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**Research Article**

# The Exposure and Health Examination Survey mother-child (ren) cohort profile: applying the exposome to the comprehension of child's health and development joining singletons and twins data

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## Abstract

The Exposure and Health Examination Survey (EXHES) cohort aims to elucidate the impact of environmental exposures (the external exposome) and their biological markers (the internal exposome) on childhood health conditions, asthma and allergies, obesity, and cognitive development in particular. Utilizing singletons and twins helped differentiate environmental effects from genetic influences due to the shared genetic background in twins. The EXHES cohort includes 2356 mother-child pairs across 10 European countries, comprising 1945 singletons and 411 twins, with data collected during the crucial first 1000 days of life. Data were gathered through epidemiological questionnaires and biomarkers, including blood, urine, hair, and breast milk from mothers, and cord blood, placenta, and cord tissues from children. Findings confirm that twin pregnancies are linked with increased risks of pregnancy complications, preterm birth, cesarean delivery, low birthweight, maternal health problems during pregnancy and a lower risk of macrosomia.

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Moreover, mothers of twins were more likely to have asthma, while higher maternal education was associated with a lower likelihood of twin births. The EXHES cohort provides a robust framework to be adopted in other studies for comparing singletons and twins to better understand how the exposome affects early child development and health outcomes. This approach offers new insights into the interplay between environmental and biological factors in shaping long-term health.

**Key words:** Mother-child; cohort study; exposome; asthma; allergies; overweight; obesity; cognitive development.

# Introduction

## Why was the EXHES cohort of singletons and twins set up?

The health status and development of human beings result from the interaction between genetic and environmental factors. However, genetic background is stable across decades. Increases observed in the evolution of diseases are due to environmental transformations. In addition, a wealth of recent epidemiological data as well as experimental studies have firmly corroborated the central tenet of the "developmental origins of health and disease" concept, commonly referred to as the Barker hypothesis.[1-5] This hypothesis posits that the environmental and maternal conditions experienced by the fetus, newborn, and young child during critical developmental timeframes in early life and particularly in the first 1000 days (from conception until 2 years of age)[6,7] can impart lasting effects on fundamental biological processes such as cellular differentiation, organogenesis, metabolic programming, and physiological regulation. These early-life influences are then believed to shape an increased risk of chronic diseases or an epigenetic transgenerational inheritance of such risks across the entire lifespan.[8-11] In particular, substantial research suggests that maternal dietary intake and nutritional status early in life are associated with altered patterns of short- and long-term disease susceptibility in offspring, encompassing metabolic, immunological, psychiatric and reproductive pathologies.[11-14]

In essence, the Barker hypothesis proposes that the developmental environment—the overall atmosphere that surrounds a fetus—encountered in the earliest stages of life has the capacity to "program" an individual's biology and health trajectory, with profound and durable implications, and that postnatally, in the presence of plentiful resources, these changes persist and may then confer a disadvantage to that individual.[1-5] Due to changes in our habits and environment, exposure to environmental contaminants is becoming increasingly complex. This needs the introduction of the exposome concept[15] and of the exposomic approach allowing for the consideration of all exposures to environmental factors, alone or in combination, starting from preconception to the present age of the children being studied. The term "all exposures" refers to a comprehensive approach that considers a wide range of stressors, including chemical, physical, biological, and psychological factors, as well as environmental influences such as diet, climate, green spaces, and social interactions—collectively referred to as the "external Exposome." Additionally, it includes all measurable biomarkers, such as metabolites and biotransformation products, which are indicators of "internal exposures," known as the "internal Exposome." Early-life, from *in utero* to childhood, represents the most sensitive developmental period where the Exposome can have lasting effects on child health trajectories.[16]

and the relationship between the exposome, namely the totality of environmental contributions, and child development, Exposure and Health Examination Survey (EXHES) study recruited both singletons and twins and followed them, since *in utero* life. Twins, both monozygotic (MZ) and dizygotic (DZ), offer a wealth of information due to their genetic and environmental similarities, as twins, in particular MZ-twins, who share identical genetic background, allow for the assessment of nongenetic influences on child's development. Epidemiological studies involving twins can highlight the significance of the exposome, beginning with *in utero* and postnatal exposures, which, whether individually or in combination, play a role in establishing and maintaining the human epigenome[17-21] and influence disease development later in life. The first aim of the present paper was to present how the EXHES cohort collected through a robust protocol comprehensive data on health, environmental, and socio-demographic factors and biological specimens to build the external and internal exposomes in order to understand its impact on childhood disease trajectories in the case of major conditions like asthma, and allergies, obesity, and cognitive development. The second aim was to describe characteristics of mothers and children of the EXHES cohort and show main differences between singletons and twins from pregnancy up to birth.

## Where is it located? Who set it up?

The (EXHES) is a prospective cohort study set up in 2017 in ten distinct European countries, by the local research teams from different university hospitals/institutions as part of the Health and Environment-wide Associations based on Large population Surveys (HEALS) project funded by the European Commission as part of FP7-ENV (www.heals-eu.eu).

# Methods

## Who is in the cohort?

The study participation was proposed to all women during their pregnancies between the second and third trimester of amenorrhea or at the birth of the child(ren) (if twins) at the hospital maternity clinic in ten distinct countries, namely (Croatia (Rijeka), Germany (Regensburg), Greece (Thessaloniki), France (Paris), Italy (Palermo), Portugal (Porto), Poland (Łódź), Slovenia (Celje), Spain (Reus) and UK (Manchester)), as illustrated in Figure 1 However, Germany conducted a separate protocol, and their data were not incorporated into the meta-analysis.

Women were excluded from participation if they were <16 years old, had multiple pregnancies, problems with communication language, or plans to relocate from the region within the subsequent three years. The study protocols were reviewed and approved by local Ethic Committees. Recruitment spanned from 2017 to 2020, during which 2356 women were enrolled in the study, comprising 1945 singletons and 411 twins. Mother-child(ren) pairs were followed-up during the first 1000 days of life (from conception to 2 years of life).

