

The Heritability of Otitis Media

A Twin and Triplet Study

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THE PREDISPOSITION OF INFANTS and young children to recurrent episodes of or prolonged time with middle ear effusion (MEE) may have a significant genetic component that can be quantified. Anatomical, physiological, and epidemiological data have suggested a heritable aspect of the disease.¹⁻¹⁴

Twin studies provide a powerful method of determining the contribution of genetics to a disease, because the potentially confounding effect of environmental factors is significantly reduced. The children in our monozygotic (MZ) or dizygotic (DZ) twin pairs were the same age, lived in the same household, and therefore presumably shared similar environments. A significantly higher concordance rate of a trait in MZ twins compared with DZ twins is suggestive of a significant genetic component. Conversely, similar concordance rates for MZ and DZ twins suggest that environmental factors play a larger role.

This article reports the results of a prospective twin and triplet study with a clinically characterized patient population designed to provide data on the heritability of MEE.

See also p 2167 and Patient Page.

Context Anatomical, physiological, and epidemiological data indicate that there may be a significant genetic component to prolonged time with and recurrent episodes of otitis media in children.

Objective To determine the genetic component of time with and episodes of middle ear effusion and acute otitis media (AOM) during the first 2 years of life.

Design Prospective twin and triplet cohort study with enrollment from 1982 through 1995.

Setting Otitis Media Research Center in the ear, nose, and throat clinic of Children's Hospital of Pittsburgh, Pittsburgh, Pa.

Patients A total of 168 healthy same-sex twin and 7 triplet sets were recruited within the first 2 months of life; zygosity results were available for 140 sets; 138 (99%) of these were followed up for 1 year and 126 (90%) for 2 years.

Main Outcome Measures Proportion of time with middle ear effusion, episodes of middle ear effusion, and episodes of AOM by zygosity status.

Results At the 2-year end point, the estimate of heritability of time with middle ear effusion was 0.73 ($P < .001$). The estimates of discordance for 3 or more episodes of middle ear effusion were 0.04 for monozygotic twins and 0.37 for dizygotic twins ($P = .01$). The estimate of discordance of an episode of AOM in monozygotic twins was 0.04 compared with 0.49 in dizygotic twins ($P = .005$).

Conclusions Our study suggests there is a strong genetic component to the amount of time with middle ear effusion and episodes of middle ear effusion and AOM in children.

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METHODS

Subjects and Enrollment

The study was conducted at the Otitis Media Research Center in the ear, nose, and throat clinic at Children's Hospital of Pittsburgh. Newborn twin and triplet sets were recruited mainly from Magee-Women's Hospital in Pittsburgh, Pa, the largest maternity hospital in the area, with a total of 68 745 births (yearly mean, 8593; range, 8256-8821) from 1990 through 1997—approximately 30% of all births in the area. From 1995 through 1997, 900 sets of twins and 108 sets of triplets were born. Some sets were enrolled from recruitment information received from Mothers of Multiples clubs in the Pittsburgh area.

In a pilot project (Twin Study I), same-sex and opposite-sex twin pairs were en-

rolled, whereas in Twin Study II, only same-sex twin and triplet sets were enrolled. A child was ineligible if he or she had any major congenital malformations, a medical condition with a predisposition for otitis media (OM) (eg, cleft palate, Down syndrome), received assisted ventilation, been cared for in an intensive care unit, or was at high risk for sensorineural hearing loss. Infants

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whose parent(s) gave informed consent were enrolled in the study, which was approved by the human rights committee at Children's Hospital of Pittsburgh and the internal review board at Magee-Women's Hospital.

Entry and Follow-up Evaluation

Information obtained at entry included history of illness and treatment received; method of feeding; type of daily care; family history with respect to OM, allergy, and recurrent infection; number and age of sibs, and socioeconomic status. An ear, nose, and throat examination, including pneumatic otoscopy by a validated otoscopist,¹⁵ and behavioral audiometry appropriate for the age of the child were performed.

