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Risk factors for perinatal arterial ischemic stroke – a large case control study

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Abstract

Aim: To identify maternal, obstetrical and neonatal risk factors related to perinatal arterial ischemic stroke (PAIS) and to understand pathophysiological concepts.

Method: For case and control ascertainment we used active surveillance in 345 pediatric hospitals and a population-based perinatal database for quality assurance of hospital care. Analysis was performed on complete cases using logistic regression. Multivariable analysis was guided by a directed acyclic graph.

Results: After exclusion of records with missing data, 134 cases and 576 controls were compared. In univariate analysis male sex, prematurity, small for gestational age (SGA), low umbilical artery pH, low 5-minute-apgar score, multiple pregnancies, hypoxia, intubation/mask ventilation, primiparity, caesarian and vaginal-operative delivery, chorioamnionitis and oligohydramnios were associated with an increased risk. Mutual adjustment yielded male sex [OR 1.81; 95 %CI 1.20-2.73], multiple birth [OR 3.22; 95 %CI 1.21-8.58], chorioamnionitis [OR 9.89; 95 %CI 2.88-33.94], prematurity [OR 1.86; 95 %CI 1.01-3.43] and SGA [OR 3.05; 95 %CI 1.76-5.28] as independent risk factors.

Interpretation: We confirmed the increased risk in males and the role of chorioamnionitis and SGA for PAIS, pointing to the importance of inflammatory processes and fetal-placental insufficiency. Multiple birth and prematurity were additional risk factors. The role of prematurity may previously have been underestimated.

What this paper adds:

- Risk factors for PAIS are discussed within the framework of a directed acylclic graph
- Chorioamnionitis and SGA clearly precede PAIS and are important independent risk factors for PAIS
- Inflammatory processes and fetal-placental insufficiency are the likely underlying causing mechanisms
- Multiple birth and prematurity were identified as additional risk factors

Perinatal arterial ischemic stroke (PAIS) has been identified as a cause of unexplained clinical conditions in newborn infants [1]. It is an important cause of chronic neurological disability, including unilateral cerebral palsy and it is the second most underlying cause of seizures in the neonate [2–4].

The etiology and the timing of onset in PAIS, however, remains unclear. Identification of risk factors helps to enhance understanding of the underlying pathophysiology as well as to characterize high risk populations.

Although previous studies have identified several risk factors, their interdependence and role in the causal pathway of PAIS are poorly understood [5–12]. Most available studies are either small, often restricted to full-term infants, lack an adequate control group or did not control for interdependencies between potential risk factors.

A recently published meta-analysis depicted some of these limitations and identified pre-eclampsia, oligohydramnios, intrapartum fever, birth asphyxia, hypoglycemia and small for gestational age (SGA), as the most likely relevant risk factors [8]. Since this study was not based on an individual patient data analysis, however, no uniform analysis could be applied. Non-uniform adjustment of the included studies may account for biased assessments.

Based on prospectively ascertained PAIS cases with extensive documentation of potential risk factors, we had the opportunity to investigate risk factors for cases both in term and preterm neonates, with four population-based controls for each case. Based on these data we validated risk factors described in previous studies and had the chance to identify hitherto unknown potential causes. In addition, we investigated potential causal pathways of risk factors in the development of PAIS.

Methods

Study Design

We conducted a case-control study, with cases recruited in the German pediatric surveillance system (ESPED) and controls from the Bavarian Working Group for Quality Assessment

(BAQ). Four controls per case were randomly selected with same birth year as the only selection criterion.

Case definition

A cerebral arterial ischemic infarction confirmed by any imaging technique within 28 days after birth diagnosed as PAIS by the responsible physician was considered as a case.

Case ascertainment

ESPED, an established active surveillance system in 345 pediatric hospitals was used for case identification. Physicians were asked to report PAIS cases on a monthly basis (including a null option) from January 1, 2015 to December 31, 2017. Report of a case prompted an anonymous questionnaire, which was answered based on pre-and postnatal medical documentation by the notifying physician. In >95% of all reported cases questionnaires were returned. Case reports were independently validated by a pediatric neurologist (LG) and three neonatologists (MK, MD, UF). A focus was on diagnostic criteria for PAIS and to differentiate PAIS from other forms of infarction (hemorrhagic stroke, cerebral venous sinus thrombosis). In case of inconclusive statements the notifying physicians was asked for further information. Our main analysis was confined to 134 of 161 reported cases with no missing values on relevant covariates (Table 1; online only).

