Birth-related characteristics, congenital malformation, maternal reproductive history and neuroblastoma: the ESCALE study (SFCE)

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Abstract

Since neuroblastoma (NB) occurs very early in children's lives, it has been hypothesized that pre- and perinatal factors may play a role in its etiology. This study investigated the role of birth characteristics, congenital malformation and maternal reproductive history in neuroblastoma. The data used were generated by the national population-based case-control study, ESCALE, conducted in France in 2003–2004. The mothers of 191 neuroblastoma cases and 1681 controls, frequency-matched by age and gender, were interviewed by telephone, using a standardized questionnaire, on several factors including pregnancy, medical history, lifestyle, childhood medical conditions and exposures. A positive association between congenital malformation and all neuroblastoma cases was observed (Odds ratio (OR) = 2.2, 95% confidence interval (95% CI): 1.1–4.5). Congenital malformations were highly associated to neuroblastoma in children aged less than 1 year (OR = 16.8, 95% CI: 3.1–90), while no association was observed in children aged 1 year or more (OR = 1.0, 95% CI: 0.3–2.9). A negative association with a maternal history of spontaneous abortions was also found (OR = 0.6, 95% CI: 0.4–0.9). The results strongly support the hypothesis that congenital anomalies may be associated with neuroblastoma, particularly in infant (less than 1 year of age).

MESH Keywords Adolescent ; Adult ; Birth Order ; Birth Weight ; Case-Control Studies ; Child ; Child, Preschool ; Congenital Abnormalities ; diagnosis ; Female ; France ; epidemiology ; Gestational Age ; Humans ; Infant ; Male ; Maternal Age ; Neuroblastoma ; diagnosis ; Pregnancy ; Questionnaires ; Reproductive History ; Socioeconomic Factors

Author Keywords child ; neuroblastoma ; congenital anomalies ; epidemiology ; risk factors.

INTRODUCTION

Neuroblastoma (NB) is a malignant embryonal tumour of the autonomic nervous system derived from neural crest cells. It has the earliest incidence peak of childhood cancers and is the most frequent cancer in infants (aged less than one year).1 The median age at diagnosis is 22 months, and 85% of cases are diagnosed before the age of 5 years.2

Little is known about the etiology of neuroblastoma and the early age at diagnosis strongly suggests that pre- and perinatal factors may play an important role in its pathogenesis. Subgroups characterized by age at diagnosis, stage and MYCN oncogene amplification status, which are known to be important prognostic factors, may be of etiologic relevance.3

The literature on pre- and perinatal factors in neuroblastoma is relatively limited and the results of the various studies are most often inconsistent. Pre-term (< 37 weeks) and post-term (> 41 weeks) birth, low birth weight (< 2500 grams) and high birth weight (> 4000 grams), have been associated with neuroblastoma, with positive or negative associations for the same risk factor, depending on the study.[4–9] Maternal reproductive factors such as fetal losses or use of sex hormones have also been associated with neuroblastoma in some studies,[10–12] while others did not show any association.[4–6,13]

Birth defect constitutes the factor that has been most consistently positively associated with NB in several studies.[13–22]

Very few papers have reported analyses by NB subgroups. Only the study by the Children's Oncology Group has suggested that associations with previous miscarriages and previous induced abortion may depend on MYCN oncogene amplification status.[10]

This study therefore investigated the role of birth-related characteristics, birth defects and maternal reproductive history in the etiology of neuroblastoma using the data generated by the ESCALE study. ESCALE is a French national population-based case-control study, which was carried out to assess the role of infectious, environmental and genetic factors in 4 childhood cancers: leukemia, lymphoma, malignant central nervous system tumor and neuroblastoma.

MATERIAL AND METHODS

Study population

Details of the study design have been provided elsewhere.[23] Briefly, the cases were children under the age of 15 years, in whom neuroblastoma had been newly diagnosed between January 1, 2003, and December 31, 2004, and who were residing in mainland France at the time of diagnosis. All the cases were directly recruited by the investigators in each French pediatric oncology hospital department within the network of the two National Registries of childhood cancer: the National Registry of Childhood Hematological Cancers (RNHE)[24] and the National Registry of Childhood Solid Tumors (RNTSE).[25]