Follow-up visits were scheduled at the Otitis Media Research Center clinic or at home at bimonthly (Twin Study I) or monthly (Twin Study II) intervals and whenever symptoms of ear disease intervened. Each visit included an extensive interval history and pneumatic otoscopy. Beginning at 7 months of age, tympanograms and acoustic reflex measurements were obtained at each visit. If a child was diagnosed as having middle ear disease, he or she was treated according to a standardized treatment protocol. At age 12 months, blood samples were obtained for zygosity testing.

Specific Methods of Testing

Acoustic Immittance Measurements. Prior to 1993, tympanometry at the Otitis Media Research Center clinic was performed on a Madsen Z073 tympanometer, and the presence of effusion was determined using an algorithm combining otoscopy and tympanometry.¹⁶ As of January 1993, a GSI-33 middle ear analyzer (Lucas-Grason-Stadler, Inc) was used for testing at the Otitis Media Research Center clinic and a GSI-38 middle ear analyzer was used for home visits to meet the specifications of the American National Standards Institute.¹⁷ An algorithm consistent with American Speech-Language-Hearing Association guidelines¹⁸ was developed by Nozza et al¹⁹ to determine the presence of effusion, categorizing middle ear status by

tympanometric width and otoscopy. If the infant was younger than 7 months old or if tympanometry could not be obtained, the diagnosis was based on otoscopy alone.

Definition of Disease. The diagnosis of acute otitis media (AOM) required the presence of effusion by otoscopy and at least 1 symptom (fever, otalgia, or irritability) and 1 sign (erythema and/or white opacification of the tympanic membrane, bulging or fullness, or otorrhea) of inflammation. 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 previously described algorithms (see "Acoustic Immittance Measurements" section). 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 diagnosed as 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, FY) and 7 plasma protein and red blood cell enzyme loci (HP, GC, PLG, PGMI, ESD, ACP, GPT).²⁰ In Twin Study II, zygosity was assessed using 6 microsatellite loci with a minimum heterozygosity of 0.7, using standard polymerase chain reaction-based methods.²¹ Each battery of markers provides a probability of excluding monozygosity of greater than 0.99. At follow-up, most participants in Twin Study I were resampled for DNA genotyping, and no discrepancies between serological and biochemical testing and DNA testing were observed. For subjects for whom permission to obtain blood samples was denied, an attempt was made to determine zygosity status using cheek scrapings.

Treatment Intervention. In Twin Study II, the treatment of first choice for a new episode of AOM or an episode of OME was a 10-day course of

amoxicillin. If this regimen was not effective for AOM, the children received amoxicillin-clavulanate. Patients allergic to amoxicillin were given alternative medication, such as cefaclor, erythromycin-sulfisoxazole, or 1 of the newer cephalosporins. If OME persisted for 4 consecutive months or 180 days (cumulative) during a 1-year period, tympanostomy tubes were recommended. Antibiotic prophylactic treatment for a 3-month period or tympanostomy tubes were recommended for children who had 4 episodes of AOM in 6 months or 5 episodes in 12 months. For children in Twin Study I, there was no treatment protocol, but they were treated in a similar fashion with amoxicillin given initially for episodes of AOM and OME.

Statistical Methods. The primary outcome was the proportion of time with MEE (proportion of time \times 100 = percentage of time). Time with MEE was estimated by dividing the length of time that the child participated in the study into intervals in which the end points of the intervals 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, the 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. The beginning and resolution of an episode of MEE was defined as a change in status from no MEE to MEE and back to no MEE. If tympanostomy tubes had been inserted, the child was not considered at risk for MEE when the tympanostomy tube was patent.

Estimates of heritability were obtained using the model proposed by DeFries and Fulker.²² An observation of each child was used once as the independent observation, and the degrees of freedom were appropriately adjusted. Because the variable of interest was the proportion of time with MEE, an arcsine transformation was applied to the data. Because twin or triplet sets in which none of the children had disease may ex-

ert 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. To accommodate the 5 sets of triplets, we fit all possible regression models from a random pairing of 2 members of the triplet set, which resulted in 243 possible regression models from the total twin or triplet populations. For the estimates of the genetic component (h^2), shared environment (c^2), and the corresponding significance levels, we used a weighted-probability estimate from the 243 samples. We also followed the strategy proposed by Cherny et al,²³ 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.