## What has been measured?

The main purpose of EXHES was to better understand common childhood health conditions, namely asthma and allergies, overweight and obesity, and cognitive impairment, to which a high public health and societal burden are associated. To this extent,

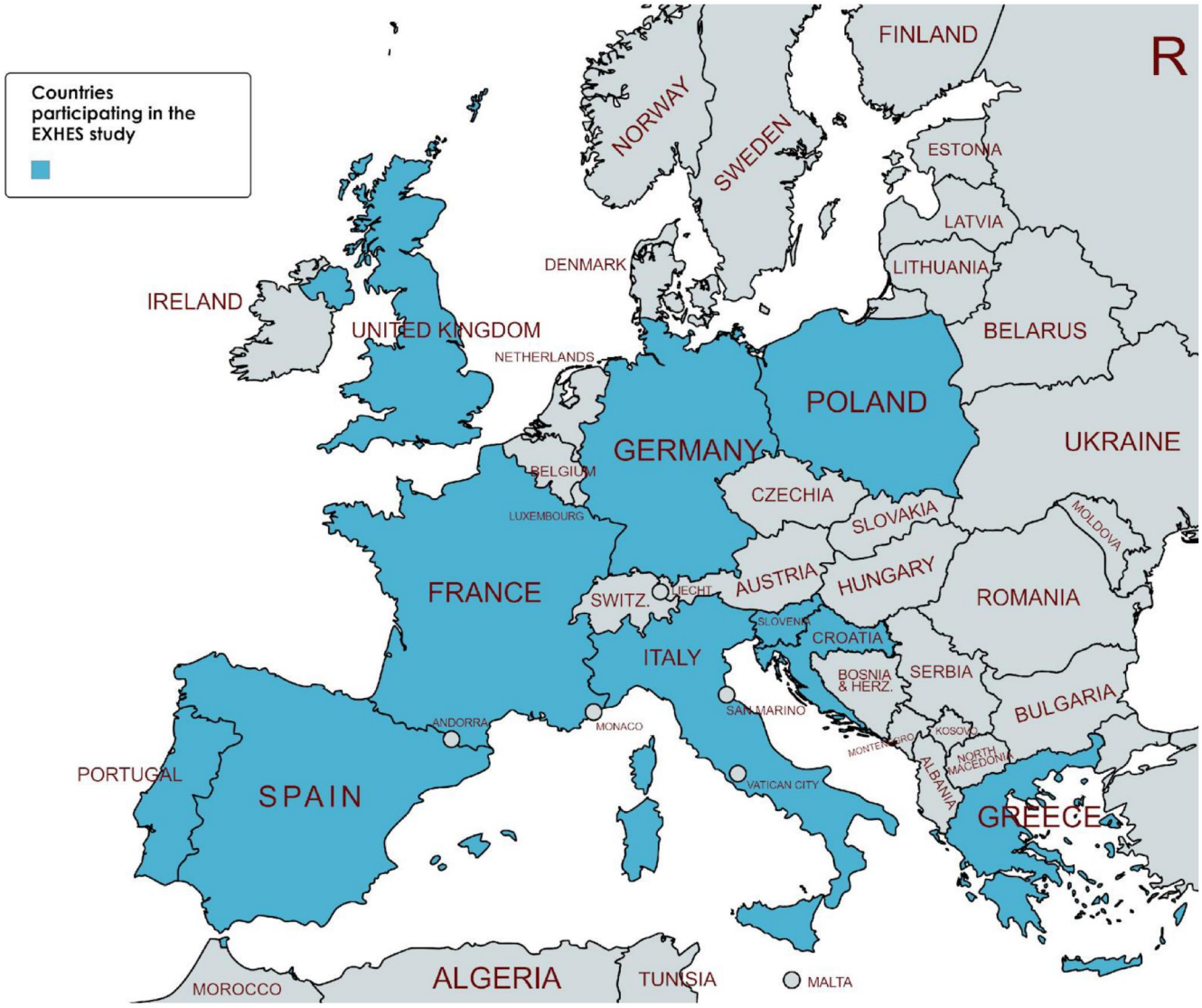


**Figure 1.** Map of the countries participating in the EHXES cohort (modified by MapChart, https://www.mapchart.net).

EXHES collected the data to implement the external and internal exposome. Standardized questionnaires used in the EDEN study (A cohort study on the Pre- and Early Postnatal Determinants of Child Health and Development)[22] and Open Access data (ie, electronic resources available to the public without financial, legal, or technical barriers)[23] were used for EXHES.

## Mother's characteristics

Initially, the study focused on the socio-demographic characteristics of the mothers and their clinical conditions, particularly the pathologies encountered during pregnancy. The information gathered encompassed detailed demographic data about the mothers, including their educational background, occupation, reproductive and pregnancy history, and lifestyle choices. Additionally, we gathered some information about the fathers, which will be used in future studies (Table1).

## Child(ren)'s development and health

Successively, EXHES closely examined the clinical and biological aspects of child development, since *in utero* life, using data from the child's medical records and epidemiological standardized questionnaires completed by parents on disease occurrence, pregnancy and perinatal outcomes, up to 2 years of age obtained through face-to-face interviews and/or clinical investigations. The main focus had been on monitoring the child's symptoms, illnesses, and the functioning of their metabolic, respiratory, and immune systems, as well as their psychomotor and cognitive development. Key indicators presented in this paper include child's sex, birth weight, birth height, head circumference, waist circumference, Apgar score, and breastfeeding.

Tables 1 and Table S1 provide a summary of the main categories of data collected at enrollment, during each follow-up visit, and at delivery.

## Child(ren)'s external exposome

External exposome (Figure 2) was assessed both through epidemiological standardized questionnaires as well as the HEALS Environmental Data Management System.[23] Detailed assessments were made of prenatal and postnatal environmental exposures, including tobacco, alcohol, diet, potentially neurotoxic chemicals, air pollution, as well as exposure to bio contaminants, allergens and finally vitamins, antioxidants and medications during pregnancy and since birth.

**Table 1.** Questionnaires data and biological samples collected during pregnancy and at birth. The EXHES study.

| | During pregnancy | At birth |
|---|---|---|
| Questionnaires | Mother | Mother (when no recruitment during pregnancy)<br>Father |
| Guthrie paper | Mother | Mother, father, cord |
| Plasma | Mother | Mother, father, cord |
| Serum | Mother (fasting and 1 h post charge) | Cord |
| Total blood | Mother | |
| Urine | Mother | Mother |
| Colostrum | – | Mother |
| Breast milk | | Mother |
| Cord samples | – | Cord |
| Meconium | – | Child |
| Placenta samples | – | Mother |
| Cord tissue samples | | Child |
| Hair | Mother | Mother, child |
| Saliva | Mother | – |

The samples were aliquoted (–).

