

The Genetic Component of Middle Ear Disease in the First 5 Years of Life

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Objective: To determine the genetic component of time with middle ear effusion (MEE) and episodes of MEE and acute otitis media.

Design: Prospective twin/triplet cohort.

Setting: Research center at a tertiary pediatric hospital.

Participants: A total of 168 healthy same-sex twin and 7 same-sex triplet sets were recruited by age 2 months.

Interventions: Longitudinal assessment of middle ear status by pneumatic otoscopy and tympanometry at monthly evaluations, and at examinations during upper respiratory tract infections or symptoms of middle ear disease.

Outcome Measures: Proportion of time with MEE and episodes of acute otitis media and MEE.

Results: Of the 140 sets for which zygosity was obtained, 114 were followed up to age 3 years and 83 sets to age 5 years. The heritability estimate for proportion of time with MEE in the first 5 years of life was 0.72 ($P < .001$). The correlation of proportion of time with MEE between children within a set was significantly higher in monozygotic sets (0.65-0.77) than in dizygotic sets (0.31-0.39) for each year to age 3 years. In the fourth and fifth years of life, the correlations decreased in both monozygotic and dizygotic twin sets.

Conclusions: Findings for the first 2 years of follow-up have been previously published and indicate a strong genetic component to the proportion of time with MEE. In the present report, which details the entire 5-year follow-up, the effect of this component appears to attenuate after the third year but its cumulative effect remains significant after 5 years.

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THE RESULTS FROM THE FIRST 2 years of follow-up of this prospective twin and triplet study, which we recently reported, suggest that there is a strong genetic component to the proportion of time children younger than 2 years spend with middle ear effusion (MEE) and the number of episodes of MEE and acute otitis media (AOM) that they experience.¹ We continued to follow up the children with monthly evaluations to age 5 years to assess their middle ear status through the time when they are the most prone to otitis media (OM).

This report presents the results of the 5-year follow-up of this prospective twin and triplet study designed to provide data on the heritability of MEE from a clinically characterized patient population.

METHODS

SUBJECTS AND ENROLLMENT

The present study was conducted at the Ear, Nose, and Throat Research Center in the Ear,

Nose, and Throat Clinic at Children's Hospital of Pittsburgh between 1982 and 2000. Newborn twin and triplet sets were recruited mainly from Magee-Women's Hospital in Pittsburgh, the largest maternity hospital in the area, with a total of 68 745 births (yearly mean, 8593 births; range, 8256-8821 births) from 1990 through 1997. From 1995 through 1997, 900 sets of twins and 108 sets of triplets were born. From 1989 through 1995 the distribution according to ethnicity was 78% white, 20% black, and 2% other for all births; 77% white, 22% black, and 1% other for twins; and 86% white and 14% black for triplets. In addition to recruitment in the newborn nursery, some sets were referred by members of Mothers of Multiples clubs in the Pittsburgh area.

Healthy same-sex and opposite-sex newborn twin pairs were enrolled in the pilot project (Twin Study I) whereas only same-sex sets of twins and triplets were enrolled in Twin Study II. A child was ineligible if he or she had any major congenital malformations or a medical condition with a predisposition for OM (eg, cleft palate or Down syndrome); had received assisted ventilation or been cared for in an intensive care unit; or was at high risk for sensorineural hearing loss. Eligible infants whose parent(s) gave informed consent

were enrolled in the study, which was approved by the human rights committee of Children's Hospital of Pittsburgh and the internal review board of Magee-Women's Hospital.

ENTRY AND FOLLOW-UP EVALUATION

At entry, information obtained included history of illness and treatment received; method of feeding and type of daily care; family history with respect to OM, allergy, and recurrent infection; number and age of siblings; and socioeconomic status. An ear, nose, and throat examination including pneumatic otoscopy by a validated otoscopist,² and behavioral audiometric evaluation appropriate for the age of the child, were performed. Follow-up visits were scheduled at bimonthly (Twin Study I) or monthly (Twin Study II) intervals and whenever symptoms of ear disease intervened. Each visit included taking an extensive interval history and a pneumatic otoscopic evaluation. If a child was diagnosed as having middle ear disease, he or she was treated according to a standardized treatment protocol as previously described by Casselbrant et al.¹ Beginning when children were 7 months old, tympanograms and acoustic reflex measurements were obtained at each visit. At age 12 months, blood samples were obtained for zygosity testing. In 1987, the protocol was changed to extend the observation time to 5 years. Thus, children enrolled prior to that change were followed up to age 3 years while children enrolled later were followed up to age 5 years.

