

Conception via *in vitro* fertilization and delivery by Caesarean section are associated with paediatric asthma incidence

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Summary

Background The association between perinatal factors and asthma inception is under rigorous investigation. Nevertheless, evidence of a correlation between asthma, conception via *in vitro* fertilization (IVF) and delivery through Caesarean section (C-section) is inconclusive.

Objective We aimed to assess the relation of asthma incidence with IVF and C-section, after controlling for several potential confounding factors.

Methods Parent-reported wheeze in the last 12 months (current), wheeze ever, physician-diagnosed asthma, method of conception, and type of delivery were recorded from questionnaires filled in by the parents of 2016 Greek children aged 9–13, (the Healthy Growth Study population). Some perinatal data were recorded from children's medical records and others were reported by parents; anthropometric measurements were also conducted in children.

Results IVF was correlated with physician-diagnosed asthma (OR = 2.25; 95% CI = 1.11–4.56), but not with current/ever wheeze after adjustment for potential confounding factors. After adjustment, C-section was also associated with asthma (OR = 1.39; 95% CI = 1.04–1.87), but not with current/ever wheeze. When the association of both IVF and C-section with asthma was examined in the same multivariate logistic regression model, it was weakened to borderline significance (OR = 2.04; 95% CI = 1–4.15 and OR = 1.34; 95% CI = 1–1.81 respectively).

Conclusions and Clinical Relevance Conception via IVF and delivery by C-section may predispose children to future asthma development. Either variable could also exert a confounding effect on the link of the other to asthma; this may partially be accountable for inconsistencies in the findings of pertinent studies.

Keywords asthma, cesarean section, epidemiology, preadolescents, wheeze

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Introduction

The prevalence of asthma in westernized affluent societies has increased markedly over the last decades [1]. Concurrently, the rate of birth by Caesarean section (C-section) is increasing at a rapid pace; it has climbed from 1% to 30–38% within 2 decades in the UK [2] and is reaching proportions of 30% in the US [3] and even 50% in some regions of Latin America [4]. Accordingly, in Greece, births by C-section constitute roughly 35% of all births [5]. On this basis, several groups have reported a positive association between asthma and delivery by C-section [6–13], albeit findings from other studies challenge this notion [14–20]. To date, two

biologically plausible mechanisms have been suggested to underlie an asthma-modifying effect of C-section. The first one –and most often cited– is an extension of the Hygiene Hypothesis; it suggests that altered gut colonization brought about by differential microbial exposure during labour, alters the profile of commensal microbiota and interferes with immune system ontogeny [11]. The second hypothesis builds on the well-documented respiratory morbidity associated with the process of C-section-conducted labour [12].

As compared to the extensive research undertaken on the effect of C-section on asthma development, evidence for a relationship between *in vitro* fertilization (IVF) and asthma, is limited [21–28]. Even though over

a million infants have been born thus far via assisted reproduction technology, questions still remain regarding potential repercussions on their health [28]. Indeed, although growth of IVF children is probably similar to those spontaneously conceived [25], neonatal complications in the form of preterm delivery, low birth weight, low Apgar score, and respiratory morbidity are more common in the former [23, 29, 30]. Moreover, IVF offspring may require hospital care more often than their naturally conceived peers, from the neonatal period and up to 7 years of age [23, 24, 26, 31, 32]. Such issues could stem from common sequelae of the IVF procedure, that is, birth multiplicity, low birth weight, and short gestational age. However, validity of potential alternative hypotheses remains to be looked upon; these could involve epigenetic modification in DNA and gene expression by drugs or hormones utilized in the IVF procedure, or a largely irrelevant to the IVF methodology genetic link between asthma and parental subfertility [21, 26]. In any event, the evidence for a link of IVF with asthma is controversial and inconclusive [21–28].

In this context, we hypothesized that both IVF and C-section are associated with paediatric asthma incidence and that the establishment of such a link could be hindered by the interference of confounders – including potential confounding effect exerted by IVF on the C-section/asthma link and *vice versa*. Therefore, we sought to investigate these associations in a population-based cohort of preadolescent children, after taking in account several confounding factors.