The eligibility criteria were the biological mother's availability for interview, a telephone in the home, the biological mother's ability to speak French and a surviving index child who was not terminally sick. Out of the 276 cases of NB diagnosed in 2003–2004, 41 were not eligible (22 children had died or were terminally sick, 14 mothers did not speak French and 5 mothers had serious psycho-social disorders). Out of the 235 eligible cases, 44 (16 percent) were not included because the mother refused to participate (n = 18) or could not be contacted (n = 28). Finally, 191 incident cases (81%) were included using the International Classification of Childhood Cancer based on the ICD-O-3 (ICD-O-3 codes 9500/3 and 9490/3).[26]

The controls were randomly selected from the general population using a sample of 60,000 phone numbers representative of the French population. Control selection was stratified using quotas ensuring frequency matching with cases by age and gender. The quotas were designed to be representative of all cancer cases in terms of age and gender. Additional quotas were designed to ensure that the control group was representative of the French population in terms of the number of children living in the household, conditionally on age group. Out of the 50,217 phone numbers called, 46,994 were non-eligible numbers (22,584 businesses or disconnected numbers, 18,456 household without children, 5,277 out of quotas, 677 non-reliable interviews). The eligibility of 862 phone numbers could not be determined. Finally, out of the 2361 remaining eligible controls, 1681 (71%) were included in the study (679 parents refused and one child had a prior history of neuroblastoma).

Data collection

All case and control mothers responded to a standardized telephone interview conducted by trained interviewers and lasting approximately 40 minutes. The cases' mothers were interviewed at least 2 months after the diagnosis. The interview focused on demographic and socioeconomic characteristics, childhood environment and lifestyle, familial and personal medical history, parental occupational history, and maternal reproductive history.

The birth-related characteristics included gestational age (pre-term less than 37 complete weeks of pregnancy, 37 to 39 weeks, 40 to 41 weeks and post-term more than 41 weeks), birth weight in quintiles and in more usual categories (< 2500, 2500 to 2999, 3000 to 3499, 3500 to 3999 and ≥ 4000 grams), maternal age at birth (less than 25 years, 25–34 years, 35 years or more) and birth order (first, second, third or more).

Mothers were asked if the index child was born with a congenital malformation and, if so, to state the malformation site by choosing from a list of organs. The malformations were coded and reclassified if necessary using the ICD-10 (codes Q00–Q99), with the coder blind to case-control status. The malformations were categorized as minor or major congenital anomalies using the European Surveillance of Congenital Anomalies (EUROCAT) recommendations.[27]

Maternal reproductive history included questions on maternal history of fetal losses defined as any pregnancy loss (spontaneous abortions, abortions and termination of pregnancy for medical reasons: congenital anomalies, chromosomal anomalies, German measles or toxoplasmosis) and the use of fertility treatments for the index child, such as ovarian stimulation, in vitro fertilization and artificial insemination.

Statistical analysis

All the analyses were performed using SAS software (version 9.1, Gary, NC, USA). Odds ratios and 95% confidence intervals were estimated using unconditional logistic regression models including the stratification variables, gender and age. Because of the particular age distribution of NB, narrow age groups were defined in order to avoid possible residual confounding by age: 2, 4, 6, 8, 10, 12, 18, 24 months and 3, 4, 5 to 6, 7 to 8, 9 to 11 and 12 to 14 years. The analysis was also restricted to the subgroup aged less than 12 years given the small number of cases aged 12 or more years.

We also stratified the analyses by age (< 1 year / ≥ 1 year), known to be an important prognostic factor which may be of etiologic relevance.

Potential confounding by socioeconomic category, maternal educational level and maternal working during pregnancy was also considered in the various analyses.

Additional analyses were conducted by subgroups of NB defined by age at diagnosis (age < one year / age ≥ one year) and MYCN oncogene amplification status (amplified (MYCN+)/non-amplified (MYCN−)).

MYCN copy numbers were determined by the national laboratories of reference.

RESULTS

Table 1 describes the study population. Of the 191 cases included, the MYCN oncogene was amplified (MYCN+) in 35 cases (18%), not amplified (MYCN−) in 144 cases (76%) and not available for 12 cases (6%).

The case and control gender distributions were similar (table 1). The control group had the same age distribution as the whole ESCALE case population, but was older, on average, than the neuroblastoma group. However, there were at least two controls for each case in each age stratum. The cases and controls did not differ significantly with respect to familial status, maternal educational level, or maternal working during pregnancy (table 1). Nonetheless, the case parents (25%) were slightly more frequently factory and agricultural workers or unemployed than the control parents (16%) (p=0.04).

