

Sibship size, birth order, and atopy in 11,371 Italian young men

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Background: Having a low number of siblings and a low birth order has been reported to be a relevant risk factor for development of atopic diseases and skin sensitization to common inhalants. Although the inverse association of atopy with sibship size has been confirmed repeatedly, the association with birth order has provided conflicting results. This possibly is due to the relatively small size of the population sample examined.

Objective: The objective of this study was to investigate the relation between sibship size, birth order, and atopy in a very large population sample, highly homogeneous for age and sex.

Methods: This was a retrospective survey of 11,371 Italian young men, 18 to 24 years old, all candidates for enrollment in the Italian Air Force. Demographic data had been collected by a standard questionnaire. Specific IgE for locally relevant airborne allergens had been tested by a multi-RAST assay (CAP-Phadiatop).

Results: The prevalence of atopy (defined as a high level of specific IgE against inhalants [cut-point >1.2 log RU]) was inversely related to the total number of siblings (25% in those with no siblings and 9% in those with five or more siblings), with a mean of a 3% decrease in prevalence for each added sibling. This relation persisted after adjustment for relevant variables such as father's education and rural and southern residence. An independent association between birth order and atopy was also observed because the decrease in atopy prevalence with increasing numbers of older siblings was significantly steeper than that found with the number of younger siblings ($X^2 = 179$, $df = 1$, $p < 0.0001$).

Conclusions: In a very large and homogeneous population sample of a Mediterranean country, not only sibship size but also birth order was significantly associated with atopy. This observation further highlights the role of family structure in the development of atopy and supports the hypothesis that cross-infections acquired early in infancy or in later childhood might prevent development of atopy later in life.

(*J Allergy Clin Immunol* 1998;101:439-44.)

Key words: Atopy, asthma, epidemiology, family, siblings, specific IgE, Italy, infections, risk factors

Abbreviations used

FU:	Fluorescence units
IAF:	Italian Air Force
log RU:	Logarithm of ratio units
WLS:	Western lifestyle

Several theories have been proposed to explain why atopic diseases are increasing in prevalence in Western countries.¹ This trend probably is not determined by variations in outdoor pollution but by some still unknown factor or factors related to the "Western lifestyle" (WLS).² Actually, changes in nutritional habits, housing characteristics, sanitation practices, hygienic standards, educational models, and so forth are aggregated within this definition and are so strictly related to each other that it is not easy to discriminate which of them plays a role in the etiology of this phenomenon.

Useful information on this point came from data suggesting that family structure plays a relevant role in the development of atopy. In a British cohort study, Strachan³ observed that the risk of having hay fever was inversely related to the overall number of siblings (sibship size "effect"); he also noted that even the position in the sibship might be relevant because hay fever was less frequent in the presence of older rather than younger siblings (birth order "effect"). The sibship size effect could be also interpreted as a proxy of other characteristics of the WLS; conversely, the relation of birth order with atopy appeared to be more suggestive. Actually, it could be explained whether common infections, when acquired very early in infancy because of unhygienic contacts with older siblings, could better "protect" from atopy.³ As a corollary, it was hypothesized that the decline in cross-infections within young families caused by decrease in family size and improvement in hygienic standards is, among the set of characteristics of the WLS, mostly responsible for the increase of atopy prevalence ("hygiene theory").⁴

More recently, the relevance of sibship size on atopy and atopic diseases has been confirmed repeatedly in studies performed in Britain and in Germany.^{5,6} On the contrary, the association of birth order with atopy or hay fever has been confirmed in two other population samples^{7,8} but was not found in a recent survey among British adult women.⁹ Thus the necessity of further

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Supported by IAF 3001-94/96.

Received for publication July 3, 1997; revised Oct. 27, 1997; accepted for publication Nov. 20, 1997.