Study design

The 'Healthy Growth Study' is a cross-sectional epidemiological study, initiated in 2007. The study population comprises schoolchildren aged 9–13, attending primary schools in municipalities within the counties of Attica, Aetoloakarnania, Thessaloniki, and Iraklio, in Greece. Sampling of schools for the recruitment of subjects was random, multistage, and stratified by parents' educational level and total student population within municipalities of each county, as previously described [33]. These regions are scattered across the national territory, representing north (i.e. Thessaloniki), central (i.e. Attica), western (i.e. Aetoloakarnania), and southern (i.e. Iraklio-Crete) Greece and their overall population constitutes 70% of the total Greek population (Census 1999). The study population was therefore largely representative of the 9- to 13-year-old Greek schoolchildren. On the basis of our sampling, 77 primary schools were chosen to be part of the study and all agreed to participate; invitation was extended to 4101 children attending these schools. Height and weight were measured and information on maternal and paternal educational level was collected from all children, as

part of an approved school-based health and nutrition education programme. A detailed letter describing the aims of the study was provided to parents or guardians of the selected children. Parents, who agreed to participate, signed a consent form; consent forms were signed for 2572 children (response rate 63%). The study plan was approved by the Greek Ministry of National Education and the Ethics Committee of the Harokopio University of Athens.

Socio-demographic, maternal and perinatal information

Socio-demographic and perinatal data were either reported by the parents or extracted from the children's birth certificates and medical records, which were examined during scheduled interviews at school. If parents were unable to attend the meeting (approximately 5%), data were collected by telephone. A standardized questionnaire was used and the members of the research team who conducted the interviews were extensively trained to minimize the interviewer's effect. Information collected from the parents included: (i) type of conception, (natural conception vs. IVF); (ii) type of delivery (normal labour vs. C-section); (iii) type of pregnancy, (single vs. multiple gestation); (iv) maternal smoking during pregnancy; (v) parity; (vi) children's feeding practices from birth to 6 months of age, (breastfeeding, mixed or exclusive vs. no breastfeeding); (vii) maternal and paternal years of education stratified into: less than 9 years, (Junior High School degree); 9–12 years, (High School degree) and more than 12 years (College/University degree with/without master/PhD diploma); (viii) family status (one-parent vs. two-parent family); (ix) mother employment status (employed vs. unemployed), and (x) people involved in the daily care of the child and their relationship with the family (only the mother vs. mother and another family member vs. mother and a non-family member). We also recorded the specific municipality and county wherein each participant resided and classified them into: urban (metropolitan), semi-urban, and rural.

Anthropometric indices (height and weight) were measured as described previously [33]. Birth weight and gestational age were recorded from birth certificates and medical records; these were also used for the classification of children into small for gestational age (SGA, < 10th percentile), appropriate for gestational age (AGA, 10th to 89th percentile), and large for gestational age (LGA, ≥ 90th percentile). Finally, interviews with parents also included asthma-related questions which were based on the ISAAC core questionnaire. More specifically, the following self-reported asthma outcomes were studied: (i) Current wheeze (Has your child had wheezing or whistling in the chest in the past 12 months?); (ii) Ever wheeze (Has your child ever had

wheezing or whistling in the chest at any point in time?); (iii) Asthma ever (Has your child ever had asthma diagnosed by a doctor?).

Statistical analysis

Potential selection bias was evaluated by Pearson's χ^2 test for categorical variables and Mann–Whitney *U*-test for non-parametric continuous variables. Univariate logistic regression analyses were employed to evaluate the effect of C-section and IVF on current/ever wheeze and physician-diagnosed asthma; multivariate logistic regression analysis whereby we adjusted for a wide array of potential confounding factors was further utilized to that end. Crude and adjusted odds ratios (OR) with 95% confidence intervals (CI) are shown. The Statistical Package for Social Sciences (SPSS), version 20.0 (IBM corporation, Armonk, NY, USA), was used for all data analyses. All reported *P*-values were two-tailed. The level of statistical significance was set at $P < 0.05$.