Control Selection

The BAQ is part of a nationwide benchmarking network for assessment of clinical performance in German hospitals. The BAQ dataset, which has previously been described [13], comprises all deliveries in obstetric units of the federal state of Bavaria. Data are routinely electronically recorded by medical and paramedical hospital staff. Four controls per case, in total 644, were randomly selected and matched on birth year only. Full information was available for 576 controls (Table 1; online only).

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Variable definition for risk factors in cases and controls

All risk factors as documented in ESPED and BAQ are shown in Table 2; online only. For all variables with a null option the data were used as recorded. For variables without null option we assumed absence of the risk factor if no information was given.

We defined SGA as birthweight below 10th percentile and large for gestational age LGA; as birthweight above 90th percentile. Preterm delivery was defined as birth prior to 37+0 completed weeks of gestation. Maternal age was classified according to general standards: mothers 18 years and younger or 35 years and older were considered as high-risk pregnancy. We defined a 5-minute-Apgar below 7 as critical and an umbilical blood pH below 7.1 as an indicator for acidosis in newborns. History of abortion and miscarriage was assumed when the number of preceding pregnancies exceeded the number of births. We defined a hypertensive pregnancy disorder by preeclampsia, eclampsia, HELLP-syndrome or documentation of pregnancy-induced hypertension only. Vaginal-operative delivery combined ventouse and forceps deliveries.

Statistical analysis

For univariate analysis, we calculated odds ratios with 95% confidence intervals and p-values based on chi square testing or fisher's exact test.

We plotted a directed acyclic graph (DAG) to illustrate the temporal sequence of risk factors to identify potential causality, mediation or reverse causality. Variable selection for the multivariable statistical models was guided by the DAG.

Three different multivariable logistic regression models were calculated. Model 1 is based on a priori considerations and a univariate p-value of at least 0.2. We included only variables definitely preceding the outcome (thus potentially causal). For Model 2 we added putative mediators such as prematurity and SGA, which may be both, independent risk factors and/or in the causal pathway of preceding risk factors. 1. Model 3 used the variables included in Model

2 with backward selection with $p<0.05$ used as the cut off for retention in order to obtain a parsimonious model.

Since PAIS cases were from all over Germany, whereas controls were from Bavaria only, we compared characteristics and their role in univariate risk analysis in Bavarian (infants reported from a Bavarian hospital) to non-Bavarian cases.data (Table 3 and table 4 online only).

Because PAIS is a rare event the OR can be considered as an indicator of relative risk. We used a significance level of 5% for all analyses without adjustment for multiple testing. All statistics have been calculated using SAS, version 9.4 (SAS Institute, Cary, North Carolina), and R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). The program code is available at https://osf.io/wxfeq/.

Anonymous reporting in ESPED makes parental consent unnecessary. Ethical approval was obtained by the ethics committee of the Ludwig-Maximilians-University, Munich, Nr 42-15 (05-04-2015).

Results

As shown in Table 1; online only, the proportion of missing values was similar in cases and controls group for all infant variables, whereas there were more missing values regarding maternal items in the ESPED dataset. In both the cases and controls the majority of the excluded subjects had only one missing variable. Thus, the analyzed dataset allows for a meaningful assessment of risk factors. The variable definition was almost identical in cases and controls (Table 2; online only).

The majority (n=117; 87%) of cases presented with clinical symptoms, whereas in 13% (n=17) PAIS was an incidental finding. Most case had seizures as leading symptom (n=69; 51%) and 86% (n=115) of the cases were diagnosed within the first week of life (median 3 days). There were substantially more males (68% vs 32%), 16% were born preterm, 9 cases were twins and 21 cases had birth asphyxia (Table 1).

Univariate Risk Factor Analysis

Table 2 summarizes the univariate analysis in 134 cases and 576 controls. Infants with PAIS were more often males and multiplets. Prematurity was associated with a 2.38 [95%CI 1.37-4.12] times higher risk for PAIS. Cases were more likely to have an Apgar score less than 7 at 5 minutes (OR 41.7; 95%CI 9.51-182.9) and an umbilical artery pH ≤ 7.1 (OR 5.10 95%CI 2.55-10.20). In 15.7% of PAIS cases hypoxia or respiratory disorder was diagnosed compared to 1.2% in the control group (OR 15.10 95%CI 6.27-36.35). In addition, more infants diagnosed with PAIS required intubation or mask ventilation during initial care (n=25; 19% vs. n=29; 5%). Maternal age and history of abortion or miscarriage did not differ significantly between groups. Mothers of infants with stroke were more likely to be primiparous. Obstetrical and peripartum characteristics associated with PAIS included caesarean and vaginal-operative delivery, chorioamnionitis and oligohydramnion.