SPECIFIC METHODS OF TESTING

Acoustic Immittance Measurements

Prior to 1993, tympanometry was performed on a Madsen Z073 tympanometer (Madsen Electronics Ltd, Oakville, Canada), and the presence of effusion was determined using an algorithm combining otoscopy and tympanometry.³ As of January 1993, a GSI-33 middle ear analyzer (Lucas-Grason-Stadler Inc, Littleton, Mass) was used for testing at the Ear, Nose and Throat Research Center and a GSI-38 middle ear analyzer (Lucas-Grason-Stadler Inc) was used for home visits to meet the specifications of the American National Standards Institute.⁴ An algorithm was developed by Nozza et al⁵ to determine the presence of effusion and to categorize middle ear status by tympanometric width and otoscopic evaluation. If the infant was younger than 7 months or if tympanometric evaluation could not be obtained, the diagnosis was based on otoscopy alone.

Definition of Disease

The diagnosis of AOM required the detection of effusion by otoscopy and at least 1 symptom (fever, otalgia, or irritability) and 1 sign of inflammation (erythema and/or white opacification of the tympanic membrane, bulging or fullness, or otorrhea). Otitis media with effusion (OME) was defined as effusion in the middle ear without the symptoms of AOM. The determination of the presence of effusion was based on the algorithms described in the "Acoustic Immittance Measurements" subsection of this article. Otoscopically, OME was diagnosed by a semiopaque tympanic membrane with decreased mobility or the presence of fluid levels or bubbles behind the membrane. Middle ear effusion was used to designate middle ear fluid in children diagnosed as having either OME or AOM. The term OM represents varying types of middle ear disease (ie, OME and AOM).

Zygosity Testing

During the pilot study (Twin Study I), zygosity was assessed using a battery of 6 red blood cell antigens (ABH, Rh, MNS, K, Kp, and FY) and 7 plasma protein or red blood cell enzyme loci

(HP, GC, PLG, PGMI, ESD, ACP, and GPT).⁶ In Twin Study II, zygosity was assessed using 6 microsatellite loci—with a minimum heterozygosity of 0.70 in children of mixed European and African descent—using standard polymerase chain reaction-based methods.⁷ Each battery of markers provides a probability of excluding monozygosity greater than 0.99. Most participants in Twin Study I were resampled for DNA genotyping, and no discrepancies between serological, biochemical, and DNA testing were observed. For subjects for whom permission to obtain blood samples was denied, zygosity status was determined using cheek scrapings.

STATISTICAL METHODS

The primary outcome was the proportion of time with MEE. Time with MEE was estimated by dividing the duration of a child's participation in the study into intervals whose end points were the midpoints between the dates of 2 successive visits. Middle ear status for the entire interval was assumed to be the same as middle ear status at the visit within the interval. In cases in which there were more than 91 days between 2 successive visits, interpolation was applied for a maximum of 45.5 days. Middle ear status was considered to be unknown for the remaining days in the interval. If tympanostomy tubes had been inserted, the child was not considered at risk for MEE when the tympanostomy tubes were patent.

Estimates of heritability were obtained using the model proposed by DeFries and Fulker.⁸ An observation of each child was used once as the independent observation, and the degrees of freedom were adjusted appropriately. We also followed the strategy proposed by Cherny et al,⁹ who recommend eliminating the proband term from the model when the estimate of the environmental effect is low to obtain a more unbiased estimate of heritability. Because the variable of interest was the proportion of time with MEE, an arcsine transformation was applied to the data. As twin or triplet sets in which none of the children had disease may exert a disproportionate influence on the regression model, sets with no disease were excluded from the DeFries-Fulker model. This had little effect on the analysis at later ages because fewer sets were excluded. As in our earlier study,¹ we used a random pairing of 2 children of the triplet sets to accommodate the sets of triplets.