Results

Control for potential selection bias

Of the 4101 children invited, 2572 agreed to participate; 2016 of them had full data on all the required variables and were included in the study, 274 were excluded due to missing data in one or more of the key variables (C-section, IVF, current/ever wheeze and asthma) and further 282 were excluded due to missing data in one or more of the minor covariates (confounders); in all, 556 children with partial data were excluded.

To assess the potential for selection bias we compared available characteristics between the subjects who consented to participate ($n = 2572$) and those who did not ($n = 1529$; Table 1); no significant difference was found. We further opted to compare the key variables in the study population ($n = 2016$) vs. the population that was excluded for lacking data on minor covariates ($n = 282$; Table 2); no significant difference was shown. We also evaluated available characteristics of the study

Table 1. Associations between available characteristics of children who did not consent to participate in the study and of those who did (χ^2 and Mann–Whitney *U*-test)

Characteristics	Children who consented to participate ($n = 2572$) vs. Children who did not consent ($n = 1529$)
Gender	$P = 0.27$
BMI	$P = 0.24$
Father educational level	$P = 0.99$
Mother educational level	$P = 0.52$

population ($n = 2016$) vs. those of the entirety of the subjects that were excluded because of partial information ($n = 556$). Significant difference was found in maternal education and parity. To better evaluate the estimates and confidence intervals of the effect sizes, we inserted the variables in a logistic regression model (Table 3). The results indicated that children whose mothers were of low educational level (≤ 9 years of studies) or had only one child, were more likely to be excluded from the analysis due to missing data ($P = 0.001$ and $P < 0.001$ respectively).

Associations of C-section and IVF with wheeze/asthma outcomes

The main descriptive characteristics of the study population are shown in Table 4. Current wheeze was reported for 74 of 2016 children (3.5%), ever wheeze for 254 (12.5%), and physician-diagnosed asthma for 273 (13.5%); 59 children (3%) were conceived via IVF and 579 (28.7%) were delivered by C-section.

The univariate and multivariate logistic regression analyses in Table 5 present the associations of IVF and C-section with parent-reported current/ever wheeze and physician-diagnosed asthma, both before and after controlling for factors which are reported to possibly perturb in assessment of asthma-related outcomes i.e. maternal prenatal smoking, gestational age, single or multiple gestation, birth weight, gender, parity, breastfeeding, parental educational level, passive smoking at home, and current BMI. We also accounted for additional parameters which could exert a potential confounding effect such as differences in municipality, county, and urbanity, as well as for factors which could affect parental health behaviour (family status, mother employment, and people involved in the daily care of the child).

In the univariate analysis, IVF was significantly correlated with asthma and ever wheeze, but it did not correlate with current wheeze. The association with asthma remained significant after controlling for the

Table 2. Associations between the key variables in the study population vs. the population with full data on these variables, but excluded due to missing data on minor covariates. χ^2 test

Key variables	Study population ($n = 2016$) vs. Subjects having full data on key variables but excluded due to missing data on minor variables ($n = 282$)
Caesarean section	$P = 0.38$
<i>In vitro</i> fertilization	$P = 0.14$
Current wheeze	$P = 0.91$
Ever wheeze	$P = 0.79$
Asthma ever	$P = 0.17$

Table 3. Crude odd ratios and 95% confidence interval (CI) for the association between available characteristics of the study population vs. those of the children who were excluded due to missing data in any variable (logistic regression)

Characteristics	Study population (<i>n</i> = 2016)	Subjects excluded due to missing data in any required variable (<i>n</i> = 556)
Urbanity (metropolitan)	Reference	OR = 0.81 CI = 0.61–1.07 <i>P</i> = 0.147
Urbanity (rural)	Reference	OR = 0.87 CI = 0.62–1.22 <i>P</i> = 0.422
Parity (uniparous)	Reference	OR = 3.54 CI = 2.84–4.41 <i>P</i> < 0.001*
Mother education (< 9 years)	Reference	OR = 1.55 CI = 1.18–2.04 <i>P</i> = 0.001*
Mother education (> 12 years)	Reference	OR = 1.08 CI = 0.84–1.39 <i>P</i> = 0.531
Race (caucasian)	Reference	OR = 1.64 CI = 0.66–4.07 <i>P</i> = 0.281
Mother ethnicity (Greek)	Reference	OR = 0.8 CI = 0.61–1.05 <i>P</i> = 0.117
BMI	Reference	OR = 0.97 CI = 0.95–1 <i>P</i> = 0.091