Directed acyclic Graph (DAG)

Figure 1 illustrates likely pathways. All variables with univariate p<0.02 were taken into account. The temporal sequence of risk factors and outcome of PAIS is arranged from left to right. The causal pathway between sex and primiparity and PAIS is unlikely to be mediated by other risk factors which is indicated by a direct arrow to PAIS. Obstetrical risk factors definitely precede PAIS and may have a direct effect as well as an indirect effect mediated by prematurity and/or SGA. Covariates of the "asphyxia at delivery complex" (low umbilical artery pH or APGAR score, hypoxia/respiratory disorder and intubation/mask ventilation) are depicted on the right, as well as caesarian or vaginal-operative delivery, because these may also be possible consequences of PAIS (indicated by arrows between PAIS and asphyxia/delivery mode).

Multivariate Risk Factor Analysis

After adjusting for all variables preceding the outcome (Model 1), male sex, multiple births and chorioamnionitis remained as risk factors associated with PAIS (Table 3).

Adjustment for preceding risk factors changed the OR for prematurity from 2.38 [95%CI 1.37-4.12] in the univariate analysis to 1.57 [95%CI 0.82-3.03] (Model 2). The OR of SGA changed only slightly following adjustment from 2.84 [95%CI 1.67-4.85] in the univariate analysis to 2.95 [95%CI 1.68-5.19]. In general, comparing results of Model 1 and 2 the ORs for other risk factors did not change substantially, except for multipltes (decreasing) and chorioamnionitis (increasing) (Table 3).

All variables significantly associated in Model 2 and prematurity remained independently associated with PAIS in multivariable backward analysis (Model 3/Table 3).

Sensitivity analysis

In order to exclude bias due to confinement to Bavarian controls we compared the characteristics of Bavarian cases to non-Bavarian cases (Table 3; online only) and performed

separate univariate analysis for these groups (Table 4; online only). Except for caesarean section all risk factors were comparable in the univariate analysis.

Discussion

Based on a substantial number of cases we identified male sex, chorioamnionitis, multipltes, prematurity and SGA as independent risk factors for PAIS.

Male sex, chorioamnionitis and SGA were also identified in a recent meta-analysis by Li et al. [8] Confirmation in our study adds to the body of evidence since these risk factors were only analyzed in three and two studies with a limited number of cases.

Lee et al. were the first to suggest chorioamnionitis as an independent risk factor [14]. The inflammatory process characterizing chorioamnionitis may promote thromboembolism and increase the risk for emboli to the fetal brain and impair the placental function leading to PAIS [7]. Since inflammatory responses have been well described as potent pro-coagulants leading to modulation of coagulation proteins and platelet activation [15]. There appears to an important role of inflammatory processes in the placental-fetal interrelationship triggering causal pathways of PAIS. This concept is further supported by the well-established association between chorioamnionitis and cerebral palsy [8, 16].

Multiple births and prematurity were identified as new independent risk factors. After adjustment for prematurity the effect estimator for multiple births decreased suggesting that part of the multiple effect is mediated by prematurity. Indeed 6 out of 9 PAIS cases associated with multiple deliveries were premature, whereas only one twin was also SGA. Although part of the effect of multiple births may be mediated by prematurity, an additional independent direct effect appears likely. Similarly, a role of multipltes for cerebral palsy, a common outcome of PAIS, has previously been suggested by others [17–19].

The unadjusted estimate for prematurity suggested a higher risk in prematurely born infants. As depicted in the DAG some of this risk might rather be related to the role of prematurity in the causal pathway of preceding risk factors. A genuine effect of prematurity is suggested in

Model 3. A role of prematurity in the pathophysiology of PAIS is also suggested by Aa et al., who claim that maturational changes of the vascular system may account for this phenomenon [20].

Regarding the observed risk for multiple pregnancies Benders et al. [21] suggested that twin-to-twin-transfusion might be causally related to PAIS. In our data, however, there was only one case with twin-to-twin transfusion syndrome.

A unique feature of our analysis is the consideration of causal pathways using the DAG approach. The importance of considering the origin, the causal pathway and the consequence of PAIS is intuitively evident. The conceptualization of potential causal pathways in DAG graphs, however, is rather new [22, 23]. Most importantly, covariates which might also be a consequence of PAIS, do not meet the prerequisites for risk factors. Several papers have included Apgar score, umbilical artery pH, birth asphyxia and the delivery mode as risk factors in their models [5–7]. These papers did not consider that indicators of perinatal asphyxia might also be a consequence of PAIS or in the causal pathway of other risk factors, as outlined in figure 1. The concept that factors, such as low Apgar scores, low umbilical artery pH or caesarean section, might rather be a consequence of PAIS, has previously been promoted by Lee et al. and Wu et al. [11, 14] We acknowledge that indicators of perinatal asphyxia and delivery mode might have some predictive value for PIAS without necessarily being true risk factors.