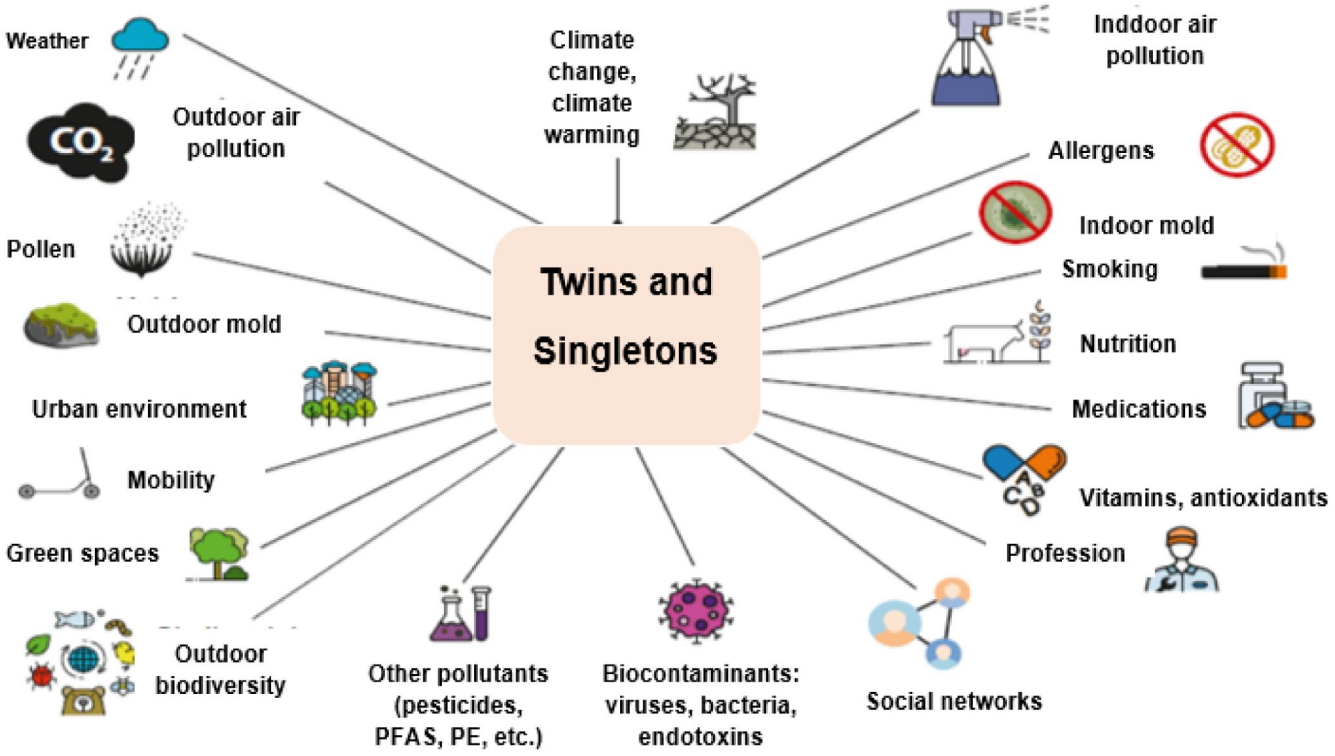


**Figure 2.** Specific and nonspecific external domains of the exposome. PFAS: per- and polyfluoroalkyl substances; PE: polyethylene.

Questionnaire data were collected also to investigate the impact of social circumstances, often linked to cumulative risks, on pregnancy outcome and subsequent child health.[24] Finally, the study considered the influence of the mother's mental health during pregnancy and the quality of family/spouse support.

The Environmental Data Management System (EDMS)[23] provides a comprehensive platform for evaluating both external specific and nonspecific exposome using open access or restricted (available upon request) data. These factors include sociodemographic characteristics, lifestyle variables such as body mass index (BMI, kg/m$^2$), smoking habits, alcohol and drug consumption, as well as air pollution, water and soil toxicants, biocontaminants, noise, climatic parameters like temperature and humidity, land cover (green, blue, gray and agricultural spaces). In particular, the EDMS captures data on air pollution metrics, including concentrations of Particulate Matter of diameter-$<10\,\mu m$ and $2.5\,\mu m$ (PM10 and PM2.5), Nitrogen Dioxide ($NO_2$), and Ozone ($O_3$). Information on pollen and spore levels, noise from traffic and railways, drinkable water quality, and pesticide residues in food was also collected. All of these exposome factors are measured and attributed to the nearest geographic location of the children's (the children's residence) when possible, allowing for identification of close exposure sources and detailed spatial analysis and modeling.[23]

## Child(ren)' internal exposome—the EXHES biobank

The types and origins of the biological samples collected during the different phases are listed in Table 1 Preparation and storage of all biological samples during the pregnancy period were

performed in each location. All biological samples were prepared and stored at −80°C in alarm-monitored freezers at each study location, ensuring consistency in storage.

### Statistical methods

In the present paper, continuous data are presented as mean values ± standard deviation (SD) and categorical data as frequency counts and percentages. The list of considered variables and their definition is shown in Table S3. Classical statistics was used to compare mothers' characteristics across countries, when data were available. The differences in descriptive variables among mothers during pregnancy and on the type of birth (singleton or twins), considering the countries heterogeneity, were assessed through a meta-analytic approach. Pooled odds ratios (OR) with 95% confidence intervals (CI) were calculated to compare categorical variables, while standardized mean differences (SMD) with 95% CI were used for continuous variables. Heterogeneity was quantified using the I2 statistic, which represents the percentage of variation across studies that is due to heterogeneity rather than chance. The meta-analysis focused on three key outcomes: prematurity, birth weight, and macrosomia. Low birthweight was defined as a weight less than 2500 g at birth, regardless of gestational age. Conversely, macrosomia refers to a high birth weight, generally over 4000 g. A value of $P < 0.05$ was considered statistically significant. All the analyses were performed using R software, version 4.3.2.

## Results

We present here descriptive data of mothers and children of the EXHES cohort up to the birth, noting that the complete dataset is not available for all countries.

### Mothers' characteristics across the countries

There were significant variations in maternal weight at 20 weeks of pregnancy (with France reporting an average weight of 57 ± 11 kg, Italy reporting 56 ± 9 kg, and Poland reporting 58.2 ± 11.4 kg) and at the end of the pregnancy (average weight of mothers ranging from 68 kg in Italy to 82.6 kg in both Croatia and Slovenia) (Table S2). Higher percentages of mothers in France (94%), Poland (97%), Slovenia (99.5%) and Croatia (97.4%) reported to be living with a partner than in Italy (70.4%). In terms of health factors, the data revealed varying prevalence rates of diabetes among mothers (ranging from 6.7% in Poland to 18% in Italy), hypertension (33% in Italy, 3.3% in Croatia, and 0.9% in Slovenia; for France and Poland the data were unavailable). The available data also highlights the utilization of fertility treatment (with Poland reporting the highest percentage (19.4%) of mothers undergoing such treatment) and medicine intake during pregnancy, particularly hormones (70.1% of mothers in France and 6.5% in Poland). Other factors such as anemia treatment, physical activity, and birth problems displayed significant variations across countries.

### Mothers' health conditions during pregnancy according to the type of birth

Table 2 presents the results of a meta-analysis examining the variations in descriptive variables among mothers during pregnancy and childbirth, based on the type of birth (singleton or twins). There were no significant differences in mother's employment (OR = 1.11, 95% CI: 0.53-2.40, $P = 0.38$) between singleton and twin births. However, a higher maternal education was inversely related to twinship OR = 0.73, 95% CI: 0.50; 0.95, $P = 0.01$). No significant differences in variables such as food allergy and diabetes before pregnancy were observed between singleton and twin births. However, twins' mothers were more likely to suffer from asthma (OR = 1.84, 95% CI: 1.34-10.07, $P = 0.01$) and anemia (OR = 1.24, 95% CI: 1.09-1.73, $P = 0.03$). Although mothers who gave birth to twins had a slightly higher weight before pregnancy (SMD = 0.12, 95% CI: −0.14 to 0.38, $P = 0.05$), this difference was not observed for BMI. Twins' mothers were more likely to have maternal health problems related to pregnancy (OR = 4.04, 95% CI: 2.11-7.75, $P = 0.01$). Overall, twins were born at an earlier gestational age, as indicated by the significant difference in weeks of amenorrhea/gestational age (SMD = −2.20, 95% CI: −2.55 to −1.85, $P = 0.01$).