Episodes of MEE and episodes of AOM were used as secondary end points in this study. The degree of discordance in monozygotic (MZ) twins and dizygotic (DZ) twins was compared using the method of Olson et al.¹⁰ This method, which provides an odds ratio measure of twin association for a dichotomous trait, is identical to the model used to test the Hardy Weinberg equilibrium in a single random sample. The odds ratio is 1.0 when there is random selection, and it assumes lower values if there is a deficiency of discordant pairs. Thus, low values indicate a high degree of concordance and high values indicate a high degree of discordance. For the purposes of this study, the presence of disease was defined as 3 or more episodes of MEE or at least 1 episode of AOM.

RESULTS

ACCRUAL AND FOLLOW-UP

A total of 168 same-sex twin pairs and 7 same-sex triplet sets (33 twin pairs in Twin Study I and 142 twin and triplet sets in Twin Study II) were enrolled from 1982 through 1995. Only same-sex twin/triplet sets were included in the analysis because of reported differences in incidence of OM between male and female children.¹¹⁻¹³ Zygosity results available for 140 twin/triplet sets were 64 (46%) DZ sets and 76 (54%) MZ sets. Twenty-three (66%) of the 35 sets

Table 1. Length of Follow-up of Twin and Triplet Sets With Known Zygosity*

	No. of Sets	Age 1 Year		Age 2 Years		Age 3 Years†		Age 4 Years		Age 5 Years‡	
		Completed <1 y	Completed ≥1 y	Completed <2 y	Completed ≥2 y	Completed <3 y	Completed ≥3 y	Completed <4 y	Completed ≥4 y	Completed <5 y	Completed ≥5 y
Twin	135	2 (1)	133 (106)	14 (11)	121 (110)	26 (22)	109 (102)	55 (48)	80 (76)	56 (49)	79 (75)
Triplet	5	0 (0)	5 (4)	0 (0)	5 (5)	0 (0)	5 (5)	1 (1)	4 (4)	1 (1)	4 (4)
Total	140	2 (1)	138 (110)	14 (11)	126 (115)	26 (22)	114 (107)	56 (49)	84 (80)	57 (50)	83 (79)
DZ	64	1 (0)	63 (53)	3 (2)	61 (57)	6 (5)	58 (56)	18 (17)	46 (44)	18 (17)	46 (44)
MZ	76	1 (1)	75 (57)	11 (9)	65 (58)	20 (17)	56 (51)	38 (32)	38 (36)	39 (33)	37 (35)

Abbreviations: DZ, dizygotic children; MZ, monozygotic children.

*The numbers in parentheses indicate the number of sets in which 1 sibling or more had middle ear effusion by end point.

†Completed 3 years or was followed up to at least 35 months of age.

‡Completed 5 years or was followed up to at least 59 months of age.

with undetermined zygosity were lost to follow-up prior to zygosity testing at age 1 year. Zygosity status was determined in 131 sets from blood and in 9 sets from cheek scrapes. Twenty-five sets with known zygosity were enrolled prior to June 1987 as part of the initial protocol and completed follow-up to age 3 years while 83 sets completed 5 years of follow-up. The duration of follow-up of children with known zygosity is shown in **Table 1**.

DISTRIBUTION OF SELECTED SUBJECT CHARACTERISTICS

Of the 140 twin/triplet sets with known zygosity, 84% were white, 13% were black, and 3% were biracial. The distribution according to ethnicity was 88% white, 10% black, and 2% biracial for the 83 sets followed up to age 5 years. At entry 54% of the sets were male, and of the sets followed up until age 5 years 56% were male. There was a slightly greater percentage of male children in the MZ sets than in the DZ sets (60% and 45%, respectively; $P=.08$). There were no statistically significant differences at entry between DZ and MZ sets regarding the following characteristics: birth order with respect to other siblings, ear disease in the natural family, ear disease in natural siblings, occupation of principal wage earner, mother's education, and number of smokers in the household.

PROPORTION OF TIME WITH MEE ACCORDING TO ZYGOSITY STATUS

Table 2 summarizes the average proportion of time with MEE by 12-month intervals for all children in DZ and MZ sets. There were no statistically significant differences between the children in the DZ and MZ groups. The average proportion of time with MEE for children in both the DZ group and the MZ group, which was 0.21 ($P=.99$) in the first year of life, decreased to 0.11 in the DZ group and 0.12 in the MZ group ($P=.57$) in the fifth year of life.