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0091-6749/98 \$5.00 + 0 1/1/87707

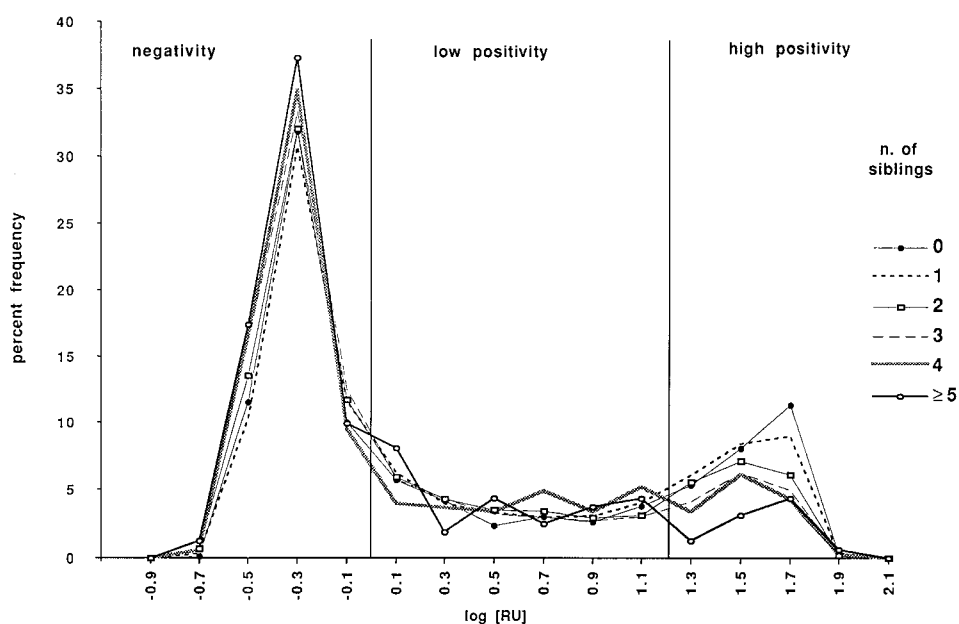


FIG. 1. Percentage frequency distribution of overall degree of serum IgE sensitization to common airborne allergens in 11,371 Italian young men according to sibship size. Peak prevalence in high-positivity area (log RU > 1.2) can be observed in the group of "only-child" subjects. This peak progressively decreases with increasing number of siblings. No major differences are observed among different groups in low-positivity area (log RU > 0; < 1.2). Sibship size is defined as the number of siblings at the time of testing.

confirmation has been emphasized.¹⁰ This demand, however, clashes with the requirement of very large population samples, combined with an objective evaluation of atopy, to distinguish with enough precision the intriguing but rather weak relation of birth order with atopy.¹⁰

The aim of this study was to investigate the association of both sibship size and birth order with atopy in a large population of over 11,371 Italian young men. To this end, we used sociodemographic data collected by compilation of standard forms together with data on serum IgE sensitization toward common airborne allergens routinely obtained by an immunoenzyme multiallergen assay according to validated methodology.¹¹

METHODS

Population sample

Data collected from a population of 11,371 Italian young men (19.7 ± 1.49 years old) were used for the current study. These subjects were candidates to be enrolled in the Italian Air Force (IAF) as permanent professionals and were examined by the medical departments of the IAF in Vigna di Valle (Rome) and in Capodichino Air Force Base (Naples) in the period from 1991 to 1996. These centers carry out several recruitment sessions each year. Data collection was built around existing examination procedures, without interference with recruiting activities, and are referred to as the complete set of applicants to five different recruitment sessions (three in Vigna di Valle [$n = 7213$] and two in Naples [$n = 4158$]). The study design was approved by the IAF Review Board authorities.

Demographic data

Demographic data such as date of birth, number of older and younger siblings, father's education, and place of residence

were acquired by an assisted compilation of a standard form. Twins of subjects examined were arbitrarily included among younger siblings. Information on the place of residence was used to build two variables (number of inhabitants and area of residence, defined as northern, central, or southern Italy).