### Children's development and health at birth according to the type of birth

Table 3 provides a comprehensive overview of data collected on the health and development of children during pregnancy and at birth across the EXHES countries.

Focusing on the child-related variables, we observed distinct patterns. Regarding sex distribution, singletons generally exhibited a more even split between males and females, while twins showed a slightly higher proportion of males in most countries. In France, 52% of singletons were male, compared to 77% of twins. In terms of birth weight, singletons consistently have higher mean weights compared to twins, with the largest difference seen in France (3.30 ± 0.52 kg for singletons vs 2.37 ± 0.63 kg for twins) and Portugal (3.23 ± 0.44 kg for singletons vs 2.21 ± 0.51 kg for twins, this difference is in accordance with the lower gestational age in twins. Similarly, singletons tend to be taller, with greater mean heights across all countries. In Poland, singletons measure 55.5 ± 2.20 cm, while twins are 47.61 ± 2.30 cm on average. Head circumference, an important indicator of brain development, also follows this trend, with singletons exhibiting larger mean measurements than twins. Waist circumference, a proxy for body composition, showed mixed results, with Italy reporting similar values between singletons 42.1 ± 4.3 cm and twins 42.2 ± 5.30 cm, while France (31.74 ± 1.67 cm for singletons vs 28.64 ± 2.24 cm for twins) and Slovenia (34.1 ± 2.22 cm for singletons vs 31.2 ± 1.48 cm for twins) demonstrates a more pronounced difference.

Apgar scores, which assess health of the neonate immediately following birth, were higher for singletons, with 98% of French singletons scoring ≥7, compared to 92% of twins. Data also revealed differences in medical interventions and pregnancy-related factors. Pregnancy-related problems, including hospitalization, were more prevalent among twins, as seen in the higher rates reported across the countries. As expected, the mode of delivery demonstrated a stark contrast, with singletons more likely to be born through vaginal delivery, while twins predominantly undergo cesarean section. Contrast was the highest in France and Portugal, where 67% of twin births were born by cesarean, compared to 22% and 23% for singletons respectively. Lastly, amniotic alterations were more frequent in twins than in singletons (Table 3). However, only 3 countries reported them.

### Children's exposome during pregnancy and at birth according to the type of birth

In terms of environmental exposures, variables such as alcohol consumption before pregnancy (OR = 1.11, 95% CI: 0.64; 1.93, $P = 0.37$), and smoking during the 3rd trimester (OR = 1.06, 95% CI: 0.68-1.66, $P = 0.88$) were not significantly associated with the type of birth (Table 2). However, mother's health problems

**Table 2.** Mothers (M) characteristics by type of birth (S = singletons and T = twins) in EXHES (meta-analysis).

| | | France | | Italy | | Spain | | Portugal | | Greece | | Poland | | Croatia | | UK | | Slovenia | | Pooled M = 2356 OR or SMD comparing twins to singletons [95% CI] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M = 421 N (%) or mean (± sd) | | M = 118 N (%) or mean (± sd) | | M = 71 N (%) or mean (± sd) | | M = 718 N (%) or mean (± sd) | | M = 52 N (%) or mean (± sd) | | M = 389 N (%) or mean (± sd) | | M = 300 N (%) or mean (± sd) | | M = 53 N (%) or mean (± sd) | | M = 234 N (%) or mean (± sd) | | | |
| | | S 359 | T 62 | S 64 | T 54 | S 68 | T 3 | S 479 | T 239 | S 50 | T 2 | S 376 | T 13 | S 276 | T 24 | S 53 | T | S 220 | T 14 | S 1945 | T 411 |
| Prior to pregnancy | | | | | | | | | | | | | | | | | | | | | |
| Maternal education | Primary and below | 11 (5.1) | 1 (4.2) | 4 (6.6) | 1 (3.8) | 15 (22) | 0 (0) | 93 (19.4) | 37 (15.5) | 0 (0) | 0 (0) | 22 (7.3) | 2 (15.4) | 2 (1) | 1 (9) | 1 (3.2) | NA | 2 (3) | 0 (0) | **REF** | |
| | Secondary | 83 (39) | 9 (38) | 29 (48) | 16 (62) | 21 (31) | 0 (0) | 179 (37.4) | 73 (30.5) | 0 (0) | 0 (0) | 93 (30.8) | 5 (38.5) | 113 (43) | 5 (45) | 7 (22.6) | NA | 52 (24) | 3 (60) | OR = 0.35 [0.33; 1.03] $I^2$ = 77% P = 0.31 | |
| | University and above | 120 (56) | 14 (58) | 28 (46) | 9 (35) | 31 (46) | 3 (100) | 207 (43.2) | 129 (54.0) | 50 (100) | 2 (100) | 187 (61.9) | 6 (46.1) | 146 (56) | 5 (45) | 20 (64.5) | NA | 155 (74) | 6 (40) | **OR = 0.73 [0.50; 0.95] $I^2$ = 0% P = 0.01** | |
| Employment | Yes | 257 (94.8) | 24 (89) | 37 (60) | 18 (69) | 56 (82) | 4 (100) | 462 (96.5) | 236 (98.7) | 43 (86) | 2 (100) | 279 (86) | 11 (85) | 235 (88) | 11 (100) | 24 (88.9) | **NA** | 186 (89) | 12 (80) | OR = 1.11 [0.53; 2.40] $I^2$ = 7% P = 0.38 | |
| BMI (before pregnancy) | Kg/m$^2$ | 23 (5) | 25 (4) | 23 (4) | 25 (6) | 25 (6) | 22 (4) | 24.68 (13.6) | 24.15 (4.6) | 24 (14) | 20 (0) | 23.71 (4.9) | 24.38 (4.1) | 24.1 (4.2) | 24.6 (4.7) | 20.89 (10.3) | **NA** | 25.7 (5.8) | 21.8 (1.8) | SMD = 0.06 [−0.18; 0.30] $I^2$ = 43% P = 0.11 | |
| Weight (before pregnancy) | Kg | 64 (13) | 69 (15) | 59 (11) | 66 (15) | 66 (16) | 61 (6) | 66 (14) | 64 (12) | 66 (11) | 51 (10) | 66 (15) | 68 (10) | 68 (13) | 71 (13) | 67 (13) | **NA** | **72 (17)** | 66 (7) | SMD = 0.12 [−0.14; 0.38] $I^2$ = 52% P = 0.05 | |
| Tabaco | Yes | 6 (2.2) | 0 (0) | 2 (3.2) | 3 (12) | 9 (14) | 0 (0) | 47 (9.8) | 23 (9.6) | 6 (12) | 0 (0) | 4 (1.1) | 0 (0) | 83 (30) | 3 (25) | 3 (5.6) | **NA** | 3 (4) | 0 (0) | OR = 1.06 [0.68; 1.66] $I^2$ = 0% P = 0.88 | |
| Alcohol | Yes | 158 (61) | 15 (58) | 9 (15) | 1 (3.8) | 22 (34) | 0 (0) | 23 (4.8) | 20 (8.5) | 37 (76) | 2 (100) | 150 (45.3) | 6 (46.2) | **NA** | **NA** | 17 (94.4) | **NA** | **NA** | **NA** | OR = 1.11 [0.64; 1.93] $I^2$ = 7% P = 0.37 | |
| During pregnancy | | | | | | | | | | | | | | | | | | | | | |
| Weeks of amenorrhea/ gestational age | Weeks | 40 (2) | 36 (3) | 39 (1) | 36 (1) | **NA** | **NA** | 38.89 (1.5) | 35.26 (2.4) | **NA** | **NA** | 38.82 (1.1) | 36.38 (1.2) | 39.6 (0.8) | 37.5 (0.9) | **NA** | **NA** | 39.3 (1.3) | 37 (1.2) | **SMD = −2.20 [−2.55; −1.85] $I^2$ = 71% P = 0.01** | |
| Maternal health problems | Yes | 105 (31) | 14 (50) | 10 (16) | 18 (67) | **NA** | **NA** | 218 (45.5) | 207 (86.6) | 12 (24) | 0 (0) | 129 (36.9) | 9 (69.2) | 109 (40) | 6 (50) | 16 (57.15) | **NA** | 41 (53) | 5 (83) | **OR = 4.04 [2.11; 7.75] $I^2$ = 63% P = 0.01** | |
| Asthma | Yes | 31 (13) | 0 (0) | 3 (4.8) | 0 (0) | NA | NA | 61 (12.7) | 26 (10.9) | 6 (12) | 0 (0) | 10 (2.8) | 1 (7.7) | 8 (3) | 0 (0) | 0 (0) | NA | 1 (1) | 0 (0) | **OR = 1.84 [1.34; 10.07] $I^2$ = 86% P = 0.01** | |
| Anemia | Yes | 46 (20) | 4 (20) | 8 (1.6) | 2 (26) | **NA** | **NA** | 90 (18.8) | 52 (21.8) | 0 (0) | 0 (0) | 32 (10.2) | 1 (7.7) | 103 (38) | 6 (50) | 22 (95.7) | **NA** | 15 (19) | 4 (67) | **OR = 1.24 [1.09; 1.73] $I^2$ = 15% P = 0.03** | |
| Diabetes | Yes | 42 (12) | 4 (13) | 3 (4.8) | 1 (3.7) | **NA** | **NA** | 51 (10.7) | 31 (13) | 12 (24) | 0 (0) | 19 (5.4) | 2 (15.3) | 25 (9) | 2 (18) | 0 (0) | **NA** | 14 (79) | 0 (0) | OR= 1.29 [0.87; 1.92] $I^2$= 0% P = 0.79 | |
| Caesarean section | Yes | 77 (22) | 20 (67) | 24 (61) | 24 (89) | **NA** | **NA** | 109 (22.8) | 159 (66.8) | 25 (52) | 2 (100) | 243 (64.6) | 12 (92.3) | **NA** | **NA** | 2 (66.7) | **NA** | 21 (27) | 8 (80) | **OR = 7.17 [5.33; 9.65] $I^2$ = 0% P = 0.04** | |