*indicates statistically significant correlation.

aforementioned confounding factors (OR = 2.25; 95% CI = 1.11–4.56), albeit the association with ever wheeze was lost. In the univariate analysis, C-section was correlated with asthma (OR = 1.44; 95% CI = 1.1–1.88; Table 5). Adjustment for the same confounders failed to thwart this association (OR = 1.39; 95% CI = 1.04–1.87; Table 5).

When IVF and C-section were both inserted in the same multivariate logistic regression model and after controlling for all potential confounders, asthma diagnosis remained positively associated with both; however, correlations were weakened (*P* = 0.05 and 0.049 respectively; Table 6). The interaction term C-section $\times$ IVF was subsequently inserted in the asthma ever model, but failed to reach significance (*P* = 0.142; OR = 0.31; 95% CI = 0.06–1.47), further supporting the independence of the C-section/IVF associations with doctor-diagnosed asthma.

Discussion

In our study, both IVF and C-section were robustly correlated with physician-diagnosed asthma, even after

adjustment for several confounders. This supports the existence of a true IVF/C-section link with asthma. Interestingly however, when both IVF and C-section were examined in the same model, their correlation with asthma incidence was weakened (*P* = 0.05 for IVF and *P* = 0.049 for C-section). This suggests that either variable could exert a confounding effect on the other, a notion that is not surprising in light of the increased probability of IVF offspring to be delivered through C-section [25]. However, the retained significance of the link of both variables with asthma indicates probable independent association.

Two recent meta-analyses have suggested a positive correlation between C-section and future asthma incidence [34, 35]. In fact, in a large study including 1 756 700 births, C-section was associated with up to 59% higher risk of asthma [6]. Nevertheless, reports have been inconsistent and several investigators have failed to show a conclusive C-section/asthma correlation [14–19]. Similarly, the evidence of an association between IVF and asthma inception is controversial. There are very few reports of such a link, the most recent being a large cross-sectional study of 2 628 728 children documenting a modest IVF-related increase in asthma incidence (OR = 1.28) [21]. Some smaller studies have reported similar [23, 24, 31] or conflicting findings [25, 27, 28].

Our data pertaining to physician-diagnosed asthma is consistent with the reports supporting a positive correlation of IVF and C-section with asthma prevalence. The association of IVF with ever wheeze was weaker and became insignificant following adjustment (*P* = 0.057). This is not surprising when the variability in the pathophysiological mechanisms underlying wheeze is taken into account. Furthermore, discrepancies could be partially ascribed to parental confusion about the clinical manifestation of wheezing and its distinction from coughing or snoring [36]. Indeed, up to 19% discordance in identification of wheeze between parents and clinicians has been reported [36]. Recall bias could further explain the rather low percentage of reported ever wheeze, in this preadolescent cohort (12.5%).

The main strengths of our study are its population-based design and the control for multiple confounders. We have made allowance for the variety in municipalities and counties wherein the children resided, so as to address possible differences in local feeding/health habits; we also accounted for urbanity to refute potential perturbation of environmental factors in our analysis [37]. We adjusted for low birth weight and short gestational age (SGA), both of which have been shown to promote asthma induction [7, 17, 18, 38–40]; furthermore, IVF is associated with preterm and SGA infants [29, 41] which are also more likely to be deliv-

Table 4. Descriptive perinatal, parental, and epidemiological characteristics of the study population presented in conjunction to the studies main outcomes (current/ever wheeze and asthma ever)