Specific IgE testing

Serum samples were obtained by whole blood and used the same day for specific IgE testing. A fluorescence, solid-phase multiallergen immunoassay (Phadiatop-CAP, Pharmacia, Uppsala) was used to evaluate the overall degree of IgE sensitization to inhalant allergens as previously validated.¹¹ The semi-quantitative interpretation of multiallergen immunoassays has been recently introduced both in epidemiologic and genetic studies on allergy.^{12, 13}

The composition of the Phadiatop-CAP solid phase has not been disclosed by the manufacturer. In a preliminary study, however, we carefully tested in parallel the sera from monosensitized subjects by single RAST-CAP and Phadiatop-CAP. In this study a good qualitative and quantitative relation was observed in subjects monosensitized to *Dermatophagoides pteronyssinus*, cat, grass, and parietaria pollen, thus providing evidence that at least these four allergens are represented in the solid phase in quantities high enough to allow an optimal range of titration (from 0.35 to >50 kU/L of specific IgE). A similar qualitative and quantitative relation was confirmed also by comparing Phadiatop-CAP with RAST-CAP in polysensitized subjects.¹¹

To allow a valuable interassay and interlaboratory standardization of specific IgE measurement (coefficient of variation <10%), the results were expressed as the logarithm of ratio units (log RU), obtained by the following formula:

$$\text{Log (fluorescence units [FU] sample/FU reference serum)} \\ = \log \text{RU}$$

TABLE I. Prevalence and crude and adjusted associations of specific IgE level (Phadiatop log RU >1.2) in 11,371 Italian young men according to sibship size, father's education, and residence*

	No.	Log RU >1.2		Crude odds ratios	Adjusted† odds ratios	95% Confidence intervals
		n	%			
Number of siblings						
0	770	192	24.9	1	1	
1	5036	1192	23.7	0.93	0.98	} ‡ 0.82,1.18 0.64,0.94 0.49,0.79 0.42,0.88 0.21,0.66
2	3838	726	18.9	0.70	0.78	
3	1190	184	15.5	0.55	0.63	
4	327	46	14.1	0.49	0.61	
≥5	161	15	9.3	0.31	0.37	
Paternal education						
University	670	196	29.3	1	1	
High school	3084	748	24.3	0.78	0.79	} ‡ 0.65,0.95 0.54,0.78 0.49,0.74 0.39,0.79
Junior high school	4433	872	19.7	0.59	0.65	
Primary	2673	461	17.2	0.50	0.60	
None	326	51	15.6	0.45	0.56	
Residence (thousands of inhabitants)						
≥256	1784	494	27.7	1	1	
≥64, <256	1106	258	23.3	0.79	0.84	} ‡ 0.70,1.00 0.65,0.86 0.59,0.78 0.49,0.69
≥16, <64	3663	732	20.0	0.69	0.75	
≥4, <16	3139	579	18.4	0.59	0.68	
<4	1597	285	17.8	0.57	0.58	
Area of residence						
North	1310	317	24.2	1	1	
Central	6249	1376	22.0	0.88	0.92	0.79,1.08
South and islands	3800	666	17.5	0.67	0.74	0.63,0.87

*A complete data set was not available for every subject.

†Adjusted for each other, source of data, and age.

‡Test for trend, $p < 0.0001$.

For the purposes of this study, values higher than log RU 1.2 have been considered as positive.¹² This cut-point is highly sensitive and specific in identifying polysensitized subjects with clinical expression (allergic rhinitis or asthma) of their atopic status.

Statistical methods

The association between each study factor and serum IgE sensitization was evaluated by estimation of the prevalence odds ratio. Confidence limits, X^2 tests, and test for trends were calculated by Epi-info.¹⁴ To estimate the independent association of each study factor on atopy, adjusted odds ratios were calculated by logistic regression analysis with BMDP program LR (BMDP Statistical Software, Inc., Los Angeles, Calif.).¹⁵ Models with categorical variables permitted an estimation of odds ratios for each exposure category with respect to the reference category. Models suitable for ordered categories permitted estimation of linear component relating exposure to atopy.