Table 2 shows the associations between birth-related characteristics and neuroblastoma. The case mothers were more often aged less than 25 years at the index child's birth than the control mothers (OR = 2.3, 95% CI: 1.5–3.6). No association between gestational age or birth weight and neuroblastoma considered as a whole was observed. However, a U-curve between birth weight and MYCN+ NB cases was observed for the highest quintile (≥ 3720 grams) (OR: 2.6, 95% CI: 0.9–7.8). A significant inverse relationship with birth order was also observed (OR = 0.6, 95% CI: 0.4–1.0 for ≥ third born). The association appeared to depend on MYCN status, with an OR of 0.4 and 95% CI of (0.3–0.8), for MYCN- cases versus an OR of 1.5 and 95% CI of (0.7–3.1) for MYCN+ cases.

Overall, congenital malformations had been diagnosed for six percent of the cases and three percent of the controls (OR = 2.2, 95% CI: 1.1–4.5) (table 3). The odds ratios increased as the number of anomalies increased (OR = 2.1, 95% CI: 1.0–4.6 and OR = 3.1, 95% CI: 0.6–17 for one malformation and two or more malformations, respectively, p_{trend} = 0.03). The association was a little more marked for major malformations (OR = 2.4, 95% CI: 1.1–5.4) than for minor malformations (OR = 1.7, 95% CI: 0.4–7.7). The strongest associations, although based on small numbers, were observed with urinary tract malformations (OR = 4.4, 95% CI: 1.1–17) and skeletal malformations (OR = 3.3, 95% CI: 1.1–9.6). Only 5 controls and none of the cases had a cardiac malformation.

Despite the small number of NB with MYCN oncogene amplification (n = 35), the association with major anomalies was slightly more pronounced for MYCN+ cases (OR = 4.4, 95% CI: 1.2–16) than for MYCN− cases (OR = 1.9, 95% CI: 0.7–5.2).

Table 4 shows the results for maternal reproductive history. Neither a maternal history of any fetal losses (OR = 0.9, 95% CI: 0.6–1.2) nor a maternal history of abortion (OR = 1.2, 95% CI: 0.8– 1.8) was associated with neuroblastoma and the results were not affected by MYCN amplification status. In contrast, a clear inverse relationship with a maternal history of spontaneous abortion was observed (OR = 0.6, 95% CI: 0.4–0.9). The ORs decreased as the number of spontaneous abortion increased (p_{trend} = 0.01). The negative association was limited to the MYCN− NB cases (OR = 0.6, 95% CI: 0.3–1.1 and OR = 0.3, 95% CI: 0.1–0.8 for one miscarriage and two or more miscarriages, respectively, p_{trend} = 0.004). While termination of pregnancy for medical reasons was not associated with neuroblastoma,

irrespective of MYCN amplification status, it is noteworthy that, although only based on one case (3%) and 14 controls (1%), an elevated OR of 3.3 (95% CI: 0.4–30) was observed in MYCN+ cases when the medical reason for termination was congenital anomalies. Finally, no association between neuroblastoma and the use of fertility treatments of any type (drug stimulation only, in vitro fertilization, artificial insemination) for the index child was evidenced (OR = 0.9, 95% CI: 0.4–2.0).

Table 5 presents the previous analyses stratified by age (< 1 year / ≥ 1 year). We did not find any association with none of the investigated perinatal factors (birth weight, gestational age, congenital malformation, spontaneous abortion and the use of fertility treatments for index child) in children aged 1 year or more. Conversely, when analyses were restricted to children aged less than one year, we observed a U-shape association between neuroblastoma and birth weight (OR = 4.8, 95% CI: 1.6–15 and OR = 2.9, 95% CI: 1.0–8.0; respectively for birth weight < 2930 grams and birth weight ≥ 3720 grams). Congenital malformations were also highly associated to neuroblastoma in children aged less than 1 year (OR = 16.8, 95% CI: 3.1–90).

Adjustment for familial situation, maternal educational level and socioeconomic categories did not modify the results. The estimates also remained unchanged after mutual adjustments for the various variables of interest, but the association with high birth order lost its significance.

Lastly, excluding the 10 cases and 37 controls whose mothers did not have the children's health records available did not change the results.

DISCUSSION

ESCALE is the first French population-based case-control study including cases of frequent childhood neoplastic diseases (leukemia, lymphoma, brain tumor and neuroblastoma) on a national scale. The main results of the analysis were the positive association with congenital malformations, especially for cases with MYCN oncogene amplification, and the negative association with a maternal history of spontaneous abortions.

The size of the study enabled detection of minimum odds ratios of 1.6, 1.9 and 2.3 for exposure prevalences in controls of 30, 10 and five percent, respectively, with a type-I error of 5 percent and power of 80 percent.