Abbreviations: NA: not applicable; OR: odds ratio; sd: standard deviation; SMD: standardized mean difference; 95% CI: 95% confidence interval.

**Table 3.** Children's characteristics during pregnancy and at birth by type of birth (S = singletons and T = twins) in EXHES (meta-analysis). The EXHES study.

| | France M = 421 N (%) or mean (± sd) | | Italy M = 118 N (%) or mean (± sd) | | Portugal M = 718 N (%) or mean (± sd) | | Poland M = 389 N (%) or mean (± sd) | | Croatia M = 300 N (%) or mean (± sd) | | Slovenia M = 234 N (%) or mean (± sd) | | M = 2180 OR or SMD comparing twins to singletons [95% CI] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Child*** | **S** | **T** | **S** | **T** | **S** | **T** | **S** | **T** | **S** | **T** | **S** | **T** | **S** | **T** |
| *Health* | 359 | 62 | 64 | 54 | 479 | 239 | 376 | 13 | 276 | 24 | 220 | 14 | **1774** | **406** |
| Sex | | | | | | | | | | | | | | |
| *F* | 166 (48) | 10 (23) | 25 (68) | 27 (61) | 210 (43.8) | 233 (49.2) | 184 (50.3) | 13 (50) | 130 (47) | 11 (46) | 112 (52.3) | 9 (0.64) | **OR = 0.26 [0.65; 1.18]** $I^2$ **= 54% P = 0.57** | |
| During pregnancy | | | | | | | | | | | | | | |
| Mother's treatment | 253 (71) | 23 (82) | 4 (11) | 12 (27) | 23 (4.8) | 99 (41.4) | 97 (27.8) | 9 (69.2) | 74 (28.6) | 6 (54.6) | 132 (60) | 4 (57.1) | **OR = 1.76 [0.19; 3.33]** $I^2$ **= 63%** **P = 0.3** | |
| Pregnancy problem | 105 (30) | 14 (48) | 6 (16) | 16 (36) | 218 (45.5) | 207 (86.6) | 129 (36.9) | 9 (69.2) | 109 (40) | 6 (50) | 125 (56.8) | 6 (85.7) | **OR = 1.14 [0.20; 2.09]** $I^2$ **= 67%** **P = 0.1** | |
| At birth | | | | | | | | | | | | | | |
| Caesarean section | 77 (22) | 20 (67) | 24 (61) | 24 (89) | 109 (22.8) | 159 (66.8) | 243 (64.6) | 12 (92.3) | NA | NA | 150 (70.8) | 10(83.3) | **OR = 7.17 [5.33; 9.65]** $I^2$ **= 0%** **P = 0.04** | |
| Amniotic alteration | 37 (10) | 0 (0) | 3 (8.6) | 7 (16) | NA | NA | 46 (12.2) | 1 (7.7) | NA | NA | NA | NA | **OR = 4.96 [3.88; 6.03]** $I^2$ **= 0%** **P = 0.001** | |
| Gestational age | 39.65 ± 1.61 | 35.72 ± 2.95 | 39.17 ± 1.08 | 37.83 ± 2.04 | 38.8 ± 1.56 | 35.26 ± 2.47 | 38.82 ± 1.10 | 36.38 ± 1.20 | 39.6 ± 0.80 | 37.5 ± 0.90 | 39.3 ± 1.20 | 37.0 ± 1.11 | **OR = 1.88 [1.40; 2.36]** $I^2$ **= 9%** **P = 0.001** | |
| Weight (kg) | 3.30 ± 0.52 | 2.37 ± 0.63 | 3.32 ± 0.49 | 2.92 ± 0.67 | 3.23 ± 0.44 | 2.21 ± 0.51 | 3.456 ± 0.50 | 2.463 ± 0.50 | 3.53 ± 0.45 | 2.74 ± 0.34 | 3.45 ± 0.41 | 2.65 ± 0.24 | **SMD = 1.72 [1.29; 2.14]** $I^2$ **= 8%** **P = 0.001** | |
| Height (cm) | 50.25 ± 2.73 | 47.41 ± 3.57 | 49.76 ± 1.92 | 47.81 ± 2.86 | 49.19 ± 2.50 | 44.26 ± 3.43 | 55.5 ± 2.20 | 47.61 ± 2.30 | 50.8 ± 2.30 | 48.0 ± 1.80 | 51.0 ± 1.95 | 48.0 ± 1.60 | **SMD = 1.62 [0.84; 2.39]** $I^2$ **= 8%** **P = 0.001** | |
| Head Circumference | 34.85 ± 1.67 | 33.23 ± 1.63 | 34.49 ± 1.40 | 33.82 ± 1.74 | 34.33 ± 1.66 | 31.89 ± 2.27 | 34.8 ± 1.50 | 32.7 ± 1.50 | 34.4 ± 1.30 | 33.4 0.90 | 34.9 ± 1.26 | 33.8 ± 1.25 | **SMD = 1.02 [0.75; 1.29]** $I^2$ **= 9%** **P = 0.003** | |
| Waist Circumference | 31.74 ± 2.70 | 28.64 ± 2.24 | 42.1 ± 4.30 | 42.2 ± 5.30 | NA | NA | NA | NA | NA | NA | 34.1 ± 2.22 | 31.2 ± 1.48 | **SMD = 0.82 [−0.02; 1.65]** $I^2$ **= 92%** **P = 0.06** | |
| Apgar (≥7) | 308 (98) | 23 (92) | NA | NA | 478 (99.8) | 470 (99.2) | NA | NA | NA | NA | 206 (99.0) | 14 (100) | **OR = 6.80 [0.26; 13.86]** $I^2$ **= 8%** **P = 0.06** | |
| Breastfeeding | 84 (92) | 12 (98) | 14 (22) | 41 (93) | 261 (97.4) | 295 (91.9) | 305 (81) | 13 (100) | 142 (97) | 10 (100) | 210 (96.3) | 8 (57.1) | **OR = −4.41 [1.66; 7.15]** $I^2$ **= 95%** **P = 0.0016** | |