Male sex and primiparity are definitely preceding risk factors. The predominance of male infants in PAIS patients has been reported in most of the published studies [7, 24–26]. The role for sex in PAIS has been linked to the general vulnerability related to male sex or the hormonal status which potentially influences the susceptibility to ischemic events in males [24, 27, 28]. Primiparity was analyzed, because others described an increased risk of PAIS in infants of primiparous women [6–8, 14]. We confirmed this in our univariate analysis, but not after multiple adjustment. Indeed, other studies have pointed to limited convincing

pathophysiological plausibility to explain the association of primiparity and PAIS and suggested that this covariate might rather be a statistical predictor than causally relevant [14].

SGA was identified as a strong risk factor. SGA, as shown in figure 1, may be an intermediate variable reflecting several preceding disorders in pregnancy [11, 29]. These preceding conditions however, do not fully explain the effect of SGA. Our data thus strengthens previously findings of SGA as independent risk factor for PAIS [6, 8, 11].

Some maternal and pregnancy disorders reported in other studies could not be confirmed by our data, such as hypertensive pregnancy disorders, oligohydramnios or gestational diabetes [8, 14, 30]. Our definition of hypertensive pregnancy disorders might not be optimal, because we did not differentiate between pre-eclampsia, HELLP and maternal hypertension in the ESPED survey. Thus, an association of pre-eclampsia and PAIS cannot be excluded, despite the lack of proof in the current study. Darmency-Stamboul et al. showed an association of PAIS and gestational diabetes, but pathophysiologic plausibility has been questioned [10]. In our data gestational diabetes was not more frequent in cases compared to controls, confirming a published meta-analysis [8]. Benders et al identified hypoglycemia as an independent risk factors for PAIS [21]. Unfortunately we could not address this risk factor in our analysis because the database for the controls did not provide this information.

Limitations

Our case ascertainment was based on surveillance. Reporting by the treating physician was not mandatory and thus might be incomplete. Incomplete reporting, however, is unlikely to differ by risk factors. We lacked cerebral imaging for case validation, but asked the physician to report the findings of the imaging. These were carefully scrutinized and in case of uncertainty of implausibility further validated from medical documentation. Recall bias is unlikely since almost all risk factors were abstracted from documentation during pregnancy or delivery. Absence of null options for some variables was identical in both data sources.

In theory there might be cases of PAIS in the control group. The probability, however, is pretty low. With an expected PAIS incidence between 1 in 5000 and 1 in 2500 infants, the probability that at least one PAIS case was included in the control group is about 12.1 % till 22.7 %. The criterion to include all variables with a univariable p-value of <0.2 in the multivariable analysis was determined arbitrarily.

Confinement to controls from Bavaria only might account for bias. However, we demonstrated only a small difference between Bavarian and non-Bavarian children accounting for almost identical univariate risk estimates. The only difference observed pertained the delivery mode which may be explained by multiple testing. Related to entire population data there's no difference in caesarean section or vaginal-operative delivery rates of Bavaria compared to other regions in Germany.

Strengths

These limitations are further offset by several strengths of our study. These include study size, population-based setting, selection of an appropriate control group and mutual adjustment guided by a priori considerations concerning causal pathways. The period of case ascertainment was confined to three years only assuring a comparable framework of clinical and health policies and available structures for imaging.

Conclusion

The role of chorioamnionitis and SGA for PAIS was confirmed pointing to the importance of inflammatory processes and fetal-placental insufficiency. Multiple birth and prematurity were identified as additional risk factors. The effect of multiple births is likely to be related to placental or prematurity linked complications. Prematurity is likely to have an additional independent effect on PAIS. Our data further support the theory of a multifactorial pathogenesis with a combination of prenatal, perinatal and neonatal risk factors to be involved in the etiology of PAIS.

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Authors` contributions statement

Anna-Lisa Sorg and Rüdiger von Kries developed the study hypotheses and drafted the first and final manuscript. Anna-Lisa Sorg was responsible for data management, the interpretation of data and performed the statistical analysis. Mark Dzietko and Ursula Felderhoff-Müser critically reviewed the manuscript.

Lucia Gerstl, Mathias Klemme, Raphael Weinberger, Mark Dzietko, Ursula Felderhoff-Müser and Rüdiger von Kries conceptualized and designed the active surveillance of perinatal stroke in ESPED, contributed to the acquisition, analysis and interpretation of data. Nicholas Lack provided the BAQ control data. Andreas Beyerlein was responsible for control selection and matching. All authors approved the final manuscript as submitted.