Case identification was based on the network of investigators working with the two French National Registries of Childhood Cancer (RNHE and RNTSE), making a selection bias due to the case identification process unlikely. Over the two-year study period, 276 NB cases were identified. The number was equivalent to the expected incidence of NB in France.25

Out of the 276 cases identified, 41 were not eligible (20 were MYCN oncogene not amplified, 11 were MYCN oncogene amplified and 10 had MYCN oncogene status not informative). Twenty-two had died or were receiving palliative care. The non-inclusion of those 22 children may have induced survival bias. Indeed, those children were younger (average age of 1.5 versus 2.4 years, p=0.01) and more often MYCN oncogene amplified (45% versus 18%, p=0.002) than included children. However, a history of malformation is unlikely to lead to a better prognosis, and there is no obvious reason for a maternal history of miscarriages being related to a poorer prognosis. In addition, the prevalence of MYCN oncogene amplification, which is an important prognostic factor, was 18 percent, similar to that of the published clinical series.2,28 Nineteen percent (n = 44) of the eligible cases did not respond. However, the age and gender distributions of the respondent and non-respondent cases were similar. The controls were randomly selected from the general population using the national telephone directory as a random basis. Unlisted numbers were computer generated prior to the random selection in order to avoid listed-control selection bias. The sampling process also made the controls similar to the French population in terms of number of children living in the household and, subsequently, in terms of birth order, when compared with the national perinatal surveys.29,30 The comparison with those surveys also showed that the controls were very similar to the French population in terms of mother's educational level, gestational age, birth weight and maternal history of voluntary abortion, miscarriage and use of assisted reproductive technologies. The controls' mothers were slightly older than in the overall population. In fact, only 10% of the control mothers were aged less than 25 years at the birth of the index child, compared to 17% and 19% in the 1998 and 2003 national surveys, respectively. Thus, the association observed between maternal age < 25 years at birth and neuroblastoma may be due to an underestimation of the number of young mothers among the controls. However, maternal age was not related to the variables of interest and adjustment on maternal age did not change any of the results.

The control mothers were very similar to the case mothers with respect to their familial situation, educational level and working during pregnancy, but their socioeconomic categories were slightly higher. However, the results remained unchanged after additional adjustment for those factors.

The use of standardized questionnaires and the similar interviewing conditions for case and control mothers reduced potential differential misclassifications. In order to facilitate recall and increase efficiency, at the beginning of the interview, the mothers were asked to fetch the index child's health record. Moreover, completion of the health record, especially for birth-related characteristics (birth weight

and gestational age), was independent of the case/control status. Only 10 case mothers (5%) and 37 control mothers (2%) did not have access to their children's health record during the interview ($p = 0.02$). In any event, the analyses conducted after excluding those cases and controls gave similar results.

The literature on birth-related characteristics is still inconsistent. Both, low birth weight[5,6,8] and high birth weight[7,9] have been positively associated with neuroblastoma, but five other studies did not detect any association.[4,10–13] As was the case in the present study, most studies have not evidenced any association with gestational age, even though others have shown a positive association[5] or a negative association[4,8] with pre-term birth (< 37 weeks). The negative association between neuroblastoma and high birth order observed in the present study is in line with the findings of two other studies.[4,13] It has been suggested that the "hygiene hypothesis" proposed with respect to childhood acute leukemia[31] should also be considered for neuroblastoma.[32,33]

A potential drawback of case-control studies is the lack of reliability, possibly differential, of maternal recall. Since the congenital malformation data were obtained by interviewing case and control mothers and not from medical records, there was an opportunity for a recall bias to occur. The results of the present study may be explained by over-declaration of malformations by case mothers or under-declaration by control mothers. However, the results remained the same when the analyses were restricted to malformations considered major by EUROCAT which are less liable to differential recall bias.[27]

Another concern is that the observed association between congenital anomalies and neuroblastoma may be the consequence of over-detection of asymptomatic anomalies in the course of disease staging. However, the medical records of 9 out of 12 cases with a malformation were checked (the 3 remaining medical records were not available). All the malformations were diagnosed before the neuroblastoma and all the diagnoses were the same as those reported by the mothers. In addition, associations remained unchanged after excusion of cases whose malformation was not validated and thus, specifically for children aged less than 1 year (any malformation: OR= 12.3 [2.1–71], major malformation: OR=23.1 [2.5–214]). Nevertheless, we can not completely rule out that some neuroblastoma cases may have been diagnosed in the process of evaluating a child with a known malformation.