	Current wheeze		Ever wheeze		Asthma ever	
	Yes (<i>n</i> = 74)	No (<i>n</i> = 1942)	Yes (<i>n</i> = 254)	No (<i>n</i> = 1762)	Yes (<i>n</i> = 273)	No (<i>n</i> = 1743)
Means of conception						
Normal	70 (94.6)	1887 (97.2)	241 (95)	1716 (97.4)	256 (93.8)	1701 (97.6)
<i>In vitro</i> fertilization	4 (5.4)	55 (2.8)	13 (5)	46 (2.6)	17 (6.2)	42 (2.4)
Type of delivery						
Normal birth	47 (63.5)	1390 (71.6)	170 (67)	1267 (72)	176 (64.5)	1261 (72.3)
Caesarean section	27 (36.5)	552 (28.4)	84 (33)	495 (28)	97 (35.5)	482 (27.7)
Maternal prenatal smoking						
No	56 (75.7)	1626 (83.7)	203 (80)	1479(84)	234 (85.7)	1448 (83)
Yes	18 (24.3)	316 (16.3)	51 (20)	283 (16)	39 (14.3)	295 (17)
Gestational age (weeks)						
< 37	18 (24.3)	359 (18.5)	56 (22)	321 (18.2)	59 (21.6)	318 (18.2)
≥ 37	56 (75.7)	1583 (81.5)	198 (78)	1441 (81.8)	214 (78.4)	1425 (81.8)
Parity						
Uniparous	42 (56.8)	944 (48.6)	119 (47)	867 (49.2)	132 (48.4)	854 (49)
Multiparous	32 (43.2)	998 (51.4)	135 (53)	895 (50.8)	141 (51.6)	889 (51)
Type of pregnancy						
Single gestation	72 (97.3)	1848 (95.2)	239 (94)	1681 (95.4)	251 (92)	1669 (95.8)
Multiple gestation	2 (2.7%)	94 (4.8)	15 (6)	81 (4.6)	22 (8)	74 (4.2)
Birth weight for gestational age						
Appropriate (10th–89th percentile)	55 (74.3)	1566 (80.6)	194 (76.4)	1427 (81)	219 (80.2)	1402 (80.4)
Small (< 10th percentile)	13 (17.6)	234 (12)	40 (15.6)	207 (11.7)	30 (11)	217 (12.4)
Large (> 90th percentile)	6 (8.1)	142 (7.3)	20 (8)	128 (7.3)	24 (8.8)	124 (7.1)
Breastfeeding						
Mixed or exclusive Breastfeeding	57 (77)	1587 (81.7)	210 (82.7)	1434 (81.4)	218 (80)	1426 (81.8)
Not Breastfeeding	17 (23)	355 (18.3)	44 (17.3)	328 (18.6)	55 (20)	317 (18.2)
Maternal education						
< 9 years	10 (13.5)	399 (20.5)	43 (17)	366 (20.8)	51 (18.7)	358 (20.5)
9–12 years	36 (48.6)	775 (39.9)	106 (41.7)	705 (40.0)	103 (37.7)	708 (40.6)
> 12 years	28 (37.8)	768 (39.5)	105 (41.3)	691 (39.2)	119 (43.6)	677 (38.8)
Paternal education						
< 9 years	16 (21.6)	488 (25.1)	64 (25.2)	440 (25)	63 (23)	441 (25.3)
9–12 years	29 (39.2)	745 (38.4)	87 (34.3)	687(39)	94 (34.5)	680 (39)
> 12 years	29 (39.2)	709 (36.5)	103(40.6)	635 (36)	116 (42.5)	622 (35.7)
Urbanity						
Metropolitan	45 (60.8)	1292 (66.5)	160 (63)	1177 (66.8)	167 (61)	1170 (67)
Semi-urban	10 (13.5)	311 (16)	43 (17)	278 (15.8)	47 (17.2)	294 (15.7)
Rural	19 (25.7)	339 (17.5)	51 (20)	307 (17.4)	59 (21.6)	299 (17.2)
Mother employment status						
Employed	48 (64.9)	1330 (68.5)	186 (73.2)	1192 (67.7)	186 (68)	1192 (68.4)
Unemployed	26 (35.1)	612 (31.5)	68 (26.8)	570 (32.3)	87 (32)	551 (31.6)

Values are expressed as *n* (%).