The difference between the association of older and younger siblings was tested by fitting a logistic model with both older and younger siblings as continuous variables, then fitting the same model by replacing the two variables with the total number of siblings. Twice the change in log likelihood was tested as a X^2 statistic on 1 degree of freedom.

RESULTS

Sibship size and atopy

The frequency distribution curves of the overall serum concentration of IgE antibody against common airborne

allergens according to the number of siblings at the time of testing are reported in Fig. 1. Interestingly, subjects with no siblings displayed a peak among the higher concentrations of specific IgE that disappeared progressively with increasing numbers of siblings. This peak occurred at log RU of 1.5 (range, 1.2 to 1.8 log RU); for simplicity, a measurement of IgE antibody greater than log RU 1.2 has been used hereafter to label subjects with a high overall degree of sensitization to airborne allergens ("high specific IgE level"). Conversely, the prevalence of subjects having a detectable but low specific IgE level resulted in approximately 23% (range, 21.4% to 24.8%) in all the subgroups, independent of the number of siblings (Fig. 1).

The univariate analysis shows that the prevalence of highly positive subjects was inversely related to the number of siblings (25% in those with no siblings, 9% in those with five or more siblings), with an average prevalence decrease of 3% for each added sibling (Table I and Fig. 2). This relation persisted in the multivariate analysis after adjustment by logistic modeling for relevant sociodemographic factors such as geographic location, number of inhabitants of the community of residence, father's educational level, and age (Table I). This same analysis presents significantly lower prevalence of atopy among subjects coming from rural areas, from southern Italy, or whose father had a low educational level.

TABLE II. Crude and adjusted associations of high specific IgE level (Phadiatop log RU >1.2) with younger and older siblings in 11,371 Italian young men*

	No.	Log RU >1.2		Crude odds ratios	Adjusted† odds ratios	95% Confidence intervals
		n	%			
Number of older siblings						
0	5600	1267	22.6	1	1	}‡ 0.73,0.92 0.52,0.74 0.42,0.70
1	3561	740	20.8	0.90	0.82	
2	1363	229	16.8	0.69	0.62	
≥3	615	83	13.5	0.53	0.54	
Number of younger siblings						
0	4330	916	21.2	1	1	}‡ 0.77,0.98 0.60,0.83 0.48,0.83
1	4542	988	21.8	1.04	0.87	
2	1817	337	18.5	0.85	0.71	
≥3	450	78	17.3	0.78	0.63	

*A complete data set was not available for every subject.

†Adjusted for each other and for source of data, age, paternal education, residence (number of inhabitants), and area of residence.

‡Test for trend, $p < 0.0001$.

Birth order and atopy

The independent role of birth order on the prevalence of atopy was ascertained, fitting the same logistic model by substituting the number of older siblings and that of younger siblings in the place of the total number of siblings. From this analysis, both the number of older and younger siblings resulted in inverse and significant association with atopy (Table II), but the association between atopy and the number of older siblings was steeper (Fig. 3) and significantly stronger ($X^2 = 179$, $df = 1$, $p < 0.0001$) than that found with the number of younger siblings.

