1998;107:213–219
8. Kehrl JH, Miller A, Fauci AS. Effect of tumor necrosis factor alpha on mitogen-activated human B cells. *J Exp Med.* 1987;166:786–791
9. Lindberg K, Rynnel-Dagoo B, Sundqvist KG. Cytokines in nasopharyngeal secretions: evidence for defective IL-1 beta production in children with recurrent episodes of acute otitis media. *Clin Exp Immunol.* 1994;97:396–402
10. Casselbrant ML, Mandel EM. Genetic susceptibility to otitis media. *Curr Opin Allergy Clin Immunol.* 2005;5:1–4
11. Casselbrant ML, Mandel EM, Fall PA, et al. The heritability of otitis media: a twin and triplet study. *JAMA.* 1999;282:2167–2169
12. Casselbrant ML, Mandel EM, Rockette HE, et al. The genetic component of middle ear disease in the first 5 years of life. *Arch Otolaryngol Head Neck Surg.* 2004;130:273–278
13. Kvaerner KJ, Tambs K, Harris JR, Magnus P. Distribution and heritability of recurrent ear infections. *Ann Otol Rhinol Laryngol.* 1997;106:624–632
14. Rovers M, Haggard M, Gannon M, Koeppen-Schomerus G, Plomin R. Heritability of symptom domains in otitis media: a longitudinal study of 1,373 twin pairs. *Am J Epidemiol.* 2002;155:958–964
15. Kvestad E, Kvaerner KJ, Roysamb E, Tambs K, Harris JR, Magnus P. Otitis media: genetic factors and sex differences. *Twin Res.* 2004;7:239–244
16. Bluestone CD, Stephenson JS, Martin LM. Ten-year review of otitis media pathogens. *Pediatr Infect Dis J.* 1992;11(suppl 8):7–11
17. Del Beccaro MA, Mendelman PM, Inglis AF, et al. Bacteriology of acute otitis media: a new perspective. *J Pediatr.* 1992;120:81–84
18. Kilpi T, Herva E, Kaijalainen T, Syrjanen R, Takala AK. Bacteriology of acute otitis media in a cohort of Finnish children followed for the first two years of life. *Pediatr Infect Dis J.* 2001;20:654–662
19. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet.* 1997;24:1–8
20. Rosenwasser LJ, Borish L. Genetics of atopy and asthma: the rationale behind promoter-based candidate gene studies (IL-4 and IL-10). *Am J Respir Crit Care Med.* 1997;156(suppl 4):152–155
21. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest.* 1998;102:1369–1376
22. Arbour NC, Lorenz E, Schutte BC, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet.* 2000;25:187–191
23. Lockett KL, Hall MC, Xu J, et al. The ADPRT V762A genetic variant contributes to prostate cancer susceptibility and deficient enzyme function. *Cancer Res.* 2004;64:6344–6348
24. Hull J, Ackerman H, Isles K, et al. Unusual haplotypic structure of IL8, a susceptibility locus for a common respiratory virus. *Am J Hum Genet.* 2001;69:413–419
25. Veenhoven R, Bogaert D, Uiterwaal C, et al. Effect of conjugate pneumococcal vaccine followed by polysaccharide pneumococcal vaccine on recurrent acute otitis media: a randomised study. *Lancet.* 2003;361:2189–2195
26. Faden H, Duffy L, Boeve M. Otitis media: back to basics. *Pediatr Infect Dis J.* 1998;17:1105, 1113
27. Plasschaert AI, Rovers MM, Schilder AG, Verheij TJ, Hak E. Trends in doctor consultations, antibiotic prescription, and specialist referrals for otitis media in children: 1995–2003. *Pediatrics.* 2006;11:1879–1886
28. Veenhoven R, van Kempen MJ, Wiertsema SP, et al. Immunogenicity of combined pneumococcal conjugate and polysaccharide vaccination in children with recurrent AOM. Presented at: 5th Extraordinary International Symposium on Recent Advances in Otitis Media, OM2005; April 24–27, 2005; Amsterdam, Netherlands
29. Musher DM, Luchi MJ, Watson DA, Hamilton R, Baughn RE. Pneumococcal polysaccharide vaccine in young adults and older bronchitics: determination of IgG responses by ELISA and the effect of adsorption of serum with non-type-specific cell wall polysaccharide. *J Infect Dis.* 1990;161:728–735
30. Harsten G, Prellner K, Heldrup J, Kalm O, Kornfalt R. Recurrent acute otitis media: a prospective study of children during the first three years of life. *Acta Otolaryngol.* 1989;107:111–119
31. Rovers MM, Schilder AG, Zielhuis GA, Rosenfeld RM. Otitis media. *Lancet.* 2004;363:465–473
32. Joki-Erkkila VP, Puhakka H, Hurme M. Cytokine gene polymorphism in recurrent acute otitis media. *Arch Otolaryngol Head Neck Surg.* 2002;128:17–20
33. Patel JA, Nair S, Revai K, et al. Association of proinflammatory cytokine gene polymorphisms with susceptibility to otitis media. *Pediatrics.* 2006;118:2273–2279