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Disclosure of conflicts of interest

All authors stated that they had no interests which might be perceived as posing a conflict or bias. As well they all disclose prior publication and submission of the manuscript.

Figure 1. Directed acyclic graph (DAG) showing causal pathways of investigated variables and PAIS. Prematurity and SGA are intermediate variables on the pathway of association of obstetrical factors and PAIS. Factors related to «asphyxia at delivery» as well as delivery modes fraught with risk are depicted as potential results of PAIS.

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Table 1. Maternal and infant characteristics of the cases (n=134)

sex [male to female ratio]	2.12 : 1
gestational age [in weeks]	39 (30; 41)
gestational age <32	2 (1.5)
gestational age 32 to <37	20 (14.9)
gestational age ≥ 37	112 (83.5)
birth weight [in Gramm]	3215 (1410; 4830)
head circumference at birth [in cm]	34 (28; 38)
maternal age [in years]	30 (20; 45)
multiples	9 (6.7)
maternal obesity	12 (9.0)
1-minute Apgar score	8 (0;10)
5-minute Apgar score	9 (0; 10)
10-minute Apgar score	10 (1; 10)
umbilical artery pH	7.25 (6.93; 7.60)
Caucasian ethnicity	127 (96)
age at time of diagnose [in days]	3 (0; 27)
*underlying diseases:**	65 (49)
no underlying diseases	59 (51)
perinatal asphyxia	21 (16)
newborn sepsis	10 (7)
heart defect	7 (5)
polyglobulia	4 (3)
meconium aspiration	2 (1)
genetic disorder	2 (1)
hematological disease	2 (1)
others	2 (1)
cerebrovascular disease	1 (0.7)
conspicuous family history†	17 (13)

Quantitative variables are expressed as median (minimum; maximum). Categorical variables are expressed as n (%).

* multiple underlying diseases are possible

† stroke, thrombosis, cardiovascular events or other conspicuous events in family history

Table 2. Univariate analyses of maternal and neonatal characteristics in 134 cases versus 576 controls

			univariate analysis	
	cases (n=134)	controls (n=576)	OR (95%CI)	*p*-value
infant characteristics				
male sex	91 (67.9)	308 (53.5)	1.84 [1.24; 2.74]	0.0024
prematurity	22 (16.4)	44 (7.6)	2.38 [1.37; 4.12]	0.0016
SGA (birth weight < 10P)	25 (18.7)	43 (7.5)	2.84 [1.67; 4.85]	<0.0001
LGA (birth weight > 90P)	15 (11.2)	51 (8.9)	1.30 [0.71; 2.39]	0.4008
umbilical artery pH ≤ 7.1	18 (13.4)	17 (3.0)	5.10 [2.55; 10.20]	<0.0001
5-minute-Apgar score < 7	17 (12.7)	2 (0.3)	41.7 [9.51; 182.9]	<0.0001
multiples	9 (6.7)	11 (1.9)	3.70 [1.50; 9.11]	0.0025
hypoxia/respiratory disorder	21 (15.7)	7 (1.2)	15.10 [6.27; 36.35]	<0.0001
intubation/mask ventilation during initial care	25 (18.7)	29 (5.0)	4.33 [2.44; 7.67]	<0.0001
maternal factors				
age ≤ 18 or age ≥ 35	30 (22.4)	162 (28.1)	0.74 [0.47; 1.15]	0.1781
history of abortions or miscarriage	29 (21.6)	133 (23.1)	0.92 [0.58; 1.451]	0.7189
gestational diabetes	13 (9.7)	31 (5.4)	1.89 [0.96; 3.72]	0.0618
hypertensive pregnancy disorders	8 (6.0)	18 (3.1)	1.97 [0.84;4.63]	0.1143
primiparity	77 (52.2)	270 (46.9)	1.53 [1.05; 2.24]	0.0272
obstetrical and peripartum characteristics				
[1]spontaneous delivery	49 (36.6)	350 (60.8)	1.0	<0.0001
vaginal-operative delivery	16 (11.9)	36 (6.2)	3.18 [1.64; 6.15]	
caesarian section	69 (51.5)	190 (33.0)	2.60 [1.73; 3.90]	
pathological Doppler sonography	5 (3.7)	7 (1.2)	3.15 [0.98; 10.09]	0.0569
chorioamnionitis	8 (6.0)	4 (0.7)	9.08 [2.69; 30.62]	0.0003
oligohydramnios	4 (3.0)	4 (0.7)	4.40 [1.09; 17.82]	0.0456
polyhydramnios	1 (0.7)	3 (0.5)	1.44 [0.15; 13.92]	0.5677
umbilical cord abnormalities	11 (8.2)	59 (10.2)	0.78 [0.40; 1.54]	0.4768

results are given as number of subjects, as odds ratio (OR) and 95% confidence interval (95%CI), p-values chi-square test or Fisher exact test