Table 5. Crude and adjusted odds ratios (OR) and 95% confidence interval (CI) for the association between conception via *in vitro* fertilization (IVF) and delivery through C-section with parent-reported current/ever wheeze and physician-diagnosed asthma (logistic regression)

	Current wheeze		Ever wheeze		Asthma ever	
	Before adjustment	After adjustment	Before adjustment	After adjustment	Before adjustment	After adjustment
Means of conception						
Natural fertilization	Reference	Reference	Reference	Reference	Reference	Reference
IVF	OR = 1.96 CI = 0.69–5.56 P = 0.206	OR = 2.72 CI = 0.77–9.56 P = 0.117	OR = 2.01 CI = 1.07–3.78 P = 0.03*	OR = 1.77 CI = 0.83–3.77 P = 0.14	OR = 2.69 CI = 1.5–4.79 P = 0.001*	OR = 2.25 CI = 1.11–4.56 P = 0.023*
Method of delivery						
Normal labour	Reference	Reference	Reference	Reference	Reference	Reference
Caesarean section	OR = 1.44 CI = 0.89–2.34 P = 0.134	OR = 1.25 CI = 0.73–2.15 P = 0.412	OR = 1.26 CI = 0.95–1.67 P = 0.102	OR = 1.21 CI = 0.89–1.64 P = 0.213	OR = 1.44 CI = 1.1–1.88 P = 0.008*	OR = 1.39 CI = 1.04–1.87 P = 0.024*

Adjusted for maternal prenatal smoking, gestational age, single or multiple gestation, birth weight, gender, parity, breastfeeding, parental educational level, passive smoking at home, current BMI, family status, mother occupation, people involved in daily childcare, municipality, county, and urbanity.

*indicates statistically significant correlation.

Table 6. Crude and adjusted odds ratios (OR) and 95% confidence interval (CI) for the association of physician-diagnosed asthma with method of conception and type of delivery, after inserting both independent variables in the same multivariate logistic regression model

Model adjusted for	Physician-diagnosed asthma After inserting both IVF and C-section in the model
Means of conception	
Natural conception	Reference
IVF	OR = 2.04 CI = 1–4.15 P = 0.05*
Method of delivery	
Normal labour	Reference
Caesarean section	OR = 1.34 CI = 1–1.81 P = 0.049*

*indicates statistically significant correlation.

IVF, *in vitro* fertilization; C-section, Caesarean section.

ered by C-section [38]. We controlled for single or multiple gestation as IVF can increase the risk of birth plurality which, in turn, predisposes for preterm delivery and low birth weight [28]; it also affects post-neonatal health of the IVF offspring [26]. All these issues are highly relevant to a potential asthma-facilitating effect of IVF; hence, accounting for them puts into perspective alternative mechanistic hypotheses, such as a genetic link of asthma with the IVF procedure and/or parental subfertility [21, 26]. We adjusted for maternal prenatal smoking, a risk factor for *in utero* bronchial developmental changes and for pre-school wheezing [39, 42, 43]; it has also been associated with emergency C-section [44]. We further controlled for passive tobacco smoke exposure at home, a well-documented risk factor for asthma development [6, 34] and for

breastfeeding, which appears to protect from asthma [45]; there is also evidence that C-section impedes initiation of breastfeeding [11, 46]. We adjusted for parity, as the presence of older siblings probably affects asthma risk [7, 16, 39]; for current BMI as obesity serves as a modifier for asthma risk [47] and for gender, as a C-section/asthma association was reported to be restricted in girls [11]. We adjusted for socio-economic (SE) status (using parent educational level as a proxy), as children from lower SE backgrounds are prone to asthma development, possibly due to adverse environmental exposures and lacking medical care [48, 49]; people of a higher SE level, may also be more likely to use assisted reproduction technology [28]. Finally, as parental educational level is relevant to health behaviour [48], control for this factor somewhat leverages the possibility that, in our study, parents of IVF children consulted a physician more often and for less serious symptoms than their counterparts. Indeed, frequent doctor visits caused by enhanced parental anxiety may entail an increased probability of an asthma diagnosis. To address this issue, we further controlled for parameters which could presumably affect this behaviour and may, to some degree, serve as a proxy for such parental uneasiness: family status, mother employment, and number of people who provide daily care for the child. Adjustment for parity may also have served towards this end, as it is conceivable that only children are targets of increased parental concern.