Episodes of MEE and AOM were used as secondary end points in this study. The degree of discordance in MZ twins and DZ twins was compared using the method of Olson et al.²⁴ In applying this method, presence of disease was defined to be greater than or equal to 3 episodes for MEE and at least 1 episode for AOM.

RESULTS

Accrual and Follow-up

A total of 168 same-sex twin pairs and 7 triplet sets (33 twin pairs in Twin Study I and 142 twin or triplet sets in Twin Study II) were recruited within the first 2 months of life from 1982 through 1995 (TABLE 1). Only same-sex twin or triplet sets were included due to reported differences in incidence of OM between males and females.²⁵⁻²⁷ Zygosity results were available for 140 twin or triplet sets: 64 (46%) DZ sets and 76 (54%) MZ sets. Of the 135 twin sets, 61 (45%) were DZ and 74 (55%) were MZ. Of the 5 triplet sets, 3 (60%) were DZ and 2 (40%) were MZ. Twenty-three (66%) of the 35 twin or triplet sets with undetermined zygosity dropped out prior to zygosity testing at 1 year of age. Zygosity status was determined in 131 twin or triplet sets from blood samples and in 9 sets from cheek scrapings.

Table 1. Distribution of Twin or Triplet Sets According to Follow-up Time and Zygosity*

	Sets Enrolled	Sets With Known Zygosity	Sets With Follow-up, No.			
			Age 1 Year		Age 2 Years	
			Completed <1 y	Completed ≥1 y	Completed <2 y	Completed ≥2 y
Twin	168	135	2 (1)	133 (106)	14 (11)	121 (110)
Triplet	7	5	0 (0)	5 (4)	0 (0)	5 (5)
Total	175	140	2 (1)	138 (110)	14 (11)	126 (115)
DZ	NA	64	1 (0)	63 (53)	3 (2)	61 (57)
MZ	NA	76	1 (1)	75 (57)	9 (9)	65 (58)

*Numbers in parentheses are number of sets in which ≥1 sib had middle ear effusion by end point. DZ indicates dizygotic children; MZ, monozygotic children; and NA, not applicable.

Of those with known zygosity, 138 (99%) twin or triplet sets were followed up to 1 year of age: 63 (98%) DZ sets and 75 (99%) MZ sets. One hundred twenty-six twin or triplet sets were followed up for at least 2 years: 61 (95%) DZ sets and 65 (86%) MZ sets ($P = .17$).

By the 2-year end point, 1 or both sibs from 17 twin or triplet sets had undergone tympanostomy tube insertion. There were 9 sets in which both sibs received tubes (6 MZ, 3 DZ) and 8 sets in which only 1 sib received tubes (3 MZ, 5 DZ). The average age at the time of tympanostomy tube insertion was 21.2 months in the DZ group and 21.4 months in the MZ group ($P = .64$).

Distribution of Selected Subject Characteristics

For the 140 twin or triplet sets with known zygosity, 54% of the sets were male and 46% were female; 84% were white. This population appears representative of the general population born at Magee-Women's Hospital. In the present study, there was a slightly greater percentage of males in MZ twin or triplet sets than in DZ twin or triplet sets (60% and 45%, respectively, $P = .08$). There were no statistically significant differences at entry between DZ and MZ twin or triplet sets in the following subject characteristics: birth order in the family, ear disease in natural family, ear disease in natural sibs, occupation of principal wage earner, mother's education, or number of smokers in the household.