[1]reference category

Table 3. Multivariable Models

	Model 1*	Model 2†	Model 3‡
	OR (95%CI)	OR (95%CI)	OR (95%CI)
male sex	1.82 [1.21; 2.75]	1.82 [1.20; 2.76]	1.81 [1.20; 2.73]
multiples	3.95 [1.56; 10.03]	3.51 [1.29; 9.55]	3.22 [1.21; 8.58]
maternal age (≤ 18 and ≥ 35)	0.75 [0.46; 1.21]	0.75 [0.46; 1.21]	
gestational diabetes	1.54 [0.73; 3.24]	1.65 [0.77; 3.52]	
hypertensive pregnancy disorders	1.78 [0.72; 4.40]	1.52 [0.59; 3.97]	
primiparity	1.35 [0.90; 2.02]	1.22 [0.81; 1.85]	
pathological Doppler sonography	2.69 [0.76; 9.48]	1.53 [0.39; 5.97]	
chorioamnionitis	7.21 [2.05; 25.30]	7.95 [2.26; 27.97]	9.89 [2.88; 33.94]
oligohydramnios	2.50 [0.51; 12.15]	2.73 [0.54; 13.88]	
prematurity		1.57 [0.82; 3.03]	1.86 [1.01; 3.43]
SGA		2.95 [1.68; 5.19]	3.05 [1.76; 5.28]

* Model 1: multiple logistic regression including all variables univariate with a p-value <0.2 and preceding PAIS

† Model 2: same variables as Model 1 plus prematurity and SGA

‡ Model 3: multivariable backward logistic regression (p <0.05 as cut off for retention), variables included were all those listed i the table

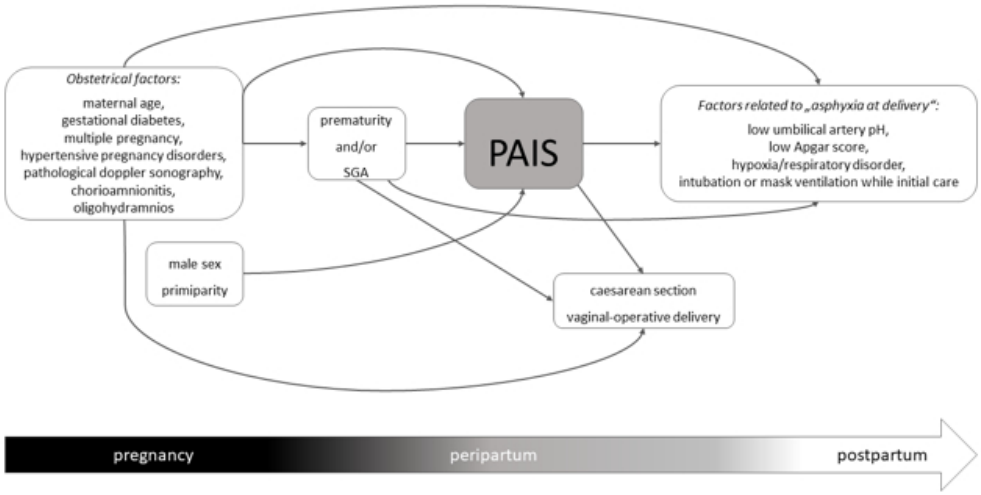

Figure 1. Directed acyclic graph (DAG) showing causal pathways of investigated variables and PAIS. Prematurity and SGA are intermediate variables on the pathway of association of obstetrical factors and PAIS. Factors related to «asphyxia at delivery» as well as delivery modes fraught with risk are depicted as potential results of PAIS.