Nevertheless, there are inherent limitations in our study because of its retrospective nature, such as potential misclassification bias. In addition, asthma is a heterogeneous disease and is therefore prone to misclassification per se [50, 51]. Furthermore, the cross-sectional design limits us from investigating temporal relationships, which are pivotal in identifying asthma

phenotypes. Therefore, conflicting results in the literature could reflect variations in definitions of phenotype and timing of onset [14, 52]. Unfortunately, we could not control for family history of asthma or atopy or directly for atopic predisposition of the children. Indeed, C-section has been proposed to increase risk for atopy *per se*, therefore leading to asthma. However, such a notion is controversial; limited evidence suggests an association of C-section with allergic diseases other than asthma [12, 35, 53] and no such association has been found with atopic dermatitis [16, 54, 55] and SPT-evidenced atopy [9, 53, 56] (albeit a risk increase for allergic rhinitis [15, 35, 57] and food allergy [35] has been documented). Therefore, it is conceivable that the C-section/asthma link may not be mediated through atopic pathways, but via inflammatory and functional control mechanisms, especially the modification in the infants indigenous intestinal microbiota by differential mode of delivery [11]. Another consideration is the rather high incidence of physician-diagnosed asthma (13.5%); nevertheless, physician diagnosis has been recommended and widely used as a method of assessing asthma status in epidemiological studies [58]. Finally, our analysis suggested a potential source of selection bias as children whose mothers were of low educational level or had only one child were more likely to be excluded from the study. However, we believe that this did not distort our findings, insofar as neither of these parameters was significantly associated with the asthma/whoeeze outcomes ($P > 0.05$; data not shown). Furthermore, even though the response rate was somewhat low (63%), no significant difference was uncovered in gender, BMI, and parental educational level between the children who consented to participate and those who did not. Accordingly, no difference in the key variables was found between the study population and the subjects excluded for partial data, further reducing the potential for selection bias.

One unaccounted confounder was elective vs. emergency caesareans. Nevertheless, no differences in the C-section/asthma association were found among children delivered with either of these [10, 11, 39] suggesting that this factor could not have significantly interfered with our analysis. The same conceivably stands true for instrumental normal delivery (utilizing vacuum, forceps, rotation, etc.). Indeed, certain studies have shown such methods to be irrelevant for future asthma induction [12, 16], whereas others suggested an asthma-favouring role [6, 8, 59]. In the first case, the confounding effect would be insignificant whereas in the second, controlling for such measures would only strengthen the correlation of CS and asthma by removing asthma-favouring normal births from the analysis.

In conclusion, in our study, IVF and C-section were correlated with asthma incidence, a link that retained its significance following adjustment for a wide array of confounders. These associations remained significant when both IVF and C-section were included in the same model, suggesting possible independent effects on asthma. Nevertheless, our study also indicates a potential confounding effect of one variable on the link of the other with asthma, which should be taken into consideration in future research.

Conflicts of interest

George Guibas, George Moschonis, Paraskevi Xepapadaki, Eirini Roumpedaki, and Odysseas Androutsos declare no relevant conflict of interest. Yannis Manios works as a part-time scientific consultant for Friesland-Campina Hellas. Nikolaos Papadopoulos has served on an advisory board for Abbott, has received grants from MSD and Deviblis (EBT), has received payment for lectures from MSD, Allergopharma, Uriach, and Glaxo-SmithKline and has received payment for development of educational presentations from MSD and Uriach.

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