DISCUSSION

We found a strong inverse relation between sibship size and serum IgE sensitization toward common airborne allergens in a very large population sample of Italian young men. Studies performed in the British^{3,5,7,9,16} and German⁶ populations already examined the influence of family structure on atopic diseases. In these studies, however, atopy was defined by self-reported hay fever,³ eczema,¹⁶ or respiratory allergy⁹ or by in vivo testing for skin sensitization.^{6,7} Our data provide the in vitro evidence that factors related to low sibship size can affect the isotype shifting of the antibody response against common inhalants, favoring IgE production. Moreover, the multi-RAST IgE testing disclosed that this association is a very specific one because it was more pronounced for higher overall degrees of IgE sensitization to inhalants (Fig. 1), which are more frequently linked to respiratory allergic symptoms.¹¹ These data further support the hypothesis that the inverse relation of sibship size with allergic diseases should be explained on an immunologic basis as previously suggested.^{2,4}

We cannot say if a small sibship size is directly related to a higher risk of becoming atopic or if we simply observed the confounding effect of many other hallmarks of the WLS. Nevertheless, the sibship size effect

was a very strong one, and it persisted after adjusting for many relevant sociodemographic characteristics (Table I); also, it was observed in a southern European country, where heating systems, climate, diet, and genetic background are rather different from those existing in Germany and Britain. Thus other factors shared in such different geographic areas and populations probably link sibship size to atopy. The exact nature of these etiologic factors, however, is not deducible by analyzing this single association only.

Interestingly, we did observe also a significantly steeper decline in the adjusted association of IgE sensitization with the number of older siblings than with the number of younger siblings (Fig. 3 and Table II). Thus the controversial inverse association between the position in the sibship and atopy (birth order effect) was confirmed by objectively testing for specific serum IgE in a population sample having the required size.

Taken together, the data given require an immunologic explanation for the combined associations, thus supporting the hypothesis of an atopy-preventing effect of early acquired infections.⁴ Actually, infections not only preferentially recirculate within large rather than small families but they are also acquired by a child precociously dependent upon the higher the number of his or her older siblings. Early infections might be necessary for physiologic development of the human immune system because animal studies showed that microbial stimulation plays a major role in the postnatal maturation of the overall immune competence in mammals, lowering the risk of developing atopy.¹⁷

It is questionable, however, if the individual susceptibility to the postulated preventive effect of infections on atopy would be limited to the very first years of life. In our study the difference between the effects of older and younger siblings on atopy was, although statistically significant, rather low. Actually, increasing numbers of younger siblings strongly influenced a child's risk of becoming atopic (Fig. 3). In the light of the hygiene

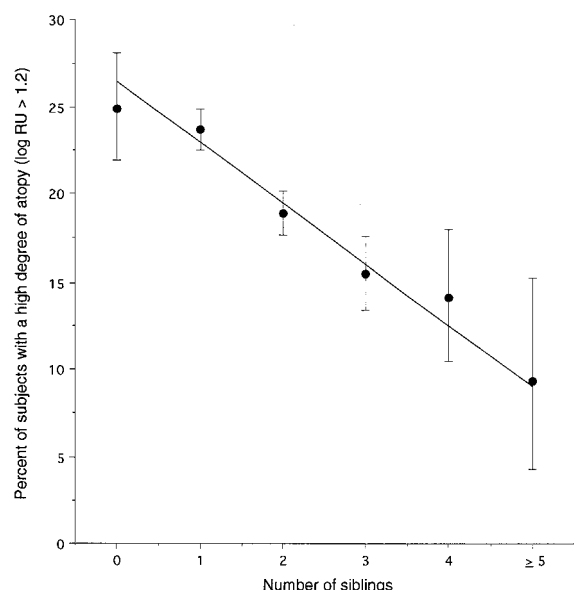


FIG. 2. Prevalence of highly atopic subjects (log RU >1.2) in relation to number of siblings in 11,371 Italian young men. Strong inverse association was observed between number of siblings at time of testing and prevalence of high atopy (test for linear trend: $p < 0.0001$).

theory, these data imply that the period of time during which the immune response might still be influenced by cross-infections would be longer than a few years after birth, as if prevention provided by infections acquired early in life from older siblings might be “boosted” by infections transmitted by younger siblings later, even during the second infancy. Thus the infection-driven prevention of IgE sensitization toward common allergens might be not a “once forever” and an “all or nothing” phenomenon but a more complex and protracted process influenced by multiple infectious events, whose protective efficacy declines slowly with age.