225x127mm (72 x 72 DPI)

Table 1; online only. Missing values

	cases (n=161)	controls (n=644)	total (n=805)
sex	-	1 (0.2%)	1 (0.1%)
gestational age	-	-	-
birth weight	1 (0.6%)	-	1 (0.1%)
umbilical artery pH	4 (2.5%)	5 (0.8%)	9 (1.1%)
Apgar score at 5 minutes	3 (2%)	2 (0.3%)	5 (0.6%)
multiples	1 (0.6%)	-	1 (0.1%)
maternal age	9 (5.6%)	-	9 (1.1%)
number of gravitas	11 (6.8%)	-	11 (1.4%)
number of parity	11 (6.8%)	-	11 (1.4%)
birth mode	3 (1.9%)	-	3 (0.4%)
intubation/mask ventilation during initial care	-	61 (9.5%)	61 (7.6%)
total number of subjects with at least one missing value (excluded from further analysis)	27 (16.8 %)	68 (10.6%)	95 (11.8%)

Table 2; online only. Variable coding in ESPED and BAQ

risk factors	CODING			
	ESPED		BAQ	
	unit / definition	null option available	unit / definition	null option available
infantile sex	female/male	n.a.	female/male	n.a.
gestational age	in weeks + days	n.a.	in weeks + days	n.a.
birth weight	in Gramm	n.a.	in Gramm	n.a.
Apgar scores	1 / 5 / 10 minutes	n.a.	1 / 5 / 10 minutes	n.a.
maternal age	at delivery in years	n.a.	at delivery in years	n.a.
umbilical artery pH		n.a.		n.a.
multiples	single/twin/high-order multiple	n.a.	number of multiples	n.a.
history of abortions or miscarriage	number of gravitas / parities	n.a.	number of gravitas / parities	n.a.
hypertensive pregnancy disorders	pregnancy-induced or pre-existing hypertension and/or preeclampsia/ eclampsia/HELLP-syndrome	no	eclampsia HELLP-syndrome hypertensive pregnancy disease	yes no no
gestational diabetes		no		no
primiparity	a parity of 1	n.a.	a parity of 1	n.a.
birth mode	query of the different options	n.a.	OPS*	n.a.
pathological Doppler sonography	conspicuous Doppler sonography	no	for cases of birth year 2015 / 2016 for cases of birth year 2017	yes no
hypoxia/ respiratory disorder	perinatal asphyxia/ stroke under hypothermia treatment for perinatal asphyxia	no	derived from ICD codes (P20 – P29)†	no
chorioamnionitis	infection / chorioamnionitis	no	suspicion of chorioamnionitis	no
umbilical cord abnormalities	umbilical cord complication + free text option to report the type of umbilical cord complication	no	umbilical cord prolapse Suspicion of other umbilical cord complications	no
oligohydramnios		no		no
polyhydramnios		no		no
intubation/ mask ventilation during initial care	mask ventilation/ intubation invasive ventilation	no no	mask ventilation intubation	yes yes

abbreviations: n.a. - not applicable /
*OPS - Operationen- und Prozedurenschlüssel: German modification of the International Classification of Health Interventions (ICHI)
† ICD - International Classification of Disease, 10. Revision, German Modification (ICD-10-GM)

Table 3; online only. Sensitivity analysis cases of Bavaria compared to cases outside of Bavaria

	cases in Bavaria (n=27)	non-Bavarian cases (n=107)	*p*-value
male sex	20 (74)	71 (66)	0.4426
gestational age [in weeks]	38.63 (33;41)	38.19 (30; 41)	0.3859
gestational age (in weeks) <32	0 (0)	2 (0)	0.6224
gestational age (in weeks) 32 to <37	3 (11)	17 (16)	
gestational age (in weeks) ≥ 37	24 (89)	88 (82)	
premature babies	3 (11)	19 (18)	0.5645
birth weight [in Gramm]	3184.6 (1950; 4460)	3116.3 (1410; 4830)	0.6523
birth head circumference [in cm]	34.6 (28.5; 39.5)	34.7 (26.5; 52)	0.6453
maternal age [in years]	30.6 (23; 44)	30.6 (20; 45)	0.5872
multiples	3 (11)	6 (5.6)	0.3846
maternal obesity	2 (7.4)	10 (9.4)	1.0000
primiparity	19 (70)	58 (54)	0.1290
spontaneous delivery	15 (56)	34 (32)	0.0120
vaginal-operative delivery	5 (19)	11 (10)	
caesarean section	7 (26)	62 (58)	
1-minute Apgar score	7.40 (2;10)	6.96 (0;10)	0.5322
5-minute Apgar score	8.70 (6; 10)	7.37 (0;10)	0.9422
10-minute Apgar score	9.37 (7; 10)	9,10 (1;10)	0.6470
umbilical artery pH	7.24 (7.02; 7.37)	7.23 (6.93; 7.60)	0.6633
Caucasian ethnicity	25 (96%)	102 (96)	0.3873
age at time of diagnose [in days]	3.26 (0; 15)	4.40 (0; 27)	0.2626
underlying diseases:*	12 (44%)	53 (50)	0.6364
conspicuous family anamnesis†	3 (11%)	14 (13)	1.0000

quantitative variables are expressed as mean (minimum; maximum). categorical variables are expressed as n (%).
p-values chi-square test or Fisher exact test for categorical variables, t-Test or Wilcoxon rank-sum test for quantitative variables