Abbreviations: NA: not applicable; OR: odds ratio; sd: standard deviation; SMD: standardized mean difference; 95% CI: 95% confidence interval.

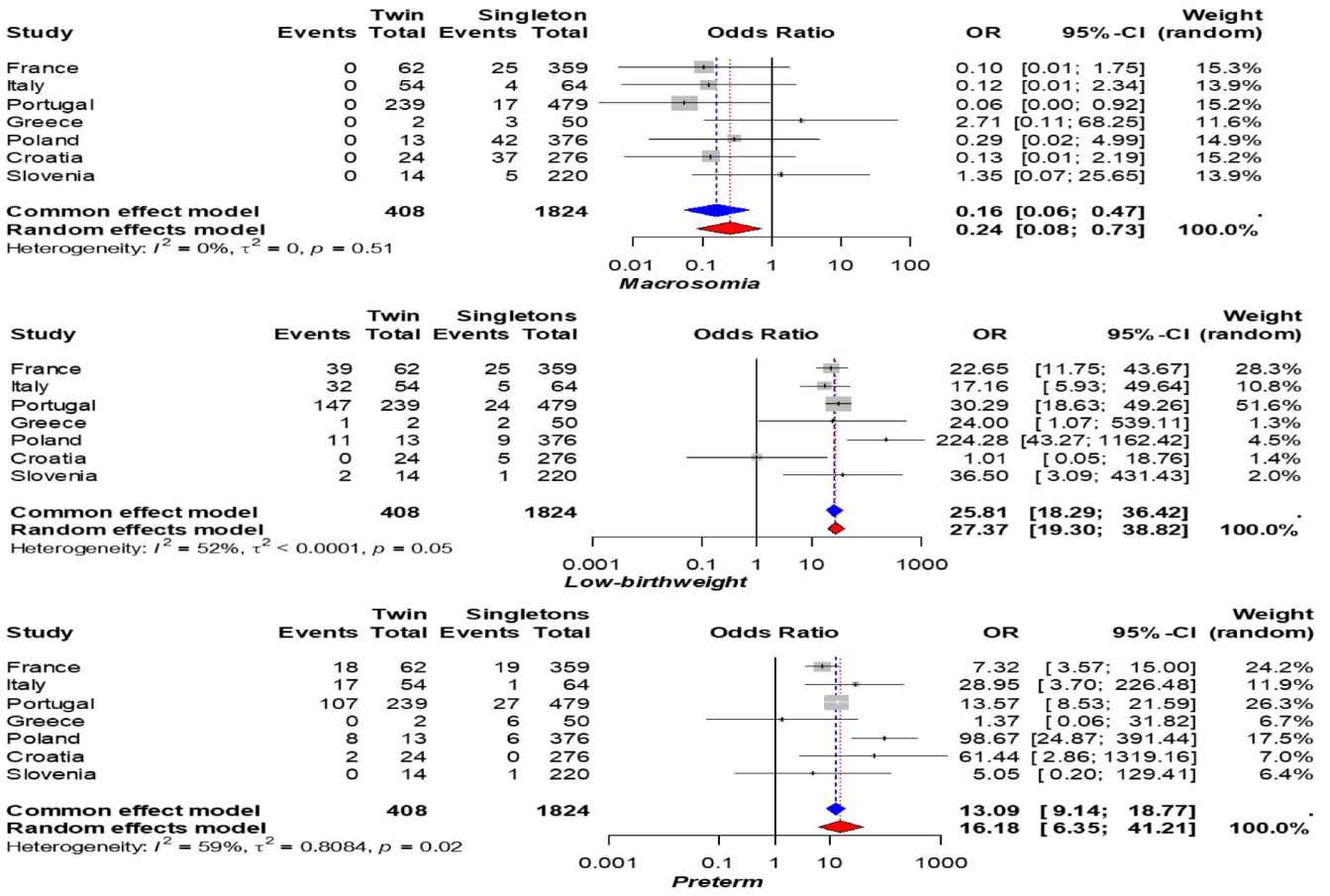


**Figure 3.** Comparative risks of adverse perinatal outcomes in twin vs singleton pregnancies: a meta-analytic perspective. OR: odds ratio; 95% CI: 95% confidence interval.

during pregnancy, possibly needing medications, were significantly more frequent in twins than in singletons (OR = 4.04, 95% CI: 2.11-7.75, $P$ = 0.01) (Table 2).