DIFFERENCES IN MEE WITHIN A TWIN/TRIPLET SET

Difference in Proportion of Time With MEE

For children who were followed up to age 3 years (≥ 35 months of age), MEE was never observed in 2 (3%) of

Table 2. Average Proportion of Time With Middle Ear Effusion in 12-Month Age Intervals

Age Interval, mo*	Proportion of Time (No. of Children)		P Value
	Dizygotic	Monozygotic	
0-11	0.207 (131)	0.212 (154)	.99
12-23	0.201 (129)	0.237 (152)	.23
24-35	0.153 (125)	0.186 (132)	.18
36-47	0.140 (107)	0.154 (97)	.82
48-59+	0.110 (94)	0.124 (78)	.57

*Intervals were defined as sequences of nonoverlapping days (eg, days 0 through 365; days 366 through 730, etc) but are listed in months for convenience.

58 DZ sets and 5 (9%) of 56 MZ sets ($P=.27$). For children who were observed with middle ear fluid, the mean difference in proportion of time with MEE between siblings was 0.14 for the 56 DZ sets and 0.09 for the 51 MZ sets ($P=.01$). An absolute difference in proportion of time with MEE of 0.25 or greater was found in 8 (14%) of the 56 DZ sets and in 2 (4%) of the 51 MZ sets ($P=.10$).

Of the children followed up to age 5 years (≥ 59 months of age), MEE was never observed in 2 (4%) of 46 DZ sets and 2 (5%) of 37 MZ sets ($P>.99$). The mean difference in proportion of time with MEE between siblings who were observed with middle ear fluid was 0.14 for the 44 DZ sets and 0.08 for the 35 MZ sets ($P=.04$). An absolute difference in proportion of time with MEE of 0.25 or greater was found in 7 (16%) of the 44 DZ sets and 5 (14%) of the 35 MZ sets ($P>.99$).

Comparison of the Correlation Within Monozygotic Sets and Dizygotic Sets

The correlation of the cumulative proportion of time with MEE between the siblings in a twin/triplet set for pairs in which at least 1 sibling had MEE is shown in **Figure 1**. The correlation of cumulative proportion of time with MEE between children in a set was significantly higher in MZ sets (0.65-0.81) than in DZ sets (0.28-0.40) by 12, 24, 36, 48, and 60 months of age ($P=.06$ for the first year and $P<.001$ for years 2-5). For selected 12-month intervals (noncumulative) the correlation of proportion of time with MEE was significantly higher in MZ sets (0.65-

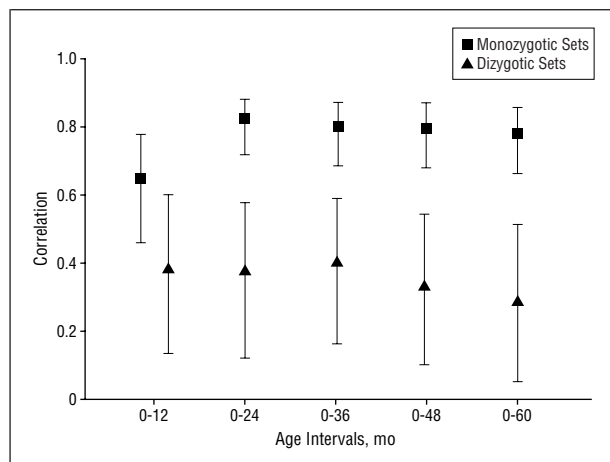


Figure 1. Correlations and confidence intervals of cumulative proportion of time with middle ear effusion (MEE) during selected month intervals for children within a twin/triplet set by zygosity in which at least 1 child had MEE. Values plotted at the 0-12 months interval are concurrent but separated on the graph for clarity. *P* values except for the 0-12 months interval (*P* = .06) were significant (*P* < .001).

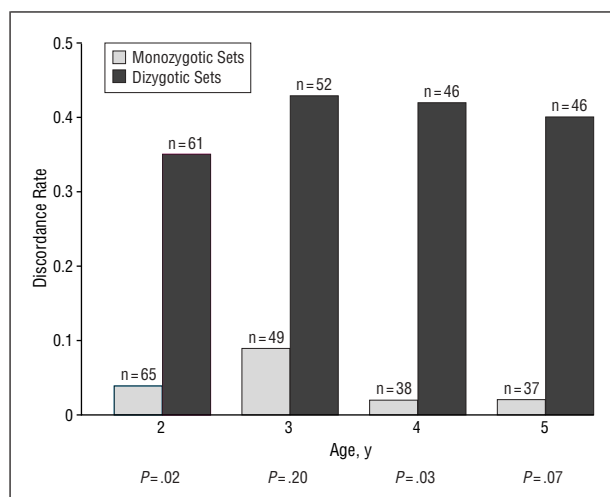


Figure 2. Discordance rate for 3 or more episodes of middle ear effusion by ages 2, 3, 4, and 5 years in monozygotic and dizygotic twin/triplet sets.