The relationship found between neuroblastoma and congenital malformation is consistent with most of the previous studies,[13–22] although no cardiovascular malformations were observed in the cases.[15,17,19,22,34,35] Those previous studies used different methods to assess congenital malformations (medical records, birth certificates and hospital records, birth defect registries and parental self-report) which may have led to various potential biases.

Our results may be an argument for the involvement of developmental genes in neuroblastoma. Indeed, the PHOX2B gene, a human development gene, has already been associated with Congenital Central Hypoventilation Syndrome (CCHS, or Ondine's curse) which, like neuroblastoma, is a disorder of autonomic nervous system development.[35] The specific association between neuroblastoma and congenital malformation in infants has also been observed in a recent study based on birth certificates and hospital records.[22]

Maternal reproductive history has rarely been investigated in the literature and the terminology used in the various studies does not always enable spontaneous abortions, abortions and termination of pregnancy for medical reasons to be distinguished. The terms used by the mothers themselves are probably not always reliable but they are likely to be similarly used by the cases and controls. Most previous studies did not find any association with fetal losses overall,[5,6,13] induced abortions,[4] or spontaneous abortions.[4] Nonetheless, one study reported a negative association between neuroblastoma and all fetal losses,[11] two studies found a positive association with prior spontaneous abortions[10,12] and one study also found a positive association with induced abortions.[10]

In contrast, in the present study, a relatively strong inverse association with maternal history of spontaneous abortions was observed and that association decreased as the number of miscarriages increased ($p = 0.01$).

Over the last 30 years, rapid advances have been made in the treatment of infertility. While the effects of infertility treatments on birth and short-term outcomes are relatively well documented, little is known about potential effects on child health after the neonatal period. Childhood cancer and especially neuroblastoma, which may be initiated during the early stages of fetal development, may be a possible adverse outcome of the use of infertility treatments. Indeed, out of five case-control studies, three reported a positive association with the use of sex hormone exposure for infertility (induction of ovulation),[37–39] one reported a non significant association[40] and one did not find any association.[12] Moreover, among six cohort studies investigating the incidence of childhood cancer in children born following infertility treatments,[41–46] two found an elevated, but non-significant, SIR for neuroblastoma[40,1,] 42 and, interestingly, one study did not find any association with childhood cancer but did detect an association with congenital anomalies.[45]

In conclusion, our results strongly support the hypothesis that congenital anomalies may be associated with neuroblastoma, particularly in children aged less than 1 year.

Ackowledgements:

This work was supported by grants from INSERM, the Fondation de France, the Association pour la Recherche contre le Cancer (ARC), the Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS), the Agence Française de Sécurité Sanitaire de l'Environnement et du Travail (AFSSET) and the association Cent pour Sang la Vie.

We are grateful to: Marie-Hélène Da Silva and Dr. Christophe Steffen (INSERM, U754), who coordinated the recruitment of the cases; Aurélie Goubin and the staff of the French National Registry of Childhood Blood Malignancies (RNHE), Sandra Guissou and Dr. Emmanuel Désandes and the staff of the French National Registry of Childhood Solid Tumors (RNTSE, who contributed to case detection and verification; Sabine Mélèze and Marie-Anne Noël (Institut CSA), who coordinated the selection of the controls and the interviews; Catherine Tricoche (Callson) and the team of interviewers, who interviewed the cases and the controls; Drs. Jean Bernard from the "Laboratoire de Pharmacologie Moléculaire" (Institut Gustave Roussy, Villejuif, France), Valérie Combaret from the "Laboratoire de Biologie Cellulaire" (Centre Léon Bérard, Lyon, France), Jérome Couturier and Olivier Delattre from the "Laboratoire de Génétique des Tumeurs" (Institut Curie, Paris, France), who determined the MYCN copy numbers; and Andrew Mullarky for his skilful revision of the manuscript.

Footnotes:

*SFCE: Société Française de lutte contre les Cancers de l'Enfant et de l'adolescent

Novelty and impact of our paper: This paper investigated the role of perinatal factors (birth weight, birth order, congenital anomalies, spontaneous abortion and the use of fertility treatments) in children with neuroblastoma in France at a national scale. We report that congenital malformations were more frequent in neuroblastoma cases than in population controls, and particularly for children aged less than 1 year which has nerver been described before.