### Overall results from the meta-analysis across EXHES countries

Figure 3 presents the results of a meta-analysis investigating the differences in several neonate-related variables between singleton and twin births. As expected; there was a trend toward a significantly higher risk of preterm birth in twins compared to singletons (OR = 16.18 (95% CI: 6.35 - 41.21), and a significantly higher risk of low birth weight in twins compared to singletons (OR = 27.37 (95% CI: 19.30 - 38.82). On the other hand, the results suggest that there was a significantly lower risk of macrosomia in twins compared to singletons, with an odds ratio of 0.24 (95% CI: 0.08-0.73).

## Discussion

The EXHES cohort has revealed significant differences and heterogeneity in various maternal and children's variables when comparing twin and singleton pregnancies across Europe. As expected, twin pregnancies were associated with a higher risk of health problems during pregnancy, cesarean section, preterm birth, a birth weight < 2.5 kg, lower anthropometric parameters, and a lower risk of macrosomia, that can be explained by a number of factors, including increased risk of maternal and fetal complications in the case of twins' pregnancy.[25-27] No association with maternal employment status was observed although occupation may be a relevant factor, potentially due to the different environmental, lifestyle, social and chemical exposures.[27-29] Unexpectedly, our results showed a significant trend toward less twin births among mothers highly educated. Reversely, in Greece higher twin rates are associated with higher maternal education, better paid parental occupations, and thus wealthier families, married maternal status, while immigrants present a lower twinning rate than Greeks.[29]

To our knowledge, the EXHES cohort is among the first European cohorts that provides comprehensive information on the Exposome of the mother-child dyad in the case of both singletons and twins. In addition to being based on a real-life population, EXHES is designed to have longitudinal follow-up extending into the childhood of the children, thereby enabling the investigation of children development in relation to their short-term and long-term exposome of the first 1000 days of life (namely prenatal, perinatal, and postnatal exposures).

In the long term, the EXHES cohort should also enable omics-based analyses, examining differences in DNA methylation patterns both between twins and singletons, as well as between monozygotic and dizygotic twins for a subset of the children, potentially providing insights into the epigenetic signatures associated with twinning status and zygosity, further elucidating the complex interplay between both chemical and environmental factors, genomic regulation, and early life development.

Although other major mother-child cohorts, including the Generation R Study,[30] the Norwegian Mother, Father and Child

Cohort Study,[31] the Canadian Initiative for Active Surveillance of the Mother-Child Cohort (CAMCCO),[32] the European HELIX cohort[33] as well as the French Mother-Child cohort (EDEN) and the national French cohort (ELFE)[22,34] have previously investigated components of the Exposome, namely specific environmental exposures, such as air pollution, diet, and chemical contaminants, in relation to various health outcomes, few cohorts have considered twinships. The twin study, or "twinship," is a powerful methodology in genetics and environmental epidemiology research. When combined with the exposome, it offers unique opportunities to understand how environmental and genetic factors interact to influence human health. Twin cohorts also enable control of genetic variability when evaluating the exposome, including the microbiome, metabolome and epigenome.

This has been developed by Drouard et al. who have revealed that twins with different lifestyles tend to be less similar in terms of both their internal and external exposome profiles, even though they share at least half of their genetic makeup as well as a common family environment.[35] Similarly, studies conducted on large MZ-twin cohorts have demonstrated that environmental factors are the major causes of chronic diseases such as leukemia and asthma or certain cancer types. Exposure-related factors, as characterized by the overarching "exposome" construct, generally account for over 90% of the risk associated with the development of these chronic health conditions.[36] Correspondingly, epidemiological studies have elucidated associations between occupational history or chemical exposures and specific disease pathologies. Graham et al. reported a robust, statistically significant link between regular vehicle maintenance and amyotrophic lateral sclerosis (ALS) incidence in discordant monozygotic twins.[37] Furthermore, the same study also found a notable association between professional paint use and the occurrence of motor neurone disease.[37]

A study conducted by Fraga et al. has revealed that epigenetic modifications may underlie the discordance observed between MZ-twins with respect to the development of common disease pathologies.[38] Importantly, these epigenetic differences may represent the mechanistic interface through which environmental factors influence phenotypic variability among genetically identical MZ-twins.[38] Research using the Swedish twin registry has supported the hypothesis that environmental factors play an important role in the development of Adolescent Idiopathic Scoliosis (AIS).[39,40] This study found a unique environmental effect size of 0.60 for AIS, indicating that environmental influences are substantial in the etiology of this condition. Additionally, studies have shown that the concordance rates among MZ-twins with AIS range from 0.73 to 0.92,[40-42] with lower concordance rates of 0.13 and 0.10 have been reported by the Danish and Swedish twin registries, respectively.[42,39]

The less than 100% concordance rates observed in MZ-twins further supports the important role of environmental, lifestyle factors in the development of several pathologies. Pietiläinen et al. showed that genetics factors account for 80% of BMI variation in adolescents aged 16-17, with twin boys being leaner than singletons, and a higher prevalence of overweighting in singletons.[25] Gordon et al. conducted a study to investigate the involvement of environmental and lifestyle factors in the development of Crohn's disease (CD) and ulcerative colitis (UC) among MZ and DZ-twins.[27] Their study showed that the environmental factor most strongly implicated in the development of CD was smoking. In contrast, for UC, breastfeeding was found to have a protective effect, while a history of frequent gastroenteritis was associated with future disease development.[27] Notably, the same study revealed that the concordance rate did not reach 100% for either CD or UC, even among MZ and DZ-twins.[27]

## What are the main strengths and weaknesses?

The primary strengths of the EXHES is its general population-based design, with the inclusion of women early in pregnancy (between the second and third trimester of gestation) in a systematic way. Additionally, the EXHES study provides a wide range of data with frequent collections, particularly in the first 1000 days of life, and offers the potential of in-depth exposome construction and phenotyping through various tools and exams for both mothers (during pregnancy, at delivery, and postpartum) and for the child. In doing it, EXHES uses standardized and tailored questionnaires to gather information. Lastly, as previously mentioned, EXHES brings population-based pairs of twins. Although there are several Twins Registries in Europe. While twin registries are valuable for their large sample sizes and genetic focus, they may lack the depth of time-specific data and the adaptability of a cohort study designed for continuous and comprehensive data collection.

We acknowledge that this study has some limitations. First, the EXHES cohort exhibits a selection bias due to its recruitment strategy, which favored urban mothers in most countries. This could limit the generalizability of the findings, as it may not accurately reflect the experiences of rural mothers. Consequently, important health disparities and unique environmental factors affecting maternal health could be overlooked. Secondly, the follow-up was run in part conducted during the COVID epidemics, which limited the participation. As a consequence, missing data and related bias are common in EXHES and do not allow a complete comparison. However, mothers were recruited at the main maternity of the involved cities in an exhaustive way during a certain period of time. In addition, these constitute the reference center for twin births that are potentially considered as at-risk outcomes.