0.77) than in DZ sets (0.31-0.39) for each year through age 3 years. However, in the fourth and fifth years of life the correlations decreased considerably. In the fourth year the correlations for MZ and DZ sets were 0.31 and 0.05, respectively (*P* = .30), and in the fifth year the correlations for MZ and DZ sets were -0.04 and -0.14, respectively (*P* = .75).

Episodes of MEE

The occurrence of 3 episodes of MEE was used as the definition of recurrent disease (**Figure 2**). Measures of discordance in MZ and DZ twin/triplet sets were obtained as defined in Olson et al.¹⁰ Based on the 126 sets that completed follow-up to age 2 years, at 2 years the discordance estimates for recurrent disease were 0.04 for MZ sets and 0.35 for DZ sets (*P* = .02). The discordance estimates based on the MZ and DZ sets followed up to age 5 years were 0.02 and 0.40, respectively (*P* = .07).

Episodes of AOM

The method of Olson et al.¹⁰ was also used to measure the discordance in MZ and DZ twin/triplet sets for episodes of AOM. Based on sets completing the respective follow-up periods the discordance estimate for 1 or more episodes of AOM was significantly higher for DZ sets than for MZ sets at age 2, 3, and 4 years. The discordance estimate for 1 or more episodes at age 5 years was 1.25 for DZ sets and 0.31 for MZ sets (*P* = .29).

Tympanostomy Tube Insertion

By the 5-year end point, at least 1 sibling from 20 twin/triplet sets had undergone tympanostomy tube insertion. There were 10 sets in which 2 siblings received tubes (7 MZ and 3 DZ sets) and 10 sets in which only 1 sibling received tubes (3 MZ and 7 DZ sets) (*P* = .20). The average age at the time of tympanostomy tube insertion was 38 months in the DZ group and 42 months in the MZ group (*P* = .65).

Estimate of Heritability

In 129 of the 140 twin/triplet sets in whom zygosity was obtained, at least 1 sibling had MEE. **Figure 3** plots the proportion of time with MEE during the 5 years of one child (y-axis) vs the proportion of time with MEE for the second child (x-axis) of a twin/triplet set for the DZ and MZ groups. If there is a genetic component of the disease, one expects the slopes of the lines fitted to the data to be similar for MZ and DZ sets, but the variability to be higher in DZ than in MZ sets. In the present study, the variability between siblings is significantly greater in DZ sets than in MZ sets (*P* < .01). When the DeFries and Fulker⁸ model was applied to the data at the 5-year end point, the estimate of heritability (*h*²) was 0.72 (*P* < .001). The proband was not included in the model because the estimate of the shared environment (*c*²) was low. The corresponding estimates for male and female children separately were 0.66 (*P* < .001) and 0.75 (*P* < .001). Although male children appear to have a lower heritability estimate, the test for interaction was not statistically significant, which indicates that the difference in the heritability estimates between male and female children may be due to chance.

COMMENT

In this prospective study of twins and triplets, the estimated heritability of proportion of time with MEE was 0.72 (*P* < .001) by age 5 years. The conclusion from the first 2 years of follow-up¹ did not change when the follow-up was extended. This suggests that genetics plays a large role in the proportion of time with MEE.