Table 2. Average Proportion of Time With Middle Ear Effusion by 3-Month Age Interval and Zygosity*

Age Interval, mo	Proportion of Time (n)†	
	Dizygotic Children	Monozygotic Children
0-3	0.03 (113)	0.07 (138)
3-6	0.15 (131)	0.11 (154)
6-9	0.28 (131)	0.27 (154)
9-12	0.25 (131)	0.30 (154)
12-15	0.24 (128)	0.29 (150)
15-18	0.23 (126)	0.24 (148)
18-21	0.18 (125)	0.21 (139)
21-24	0.15 (123)	0.20 (130)

*The intervals were defined in terms of nonoverlapping days (eg, 0-91 days, 92-182 days) but for convenience are listed here in months. Average is weighted by time at risk in given interval.

†N indicates number of children.

Proportion of Time With MEE According to Zygosity Status

TABLE 2 summarizes the average proportion of time with MEE by 3-month age intervals for the 131 children in DZ sets and the 154 children in MZ sets. Although an estimate is presented in the interval from 0 to 3 months, it should be viewed with caution, because the average age at entry was 2 months, and the first interval is likely to include only 1 month of observation. After 3 months of age, the proportion of time with MEE increased; for both groups of children, the maximum value occurred in the intervals from 6 to 15 months, when it ranged from 0.24 to 0.30. For the first year of life, the average proportion of time with MEE in the DZ group and MZ group was 0.21 ($P = .99$). The average proportion of time with MEE for the first 2 years was 0.20 in the DZ group and 0.23 in the MZ group ($P = .72$).

Differences in MEE Within a Twin or Triplet Set

Difference in Proportion of Time With MEE. No episodes of MEE were observed by 1 year of age in any child in 11 (17%) DZ sets and in 18 (24%) MZ sets ($P = .46$). In the remaining sets, there was no difference between members of the set in the proportion of time with MEE by the 1-year end point in 4 (8%) DZ sets and 11 (19%) MZ sets ($P = .14$). An absolute difference in proportion of time with MEE of greater

than or equal to 0.25 was found in 14 (26%) DZ sets and 9 (16%) MZ sets ($P = .24$). The mean difference in the proportion of time with MEE between sibs within a set was 0.17 for the 53 DZ sets and 0.13 for the 58 MZ sets ($P = .39$).

Middle ear effusion had not been observed in 5 (8%) of 64 DZ sets and 9 (12%) of 76 MZ sets ($P = .61$) by 2 years of age. In the remaining sets, there was no difference in the proportion of time with MEE in 3 (5%) DZ sets and 9 (13%) MZ sets ($P = .20$). An absolute difference in proportion of time with MEE of greater than or equal to 0.25 was found in 12 (20%) DZ sets and 4 (6%) MZ sets ($P = .03$). The mean difference in proportion of time with MEE between sibs within a set was 0.15 for the 59 DZ sets and 0.10 for the 67 MZ sets ($P = .02$).

Comparison of the Correlation Within MZ and DZ Sets. The correlation of the proportion of time with MEE between 2 sibs in a twin or triplet set for selective cumulative time intervals is shown in FIGURE 1. Estimates of correlation during the first interval are more variable, because there was often only 1 visit made during the first 3 months. The other time intervals show

a correlation between sibs in the range of 0.34 to 0.39 in DZ sets and of 0.63 to 0.83 in MZ sets. The correlations were consistently higher in the MZ twin or triplet sets than in the DZ sets, regardless of length of follow-up, and were significantly different from each other at 6 ($P = .04$), 12 ($P = .04$), 18 ($P < .001$), and 24 ($P < .001$) months.

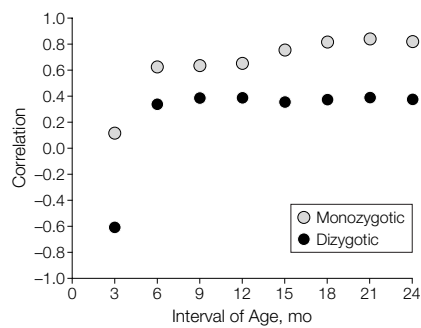
Recurrent Episodes of MEE. The occurrence of 3 episodes of MEE by 2 years of age was used as the definition of recurrent disease. Measures of discordance in MZ and DZ twin or triplet sets as defined by Olson et al²⁴ were obtained. At 2 years, the discordance estimate for recurrent episodes of MEE was 0.04 for MZ and 0.37 for DZ twin or triplet sets ($P = .01$), based on the 126 twin or triplet sets that completed follow-up to age 2 years.