As a general comment, using twin data in a context where there are distributional differences between twin and singleton cohorts like in EXHES requires a careful, methodologically sound approach. However, although twin cohorts differ from singleton populations in key characteristics (eg, birth weight, gestational age, early-life exposures), they remain a powerful resource for understanding the etiology of complex diseases such as asthma and allergies, overweight and neurodevelopmental troubles in children. Their main strength lies not in providing generalizable prevalence estimates, but in offering unique analytic opportunities to disentangle genetic, shared environmental, and individual-specific effects. By comparing monozygotic and dizygotic twins or leveraging within-pair differences—especially in discordant monozygotic twins—researchers can control for confounding by shared genetics and family environment. This allows for more robust causal inference and investigation of gene-environment interactions, epigenetic effects, and exposure-outcome relationships with reduced bias. To address the nonrepresentativeness of twins relative to the general population, findings from twin cohorts should be complemented by analyses in singleton cohorts, or adjusted statistically to account for distributional differences. Twins are thus best used as mechanistic or analytic subcohorts, rather than standalone populations for population-level inference. In sum, despite inherent limitations, twin data are invaluable for advancing mechanistic understanding of environmental health effects and should be strategically integrated into broader research frameworks.

The limited size of the study cohort and the presence of missing values for certain variables restrict the ability to investigate some associations and extreme values of continuous interval variables, resulting in wider confidence intervals around the estimates. The small sample size for twins reduced the statistical power to detect and analyze uncommon outcomes, while the missing data introduced uncertainty and decreased the effective sample size for analyses involving those variables. Consequently, the precision and statistical significance of the findings were diminished, particularly for rare events or extreme values of continuous variables. However, our paper is intended to provide a preliminary description of differences between twins and singletons.

Further statistical analyses will follow. In this context, two types of power calculations will be conducted. First, a traditional power analysis will estimate the minimum detectable effect size for a predefined association—such as a significant increase in asthma risk associated with $PM_{2.5}$ exposure—given the available sample size, variability, and significance level. Second, a power for discovery analysis will be used in high-dimensional settings such as exposome-wide association studies (ExWAS) and metabolome-wide association studies (MWAS), to assess the expected ability to detect true associations among hundreds of environmental exposures and biological features (eg, metabolites) with asthma. This approach accounts for multiple testing and controls for the false discovery rate, providing a more realistic picture of discovery potential in omics-scale datasets.

### Ongoing research

The EXHES study will continue to follow the children to study the impact of the exposome on their health. The combination of twin and singleton cohorts, when enriched with external and internal exposomic data, will allow for a multi-scale systems approach to environmental health. Twin studies serve as a powerful tool to uncover causal pathways and biological embedding, while singleton cohorts ground findings in population health relevance. Together, they provide a robust framework to move from association to causation, and from exposure assessment to actionable insight in the era of climate change and the allergy epidemic. More in detail, these complementary cohorts enable a multilayered exploration of environmental impacts on health. Twin studies, especially using monozygotic pairs, offer a powerful design to isolate environmental effects from genetic and early-life confounding, enhancing causal inference. In parallel, singleton cohorts provide broader population-level insights into exposure–outcome associations, critical for public health relevance and generalizability. By integrating external exposome data (eg, air pollution, urban environment) with internal markers (eg, metabolomics, epigenetics), the cohorts allow for multi-omics mediation analyses, shedding light on biological mechanisms linking exposures to disease. Moreover, gene–environment interaction analyses can uncover genetically vulnerable subpopulations, informing targeted prevention. Together, these approaches support robust, mechanistically informed, and policy-relevant research on the health impacts of environmental exposures.

## Conclusions

The EXHES study provides a detailed protocol allowing the implementation of a dataset that highlights the differences and commonalities in maternal characteristics, health conditions, and children's outcomes across multiple European countries. The cohort's data allows key insights into maternal health, lifestyle, and environmental exposures during pregnancy and their potential long-term impacts on child health, especially when comparing singletons and twins.

As expected, twin pregnancies were associated with a higher prevalence of pregnancy complications, such as preterm birth, lower birth weight, and maternal health issues. Twins also displayed lower anthropometric measures at birth compared to singletons, which can be attributed to shared intrauterine environments and the increased risks inherent in multiple pregnancies.

The study underscores the potential for exposome research, particularly the value of twin studies in understanding how environmental exposures and genetics interact to influence long-term health. The findings suggest that future follow-up studies should focus on the association between early-life environmental exposures (including multiple chemical exposures) and the development of conditions such as asthma, obesity, and neurodevelopmental disorders

While the study provides valuable data from a general population cohort, its limitations include selection bias due to the urban recruitment strategy and the presence of missing data, which may impact the statistical power and generalizability of the results.

The EXHES study plans to extend its follow-up into childhood and adulthood, which will enable deeper analysis of the exposome's influence on health trajectories. The inclusion of omics data, particularly DNA methylation, offers promising avenues for understanding the epigenetic effects of early-life exposures.

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## Author contributions

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## Supplementary material

Supplementary material is available at *Exposome* online.

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## Conflicts of interest

The authors have no conflict of interest. Dimosthenis Sarigiannis holds the position of Editorial Board Member and did not participate in the peer-review or make any editorial decisions for this manuscript.

## Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

## Ethics committee

**France**
Ethic Committee Name: The OUEST IV—NANTES personal protection committee
Approval Code: TLT/BB CPP N°458/2017
Approval Date: Nantes, July 13, 2017

**Italy**
The study was approved by the local Institutional Ethics, Committee (Palermo 1, Italy, No. 07/2017). All the participants' mothers were informed about all aspects of the research, and provided their written consent before study entry.

**Spain**
Ethic Committee Name: Comité d'Ètica d'Investigació Clínica (CEIC) del Hospital Sant Joan de Reus. Ethical Committee of Clinical Research of the Hospital "Sant Joan" of Reus
Approval Code: 16-04-28/4aclapoj2
Approval Date : April 28, 2016

**Portugal**
Ethic Committee Name: Comissao de Etica par a saude (Conselho de Administracao)
Approval Code: 2016-196 (166-DEFI/155-CSES)
Approval Date: June 28, 2017

**UK**
REC name: North West—Greater Manchester East Research Ethics Committee
REC reference: 17/NW/0153
Date of REC Opinion: April 20, 2017
IRAS ID: 187679

**Poland**
Bioethical Committee of the Nofer Institute of Occupational Medicine, Lodz, Poland (Decision No. 7/2016; April 1, 2016)

**Slovenia**
Ethics Committee Name: Research Ethics and Deontology Committee of the Aristotle University of Thessaloniki
Approval Code: 140540/2018
Approval Date: November 13, 2018

**Greece**
Ethics Committee Name: Aristotle University of Thessaloniki Committee of Biothics and Code of Conduct
Approval Code: 140540/2018
Approval Date: November 13, 2018

**Croatia**
Ethic Committee Name: The Ethics Committee of the University Hospital Centre Rijeka
Approval Code: Klasa: 003 - 05/16-1/29 Ur.broj: 2170-29-2/1 - 16-2
Approval Date: Rijeka June 08, 2016.

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