Nevertheless, the inverse association of birth order with atopy, considered alone, could also be explained if the newborn’s propensity to become atopic had nothing to do with infections but was a function of the number of the mother’s previous pregnancies. It has been recently observed that priming of T lymphocytes toward both food and airborne allergens can occur during fetal life.¹⁸ The placenta itself might participate in the regulation of the fetal immune system maturation.¹⁹ Therefore a mother’s immune system already “primed” by many pregnancies might interact in a qualitatively slightly different way, with her fetus tuning the first immune response toward environmental allergens. Nevertheless, in utero events obviously cannot explain the inverse association of the number of younger siblings with atopy. More studies are needed, however, before excluding that noninfectious-mediated, maternal-fetal interactions might interact with prenatal or postnatal infectious events shaping the observed complex relation between family structure and the development of atopy.

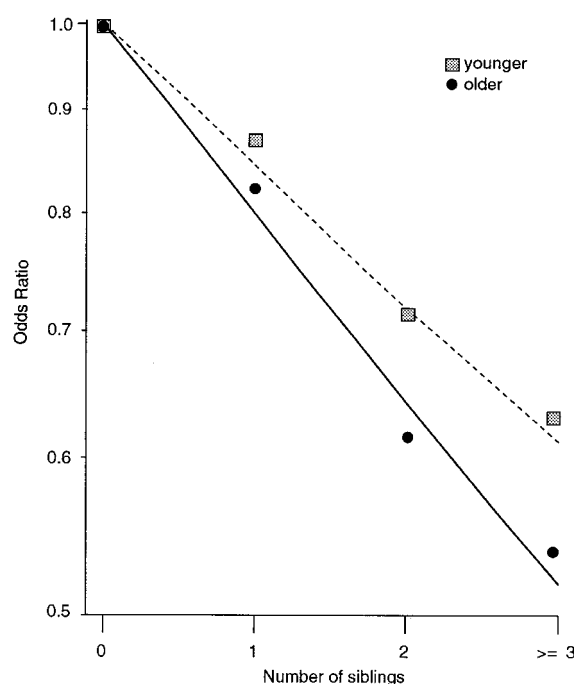


FIG. 3. Relation between number of older and younger siblings and high atopy (log RU >1.2) in 11,371 Italian young men after adjustment for each other and relevant sociodemographic factors. The linear trend of number of older siblings is statistically different from that of younger siblings ($X^2 = 179$, $df = 1$, $p < 0.0001$). Having older siblings was more protective than having younger siblings.

A fully comprehensive analysis of the data reported here may further improve their interpretation. Indeed, we noted a lower prevalence of atopy in rural and southern areas of our country (Table I). In Italy, many infections that are influenced by hygienic standards follow the same gradients but in the opposite direction. For example, hepatitis A virus infection, a good marker of poor hygienic standards, is more frequent in small towns and villages and in the southern regions.²⁰ This is consistent with the recently observed, direct evidence of an inverse relation between previous exposure to hepatitis A virus and atopy in a population of Italian military students.¹² Consequently, the hygiene theory is one that provides a unifying model, fitting all data reported in this study. In keeping with this interpretation, two recent studies performed in Japanese²¹ and Guinea-Bissau²² children reported an inverse relation of atopy, respectively, with tuberculin reactivity or a history of natural measles infection.

In conclusion, our study demonstrates the relevance of birth order, in addition to that of sibship size, on the development of IgE sensitization toward common airborne allergens and encourages further work aimed at verifying whether the complex interplay between family structure and atopy is sustained by an atopy-preventive effect of common infections rather than by other, non-infectious causes.

We are most grateful to David P. Strachan for his advice on the statistical test for difference between the effects of older and younger siblings on atopy and to Michele Fortini, Roberto Vitalone, Angelo Di Pietro, Marco Rampoli, and Antonio Rossi for excellent technical assistance.

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