Abbreviations

MYCN+: amplification of MYCN oncogene
MYCN−: Non-amplification of MYCN oncogene
NB: neuroblastoma
OR: Odds Ratio
95% CI: 95% Confidence Interval

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Table 1
Sample description for cases and controls

	Cases n = 191 (%)	Controls n = 1681 (%)	p
MYCN oncogene status			
Amplified (MYCN +)	35 (18)	-	
Non-amplified (MYCN −)	144 (76)	-	
Non-informative (NI)	12 (6)	-	
Gender			**ns**
Male	97 (51)	932 (55)	
Age (year)			**$<10^{-4}$**
< 1	74 (39)	187 (11)	
1	36 (19)	182 (11)	
2	30 (16)	153 (9)	
3	13 (7)	166 (10)	
4	11 (6)	145 (9)	
5–6	16 (8)	228 (14)	
7–8	3 (2)	163 (10)	
9–11	6 (3)	225 (13)	
12–14	2 (1)	232 (14)	
Familial situation [1]			**ns**
Couple	180 (94)	1541 (92)	
Single	8 (4)	75 (4)	
Separated or widowed	3 (2)	65 (4)	
Socioeconomic categories [1]			**0.04**
Intellectual and scientific jobs, managers and intermediate professions	74 (39)	713 (42)	
Administrative and sales workers	49 (26)	477 (28)	
Service workers	21 (11)	215 (13)	
Factory and agricultural workers, unemployed	47 (25)	274 (16)	
Maternal education [1]			**ns**
≤ High school graduation	102 (53)	979 (58)	
> High school graduation	89 (47)	701 (42)	
Maternal working during pregnancy [1]			**ns**
No	53 (28)	531 (32)	
Yes	138 (72)	1150 (68)	

ns: $p > 0.05$
1 Adjusted for age and gender

Table 2

Birth-related characteristics and neuroblastoma

		Neuroblastoma								
		All neuroblastoma			MYCN non-amplified			MYCN amplified		
	Controls n = 1681	n = 191	OR[1]	95% CI[2]	n = 144	OR	95% CI	n = 35	OR	95% CI
Maternal age at birth (year)										
<25	163	35	**2.3**	(1.5–3.6)	25	**2.2**	(1.3–3.7)	5	**1.7**	(0.6–4.7)
25–34	1235	124	**1.0**	reference	97	**1.0**	reference	22	**1.0**	reference
35 +	283	32	**0.9**	(0.5–1.4)	22	**0.8**	(0.5–1.3)	8	**1.2**	(0.5–2.8)
Birth weight (gram) [c]										
<2500	94	15	**1.8**	(0.8–3.8)	9	**1.6**	(0.6–4.0)	5	**2.2**	(0.5–11)
2500–2999	304	39	**1.3**	(0.8–2.1)	34	**1.6**	(0.9–2.6)	5	**1.1**	(0.3–3.4)
3000–3499	671	62	**1.0**	reference	48	**1.0**	reference	9	**1.0**	reference
3500–3999	464	53	**1.2**	(0.8–1.8)	41	**1.2**	(0.7–1.9)	11	**1.8**	(0.7–4.5)
≥4000	147	21	**1.6**	(0.9–2.8)	12	**1.2**	(0.6–2.4)	5	**2.6**	(0.8–8.2)
Birth weight in quintiles (gram) [c]										
<2930	334	50	**1.4**	(0.8–2.3)	40	**1.8**	(1.0–3.2)	9	**1.3**	(0.4–4.6)
2930–3199	316	29	**0.8**	(0.4–1.3)	26	**1.0**	(0.5–1.9)	3	**0.7**	(0.2–3.0)
3200–3449	353	35	**1.0**	reference	25	**1.0**	reference	5	**1.0**	reference
3450–3719	338	30	**0.9**	(0.5–1.6)	22	**1.0**	(0.5–1.8)	7	**1.7**	(0.5–5.4)
≥3720	339	46	**1.2**	(0.7–2.0)	31	**1.1**	(0.6–2.0)	11	**2.6**	(0.9–7.8)
Duration of gestation (week) [d]										
<37	111	17	**1.0**	(0.5–2.0)	9	**0.7**	(0.3–1.6)	6	**2.8**	(0.8–9.5)
37–39	493	61	**1.0**	(0.7–1.4)	50	**1.0**	(0.7–1.8)	9	**0.9**	(0.4–2.2)
40–41	1022	108	**1.0**	reference	81	**1.0**	reference	20	**1.0**	reference
>41	40	3	**0.8**	(0.2–2.7)	2	**0.6**	(0.1–3.0)	0	-	-
Birth order (index child)										
1	708	96	**1.0**	reference	76	**1.0**	reference	14	**1.0**	reference
2	608	62	**0.7**	(0.5–1.1)	49	**0.7**	(0.5–1.1)	10	**0.9**	(0.4–2.1)
3+	365	33	**0.6**	(0.4–1.0)	19	**0.4**	(0.3–0.8)	11	**1.5**	(0.7–3.1)