34. Bayley JP, Ottenhoff TH, Verweij CL. Is there a future for TNF promoter polymorphisms? *Genes Immun.* 2004;5:315–329
35. Nieters A, Brems S, Becker N. Cross-sectional study on cytokine polymorphisms, cytokine production after T-cell stimulation and clinical parameters in a random sample of a German population. *Hum Genet.* 2001;108:241–248
36. Schluter B, Raufhake C, Erren M, et al. Effect of the interleukin-6 promoter polymorphism (-174 G/C) on the incidence and outcome of sepsis. *Crit Care Med.* 2002;30:32–37
37. Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. *J Biol Chem.* 2000;275:18138–18144
38. Mukhopadhyay S, Herre J, Brown GD, Gordon S. The potential for Toll-like receptors to collaborate with other innate immune receptors. *Immunology.* 2004;112:521–530
39. Lim S, Crawley E, Woo P, Barnes PJ. Haplotype associated with low interleukin-10 production in patients with severe asthma. *Lancet.* 1998;352:113
40. Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the interleukin-10 5′ flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. *Arthritis Rheum.* 1999;42:1101–1108
41. Khan AQ, Shen Y, Wu ZQ, Wynn TA, Snapper CM. Endogenous pro- and anti-inflammatory cytokines differentially regulate an in vivo humoral response to Streptococcus pneumoniae. *Infect Immun.* 2002;70:749–761
42. Rovers MM, Zielhuis GA, Ingels K, van der Wilt GJ. Day-care and otitis media in young children: a critical overview. *Eur J Pediatr.* 1999;158:1–6

PEOPLE IN ETHNICALLY DIVERSE SETTINGS DON'T CARE ABOUT EACH OTHER

"Diversity was once just another word. Now it's a fighting word. One of the biggest problems with diversity is that it won't let you alone. Corporations everywhere have force-marched middle managers into training sessions led by 'diversity trainers.' Most people already knew that the basic idea beneath diversity emerged about 2000 years ago under two rubrics: Love thy neighbor as thyself, and Do unto others as they would do unto you. Then suddenly this got rewritten as 'appreciating differentness.' George Bernard Shaw is said to have demurred from the Golden Rule. 'Do *not* do unto others as you would have them do unto you,' Shaw advised. 'Their tastes may not be the same.' No such voluntary opt-out is permissible in our time. The parsons of the press made diversity into a secular commandment; do a word-search of 'diversity' in a broad database of newspapers and it might come up 250 million times. In the Supreme Court term just ended, the Seattle schools integration case led most of the justices into arcane discussions of diversity's legal compulsions. More recently it emerged that the University of Michigan, a virtual mecca of diversity, announced it would install Muslim footbaths in bathrooms, causing a fight. Now comes word that diversity as an ideology may be dead, or not worth saving. Robert Putnam, the Harvard don who in the controversial best-seller *Bowling Alone* announced the decline of communal-mindedness amid the rise of home-alone coach potatoes, has completed a mammoth study of the effects of ethnic diversity on communities. His researchers did 30 000 interviews in 41 US communities. Short version: People in ethnically diverse settings don't want to have much of anything to do with each other. 'Social capital' erodes. Diversity has a downside. Prof Putnam isn't exactly hiding these volatile conclusions. . . . He wasn't happy with what he found but didn't mince words describing the results: 'Inhabitants of diverse communities tend to withdraw from collective life, to distrust their neighbors, regardless of the color of their skin, to withdraw even from close friends, to expect the worst from their community and its leaders, to volunteer less, give less to charity and work on community projects less often, to register to vote less, to agitate for social reform more, but have less faith that they can actually make a difference, and to huddle unhappily in front of the television.' The diversity nightmare gets worse: They have little confidence in the 'local news media.' This after all we've done for them."

Henninger D. *Wall Street Journal*. August 16, 2007
Noted by JFL, MD

Genetic Polymorphisms in Immunoresponse Genes *TNFA*, *IL6*, *IL10*, and *TLR4* Are Associated With Recurrent Acute Otitis Media

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