*multiple underlying diseases are possible

†stroke, thrombosis, cardiovascular events or other conspicuous events in family history

Table 4; online only. Sensitivity analysis - cases from the region of Bavaria vs. cases outside from Bavaria

	BAVARIA				*Population except Bavaria*			
a	*univariate analysis*				*univariate analysis*			
	cases (n=27)	controls (n=94)	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value	cases (n=107)	controls (n=482)
infant characteristics								
male sex	20	53	2.21 [0.85; 5.71]	0.0977	1.76 [1.13; 2.72]	0.0113	71	255
prematurity	3	9	1.18 [0.30; 4.71]	0.7288	2.76 [1.51; 5.04]	0.0007	19	35
SGA (birth weight < 10P)	2	6	1.17 [0.22; 6.18]	1.000	3.20 [1.81; 5.65]	<0.0001	23	38
LGA (birth weight > 90P)	2	8	0.86 [0.17; 4.31]	1.000	1.42 [0.73; 2.73]	0.3031	13	43
umbilical artery pH ≤ 7.1	3	1	11.63 [1.18; 116.790]	0.0344	4.75 [2.27; 9.94]	<0.0001	15	16
Apgar score at 5 minutes < 7	3	0	-	0.0102	36.13 [8.08; 161.62]	<0.0001	14	2
multiples	3	2	5.75 [0.91; 36.37]	0.0730	3.12 [1.09; 8.97]	0.0263	6	9
hypoxia/ respiratory disorder	2	0	-	0.0483	14.65 [5.98.27; 35.89]	<0.0001	19	7
intubation/mask ventilation during initial care	2	3	2.43 [0.38; 15.33]	0.3097	4.80 [2.62;8.82]	<0.0001	23	26
maternal factors								
age ≤ 18 or age ≥ 35	8	26	1.10 [0.43; 2.82]	0.8409	0.66 [0.40; 1.10]	0.1059	22	120
history of abortions or miscarriage	7	13	2.18 [0.77; 6.18]	0.1358	0.78 [0.47; 1.30]	0.3429	10	27
gestational diabetes	3	4	2.81 [0.59; 13.43]	0.1846	1.74 [0.93; 1.38]	0.1488	13	31
hypertensive pregnancy disorders	2	2	3.68 [0.49; 27.44]	0.2150	1.73 [0.66; 4.53]	0.2589	6	16
primiparity	19	42	2.94 [1.17; 7.38]	0.0186	1.32 [0.87; 2.01]	0.1962	58	228
obstetrical and peripartum characteristics								
[1]spontaneous delivery	15	59	1.0	0.0178	1.0	<0.0001	34	291
vaginal-operative delivery	5	3	6.55 [1.41; 30.54]		2.85 [1.32; 6.16]		11	33
caesarean section	7	32	0.86 [0.32; 2.33]		3.36 [2.12; 5.33]		62	158
pathological Doppler sonography	0	2		1.000	4.67 [1.33; 16.45]	0.0176	5	5
chorioamnionitis	3	1	11.63 [1.16; 116.79]	0.0344	7.83 [1.84; 33.27]	0.0064	5	3
oligohydramnios	1	0	-	0.2231	3.45 [0.76; 15.63]	0.1168	3	4
polyhydramnios	1	0	-	0.2231	-	1.0	0	3
umbilical cord abnormalities	1	13	0.24 [0.03; 1.92]	0.1888	0.78 [0.40; 1.54]	0.4779	11	59

results are given as number of subjects, as odds ratio (OR) and 95% confidence interval (95%CI), p-values chi-square test or Fisher exact test

[1]reference category

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Included on page:
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any pre-specified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3,4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	
		Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	4
		Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed	
		Case-control study—For matched studies, give matching criteria and the number of controls per case	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4,5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4,5
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	4,5, table 1, online only
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5,6

Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5,6
		(*b*) Describe any methods used to examine subgroups and interactions	5,6
		(*c*) Explain how missing data were addressed	5,6, table 1, online only
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	
		Case-control study—If applicable, explain how matching of cases and controls was addressed	5
		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Continued on next page			
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7,8 Table 1
		(b) Indicate number of participants with missing data for each variable of interest	table 1, online only
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	na
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	7,8 table 2
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7,8 table 2,3
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8,9
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

	Item No	Recommendation
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		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
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Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
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		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed			
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		(*b*) Report category boundaries when continuous variables were categorized			
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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses			
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Generalisability	21	Discuss the generalisability (external validity) of the study results			
Other information					
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based			