1 Odds ratios adjusted for age and gender;

2 95% CI: 95% Confidence Interval

Table 3

Congenital malformations and neuroblastoma

		Neuroblastoma								
		All neuroblastoma			MYCN non-amplified			MYCN amplified		
	Controls n = 1681	n = 191	OR[1]	95% CI[2]	n = 144	OR	95% CI	n = 35	OR	95% CI
Any malformations										
No	1624	179	**1.0**	reference	136	**1.0**	reference	32	**1.0**	reference
Yes	57	12	**2.2**	(1.1–4.5)	8	**2.0**	(0.9–4.7)	3	**3.3**	(0.9–12)
1	51	10	**2.1**	(1.0–4.6)	7	**2.0**	(0.8–4.9)	2	**2.7**	(0.6–12)
2/over	6	2	**3.1**	(0.6–17)	1	**2.3**	(0.3–20)	1	**6.4**	(0.7–62)
			p_{trend} = 0.03			p_{trend} = 0.12			p_{trend} = 0.04	
Minor malformations	19	2	**1.7**	(0.4–7.7)	2	**2.4**	(0.5–11)	0	**-**	-
Major malformations	38	10	**2.4**	(1.1–5.4)	6	**1.9**	(0.7–5.2)	3	**4.4**	(1.2–16)
Malformation location										
Heart	5	0	**0**	-	0	**-**	-	0	**-**	-
Digestive system	2	1	**4.6**	(0.3–69)	1	**6.8**	(0.5–102)	0	**-**	-
Urinary system	10	4	**4.4**	(1.1–17)	3	**4.4**	(1.0–20)	1	**6.4**	(0.6–64)
Genital organs	6	1	**1.7**	(0.2–15)	0	**-**	-	1	**5.4**	(0.5–54)
Skeleton	22	5	**3.3**	(1.1–9.6)	3	**3.0**	(0.8–11)	1	**3.6**	(0.4–31)
Minor	8	2	**5.1**	(0.9–29)	2	**7.7**	(1.3–44)	0	**-**	-
Major	14	3	**2.6**	(0.7–10)	1	**1.3**	(0.2–11)	1	**6.1**	(0.6–58)
Others	2	14	**0.7**	(0.1–5.7)	1	**<0.001**	(<0.001–>999)	1	**5.2**	(0.5–51)

1 Odds ratios adjusted for age and gender;

2 95% CI: 95% Confidence Interval

Table 4

Maternal reproductive history and neuroblastoma

		Neuroblastoma								
		All neuroblastoma			MYCN non-amplified			MYCN amplified		
	Controls n = 1681	n = 191	OR[1]	95% CI[2]	n = 144	OR	95% CI	n = 35	OR	95% CI
Any fetal losses										
No	1031	129	**1.0**	reference	101	**1.0**	reference	20	**1.0**	reference
Yes	650	62	**0.9**	(0.6–1.2)	43	**0.8**	(0.5–1.2)	15	**1.3**	(0.6–2.6)
1	403	44	**1.0**	(0.7–1.5)	30	**0.9**	(0.5–1.4)	10	**1.4**	(0.6–3.1)
2+	247	18	**0.7**	(0.4–1.1)	13	**0.6**	(0.3–1.2)	5	**1.1**	(0.4–3.2)
Spontaneous abortions										
No	1239	160	**1.0**	reference	124	**1.0**	reference	26	**1.0**	reference
Yes	442	31	**0.6**	(0.4–0.9)	20	**0.5**	(0.3–0.8)	9	**1.0**	(0.5–2.2)
1	303	24	**0.7**	(0.4–1.1)	16	**0.6**	(0.3–1.1)	6	**1.0**	(0.4–2.6)
2+	138	7	**0.4**	(0.2–0.9)	4	**0.3**	(0.1–0.8)	3	**1.0**	(0.3–3.4)
Abortions										
No	1429	158	**1.0**	reference	119	**1.0**	reference	29	**1.0**	reference
Yes	252	33	**1.4**	(0.9–2.2)	25	**1.4**	(0.9–2.4)	6	**1.3**	(0.5–3.3)
1	209	27	**1.3**	(0.8–2.2)	19	**1.3**	(0.7–2.2)	6	**1.6**	(0.6–3.9)
2+	43	6	**1.7**	(0.7–4.5)	6	**2.4**	(0.9–6.4)	0	**-**	-
Termination of pregnancy										
No	1594	185	**1.0**	reference	140	**1.0**	reference	33	**1.0**	reference
Yes	87	6	**0.7**	(0.3–1.7)	4	**0.6**	(0.2–1.9)	2	**1.3**	(0.3–5.6)
For malformations	14	2	**1.1**	(0.2–5.6)	1	**0.8**	(0.1–6.4)	1	**3.3**	(0.4–30)
Other	73	4	**0.6**	(0.2–1.7)	3	**0.6**	(0.2–2.1)	1	**0.8**	(0.1–6.0)
Use of fertility treatments (index child)										
No	1599	182	**1.0**	reference	139	**1.0**	reference	32	**1.0**	reference
Yes	82	9	**0.9**	(0.4–2.0)	5	**0.7**	(0.3–1.8)	3	**1.6**	(0.5–5.6)
Stimulation only	41	5	**1.0**	(0.4–2.9)	3	**0.8**	(0.2–2.8)	2	**2.6**	(0.6–12)
In vitro fertilization	22	3	**1.3**	(0.4–4.6)	1	**0.6**	(0.1–4.4)	1	**1.8**	(0.2–15)
Artificial insemination	22	2	**0.8**	(0.2–3.9)	1	**0.5**	(0.1–4.2)	0	**-**	-