The strength of the correlation between children of MZ twin/triplet sets compared with children of DZ sets with regard to MEE was dependent on the duration of follow-up. During the first 3 years of follow-up there was a very strong correlation between the children in a twin/triplet set, but during years 4 and 5 the correlation decreased. This decrease in years 4 and 5 may be ex-

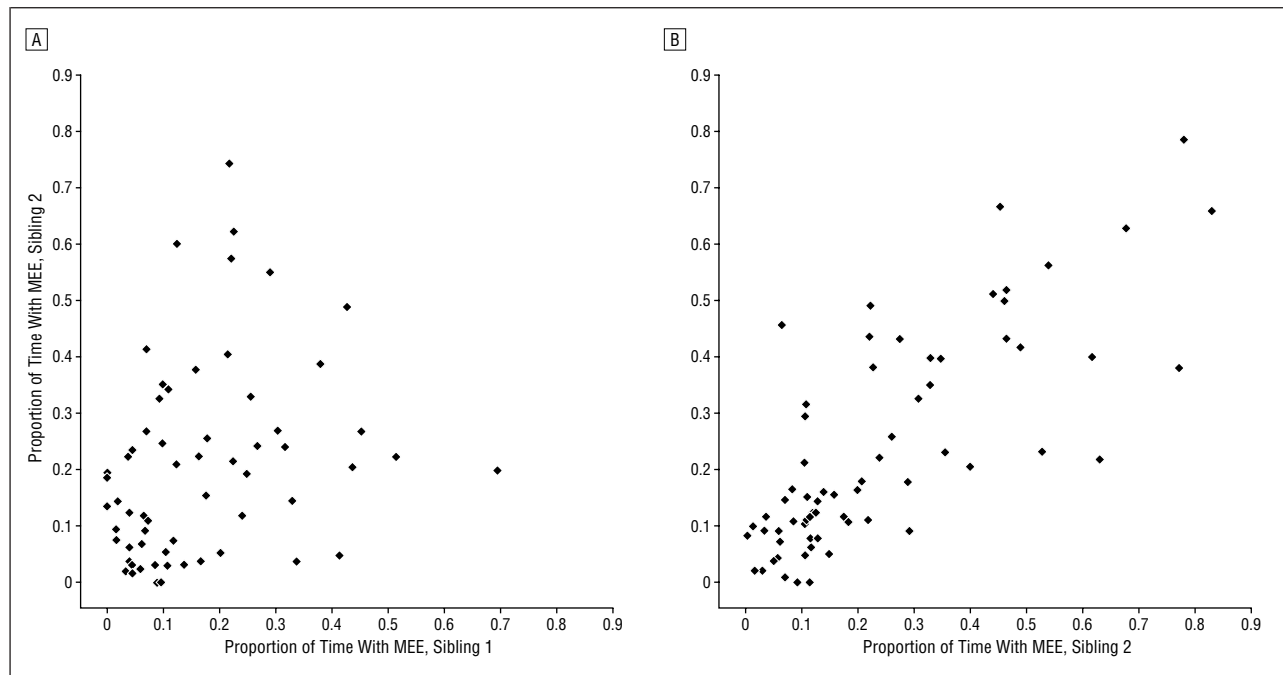


Figure 3. Scatterplot of proportion of time with middle ear effusion (MEE) by 60 months of age between children within a twin/triplet set (sibling 1 vs sibling 2) restricted to sibling pairs in which at least 1 child had MEE. A, Dizygotic twin/triplet sets. B, Monozygotic twin/triplet sets.

plained in part by the fact that the proportion of time with MEE decreased for both groups of children, as shown in Table 2. Also, fewer children were followed up in years 4 and 5 since the children enrolled before June 1987 were released from the study at age 3 years.

The investigation of end points such as number of episodes of MEE and AOM support the hypothesis of greater discordance in DZ twins than in MZ twins. The distribution of tympanostomy tube insertion, which showed a trend toward a higher correlation in MZ than DZ twin/triplet sets, also supports the hypothesis.

The results from the first 2 years of follow-up showed some tendency toward a lower heritability estimate in male (0.64) than in female (0.79) children, which remained unchanged after 5 years of follow-up. However, the differences in heritability between male and female children were not significantly different. Kvaerner et al¹⁴ showed a greater difference, with a heritability estimate for liability to AOM of 0.74 in girls and 0.45 in boys in a retrospective study of 2750 Norwegian twin pairs based on self-report.

One of the advantages of the present study is the prospective design, which allowed for accurate assessment of disease state, eliminating dependency on recalled information. Also, the twin model permits better control of the effect of environmental factors on disease as the primary comparison is between the siblings in a twin/triplet set.