Recurrent Episodes of AOM. The method of Olson et al²⁴ was also used to measure the discordance in MZ and DZ twin or triplet sets of children having at least 1 episode of AOM.

At 2 years, the discordance estimate for episodes of AOM was 0.04 for MZ and 0.49 for DZ twin or triplet sets ($P = .005$).

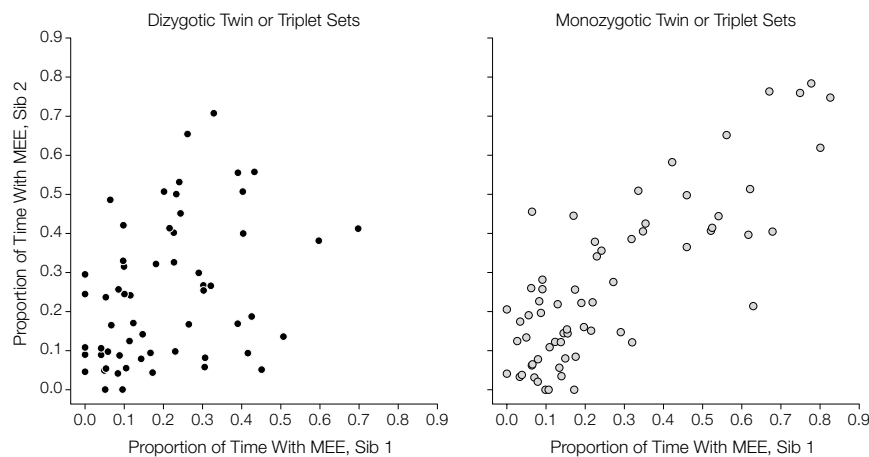
Estimate of Heritability. By 1 year, there were 111 twin or triplet sets in which at least 1 child had 1 or more episodes of MEE; by 2 years, this was true for 126 twin or triplet sets. FIGURE 2 plots the proportion of time with MEE during the first 2 years of 1 child (x-axis) vs the proportion of time with MEE for a second child (y-axis) of a twin or triplet set. The slopes of the lines fitted to the data would be expected to be similar for MZ and DZ twin or triplet sets, but if there is a genetic component of disease, one would expect the variability to be higher in DZ twins or triplets than in MZ twins or triplets. In the present study, the variability of sibs in DZ sets was significantly greater than the variability of sibs in MZ sets ($P = .007$). When the DeFries-Fulker²² model was applied to the data at the 2-year end point, the estimate of heritability (h^2) was 0.76 ($P = .02$) and the estimate of shared environment (c^2) was -0.03 . Because c^2 was not significant, it was elimi-

Figure 1. Correlation of Time With MEE Between 2 Sibs in a Twin or Triplet Set



Correlation of cumulative proportion of time with middle ear effusion (MEE) by ages 3, 6, 9, 12, 15, 18, 21, and 24 months for children within a twin or triplet set by zygosity. Restricted to twin or triplet sets in which at least 1 child had MEE.

Figure 2. Proportion of Time With MEE by 24 Months of Age for Sib 1 vs Sib 2 Within a Twin or Triplet Set



Scatterplot of proportion of time with middle ear effusion (MEE) by 24 months of age between children within a twin or triplet set (sib 1 vs sib 2). Restricted to sib pairs in which at least 1 child had MEE. Left, Dizygotic twin or triplet sets; right, monozygotic twin or triplet sets.

nated from the model, and the resultant estimate of h^2 was 0.73 ($P < .001$). The corresponding estimates for males and females separately were 0.64 ($P < .001$) and 0.79 ($P < .001$), respectively. Although males appear to have a lower heritability estimate, the test for interaction was not statistically significant ($P = .36$), indicating that the difference in the heritability estimates between males and females may be due to chance.