1 Odds ratios adjusted for age and gender;

2 95% CI: 95% Confidence Interval

Table 5

Perinatal factors (birth weight, gestational age, congenital malformation, spontaneous abortion and use of fertility treatments) and neuroblastoma: analyses stratified by age

	Children < 1 year				Children > 1 year			
	Cases (n=74)	**Controls (n=187)**	**OR[1]**	**95% CI[2]**	**Cases (n=117)**	**Controls (n=1494)**	**OR**	**95% CI**
Birth weight in quintiles (gram)								
<2930	19	31	**4.8**	(1.6–15)	31	303	**1.0**	(0.5–1.8)
2930–3199	15	38	**2.3**	(0.8–6.8)	14	278	**0.5**	(0.3–1.0)
3200–3449	7	45	**1.0**	reference	28	308	**1.0**	reference
3450–3719	12	30	**3.0**	(1.0–9.4)	18	308	**0.6**	(0.3–1.1)
≥3720	20	43	**2.9**	(1.0–8.0)	26	296	**0.9**	(0.5–1.7)
Birth weight (gram)								
<2500	4	13	**1.3**	(0.3–5.5)	11	81	**1.9**	(0.8–4.9)
2500–2999	18	28	**2.9**	(1.2–6.9)	21	276	**1.0**	(0.5–1.7)
3000–3499	19	77	**1.0**	reference	43	594	**1.0**	reference
3500–3999	23	51	**2.0**	(0.9–4.4)	30	413	**0.9**	(0.6–1.6)
≥4000	9	18	**2.7**	(0.9–7.7)	12	129	**1.3**	(0.7–2.6)
Gestational age (week)								
<37	6	13	**0.9**	(0.2–3.4)	11	98	**1.0**	(0.5–2.3)
37–39	31	71	**1.3**	(0.7–2.6)	30	422	**0.8**	(0.5–1.3)
40–41	36	100	**1.0**	reference	72	922	**1.0**	reference
>41	1	3	**0.9**	(0.1–11)	2	37	**0.8**	(0.2–3.4)
Congenital malformations								
No	67	158	**1.0**	reference	112	1439	**1.0**	reference
Yes	7	2	**16.8**	(3.1–90)	5	55	**1.0**	(0.3–2.9)
minor	0	1	-	-	2	18	**1.9**	(0.4–8.6)
major	7	1	**31.3**	(3.6–272)	3	37	**0.7**	(0.2–3.0)
Spontaneous abortions								
No	66	144	**1.0**	reference	94	1095	**1.0**	reference
Yes	8	43	**0.4**	(0.1–0.8)	23	399	**0.7**	(0.4–1.1)
Use of fertility treatments (index child)								
No	72	177	**1.0**	reference	110	1422	**1.0**	reference
Yes	2	10	**0.5**	(0.1–2.3)	7	72	**1.2**	(0.5–2.7)

1 Odds ratios adjusted for age and gender;

2 95% CI: 95% Confidence Interval