One limitation of the study is that the children were examined together by the same examiner, and not by 2 examiners blinded to the children's zygosity status. However, to minimize this effect, we used an algorithm incorporating tympanometry, a more objective measure of middle ear status, and otoscopy to determine the presence or absence of MEE. Another limitation is the fact that there is no single accepted definition of ear disease during the first 5 years of life, which is an issue in genetic stud-

ies of ear disease. According to the initial protocol the follow-up was 3 years. We extended the follow-up to 5 years to evaluate the child's total experience with OM and to assess whether there were any changes over time. Thus, some of the children were released after 3 years of follow-up, which could have reduced the power of the 5-year analysis. However, the hereditary component remained significant for the 5 years of follow-up.

The DeFries-Fulker model⁸ was used in the analysis of the present study. Other methods to estimate heritability have been proposed that have better statistical properties, but the DeFries-Fulker model has the advantage of being presented in the familiar framework of a linear regression model. It has been shown that there is good agreement between the DeFries-Fulker model and the primary alternative method of a variance component model.⁹

This prospective twin study suggests that there is a strong genetic component to OM. The next logical step would be to identify the genes that confer the susceptibility to OM, but as yet, such genes are not known. Once susceptibility genes have been identified, this knowledge could be used to develop molecular assays. Such assays would be important tools for physicians to determine whether a particular child is at increased risk for OM. Also, the identification of the susceptibility genes may enable us to better understand the pathogenesis of OM, and lead to the development of more innovative and satisfactory methods for its prevention and treatment.

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REFERENCES

1. Casselbrant ML, Mandel EM, Fall PA, et al. The heritability of otitis media: a twin and triplet study. *JAMA*. 1999;282:2125-2130.
2. Stool SE, Flaherty MR. Validation of diagnosis of otitis media with effusion. *Ann Otol Rhinol Laryngol*. 1983;92(suppl 107):5-6.
3. Cantekin EI. An algorithm for diagnosis of otitis media with effusion. *Ann Otol Rhinol Laryngol*. 1983;92(suppl 107):6.
4. American National Standards Institute. Aural acoustic immittance. In: *American National Standard Specifications for Instruments to Measure Aural Acoustic Impedance and Admittance (ANSI S3.39-1987)*. New York, NY: ANSI; 1988.
5. Nozza RJ, Bluestone CD, Kardatzke D. Sensitivity, specificity, and predictive value

- of immittance measures in the identification of middle ear effusion. In: Bess FH, Hall JW, eds. *Screening Children for Auditory Function*. Nashville, Tenn: Bill Wilkerson Center Press; 1992:315-329.
6. Ferrell RE, Bertin T, Young R, Barton SA, Murillo F, Schull WT. The Aymnara of Western Bolivia, part 4: gene frequencies for eight blood groups and 19 serum protein and erythrocyte enzyme systems. *Am J Hum Genet*. 1978;30:539-549.
7. Deka R, Shriver MD, Yu LM, Ferrell RE, Chakraborty R. Intra- and inter-population diversity at short tandem repeat loci in diverse populations of the world. *Electrophoresis*. 1995;16:1659-1664.
8. DeFries JC, Fulker DW. Multiple regression analysis of twin data. *Behav Genet*. 1985;15:467-473.
9. Cherny SS, DeFries JC, Fulker DW. Multiple regression analysis of twin data: a model-fitting approach. *Behav Genet*. 1992;22:489-497.
10. Olson JM, Witte JS, Elston RC. Association within twin pairs for a dichotomous trait. *Genet Epidemiol*. 1996;13:489-499.
11. Teele DW, Klein JO, Rosner, et al. Epidemiology of otitis media during the first seven years of life in children in greater Boston: a prospective, cohort study. *J Infect Dis*. 1989;160:83-99.
12. Pukander J, Sipila M, Karam P. Occurrence of and risk factors in acute otitis media. In: Lim DJ, Bluestone CD, Klein JO, Nelson, eds. *Proceedings of the Third International Symposium on Recent Advances in Otitis Media With Effusion*. Philadelphia, Pa: Decker Inc; 1984:9-12.
13. Stenstrom C, Ingvarsson L. Otitis-prone children and controls: a study of possible predisposing factors, part 1: heredity, family background and perinatal period. *Acta Otolaryngol (Stockh)*. 1997;117:87-93.
14. Kvaerner KJ, Harris JR, Tambs K, Magnus P. Distribution and heritability of recurrent ear infections. *Ann Otol Rhinol Laryngol*. 1997;106:624-632.

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