COMMENT

In this prospective twin study with a highly clinically characterized population, the estimated heritability of proportion of time with MEE was 0.73 ($P < .001$) by age 24 months, and the strong correlation between members of MZ twin or triplet sets compared with DZ sets in regard to experience with MEE was not dependent on length of time of follow-up (Figure 1). This suggests that genetics plays a large role in MEE. Previous studies have indicated that hereditary factors play a role in the development of OM. For example, the degree of pneumatization of the mastoid process, a trait believed to be linked causally to OM, was found to be more similar in MZ than in DZ twins.^{1,2} Racial differences in eustachian tube anatomy and function have also been reported: the shorter, straighter eustachian tube found in American Indians is associated with a higher incidence of chronic middle ear disease.^{3,4} The frequency of HLA-A2 antigens was significantly higher in children with recurrent AOM than in children with OME, yet that of HLA-A3 antigens was lower in children with recurrent AOM than in healthy children.^{5,6} Also, the genetically determined IgG2 marker IgG2m (23) has been shown to be significantly associated with recurrent AOM.^{7,8}

Several epidemiologic studies have also indicated strong support for a hereditary component of OM. A study of Apache Indian children adopted into middle-class homes outside the reservation reported that although the incidence of most infectious diseases decreased, that of OM remained com-

parable to the incidence reported for children on the reservation.⁹ Familial clustering was found in studies of OME from Pittsburgh,¹⁰ of AOM and chronic OME from Minnesota,¹¹ and of AOM from Sweden,¹² suggesting a genetic component to the disease. Rich et al¹³ estimated that the genetic component accounted for up to 60% of the liability for OM in the presence of "permissive environment." A retrospective self-reporting study in 2750 Norwegian twin pairs estimated the heritability for liability to AOM at 0.74 in females and 0.45 in males.¹⁴ The authors of that study found that individual environmental factors accounted for 26% of the susceptibility to AOM in both males and females and common environmental factors accounted for the remaining 29% of the susceptibility to AOM in males. We found a trend toward a lower heritability estimate in males (0.64) than in females (0.79). However, the difference in heritability between males and females was not statistically significant. Environmental factors accounted for 8% of the susceptibility to time with MEE in males but were slightly negative in females.

Investigation of secondary end points, such as number of episodes of MEE and number of episodes of AOM, supports the hypothesis of greater discordance in DZ twins than in MZ twins.

There are several strengths of the present study. First, the prospective design allowed for accurate assessment of disease state, eliminating dependency on recalled information. Second, the twin model permits better control of the effect of environmental factors on disease. Also, the children were treated for MEE according to a standardized treatment protocol, thereby reducing as much as possible any potential impact of different treatment interventions on duration and recurrence of disease. In addition, this study summarizes the correlations of MEE between sibs over time (Figure 1), because the magnitude of the genetic component of disease may be a function of age as well as outcome measure. The correlation between MZ twins is consistently higher than the correla-

tion between DZ twins, regardless of the length of follow-up.

There are limitations to the study. The children were examined together by the same examiner, not by 2 examiners blinded to their zygosity status. However, to minimize this effect, we used an algorithm incorporating a more objective measure of middle ear status (tympanometry) with otoscopy to determine the presence of MEE. Also, that there is no single accepted definition of ear disease during the first 2 years of life is an issue in genetic studies of ear disease.

The DeFries-Fulker model 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. There is good agreement between the DeFries-Fulker model and the primary alternative method of a variance component model.²³

CONCLUSION

These results suggest that there is a strong genetic component to the amount of time with MEE and episodes of MEE and AOM in children. This finding may influence the primary care physician to identify the sibs and offspring of affected patients as high-risk cases. Closer surveillance of patients at risk for MEE could result in earlier detection and treatment of the disease, as well as prevention of possible developmental problems.

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Let your bookcases and your shelves be your gardens and your pleasure-grounds. Pluck the fruit that grows therein, gather the roses, the spices and the myrrh.

—Judah Ibn Tibbon (1120-1190)