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Delay in blood sampling for routine newborn screening is associated with increased risk of schizophrenia

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ABSTRACT

Background: The Danish Neonatal Screening Biobank, containing dried blood spot samples from all newborn in Denmark, is a unique source of data that can be utilized for analyses of genetic and environmental exposures related to schizophrenia and other mental disorders. In previous analyses, we have found that early and late blood sampling, compared to sampling at day 5, was associated with increased risk of schizophrenia. As delay in sampling of blood for neonatal screening cannot in itself influence the risk of schizophrenia, it must be seen as a proxy for unknown underlying causes responsible for this association. Therefore, we investigated whether the increased risk can be explained by other risk factors for schizophrenia.
Methods: A case–control design was applied. A total of 846 cases with schizophrenia were selected from the Danish Psychiatric Case Register. One control was selected for each case, matched on sex and exact date of birth.
Results: Both early and late blood sampling was associated with increased risk for schizophrenia. Compared to blood sampling at day 5, sampling at days 0 to 4 after birth was associated with an incidence rate ratio (IRR) of 1.46 (95% CI 1.15–1.87) for development of schizophrenia, and sampling at days 6 to 9 and at days 10 to 53 was associated with an IRR of 1.5 (95% CI 1.13–1.98) and 3.00 (95% CI 1.59–5.67), respectively. After adjusting the estimates for place of birth, both parents' psychiatric illness, maternal and paternal age, parents' country of origin, child admission, and parental education and income, the estimates were slightly different. Thus, blood collection at 0–4 days was associated with an IRR of 1.27 (95% CI 0.94–1.71), 6–9 days 1.31 (95% CI 0.94–1.84) and 10+ days 3.52 (95% CI 1.50 to 8.24).
Discussion: After adjusting risk estimates for well-known risk factors, delay in sampling of blood for neonatal screening was associated with unexplained increased risk of schizophrenia. Thus, a key finding is that age at test is a proxy for unobserved risk factors for schizophrenia due to unexplained reasons for late blood sampling. Date of sampling will be included in future analyses of genetic and environmental risk factors.

1. Introduction

The search for genetic and environmental risk factors for schizophrenia is rapidly going forward (Ripke et al., 2013; Brown, 2011; Smoller et al., 2013). Epidemiological studies have revealed a range of environmental and genetic factors associated with increased risk for schizophrenia. Among these factors are family history of schizophrenia and other mental illnesses, specific genetic variations (Borglum et al., 2013; Demontis et al., 2011; Nyegaard et al., 2010), parental immigration(Pedersen et al., 2012; Cantor-Graae and Pedersen, 2013), urban birth and upbringing (Pedersen and Mortensen, 2001a,b), advanced paternal age (McGrath et al., 2014), intrauterine growth retardation, preterm birth (Byrne et al., 2007), obstetric complication (Cannon et al., 2002), and infections during pregnancy (McGrath et al., 2013; Mortensen et al., 2010, 2011; Pedersen et al., 2011).

The Danish Neonatal Screening Biobank, containing dried blood spot samples (DBSS) from all newborn in Denmark since 1981, is a unique source of data that can be utilized for analyses of genetic and environmental exposures related to schizophrenia and other mental disorders (Norgaard-Pedersen and Hougaard, 2007).

We have planned to utilize this source of data to explore environmental exposures during pregnancy and neonatal period and genetic variations that can be hypothesized to be associated with increased risk for mental disorders. The DBSS are stored at −24 °C, which preserves numerous compounds very well. DNA can be extracted from

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the specimens and whole genome amplified, and it has been shown that the amplified samples from Danish Neonatal Screening Biobank yield as high quality results as conventionally isolated DNA samples for genotyping (Hollegaard et al., 2007, 2013). Environmental factors can be traced in DBSS as well. Data from the Danish Neonatal Screening Biobank was the basis for analyses of Vitamin D (McGrath et al., 2010) and antibodies against infections such as Toxoplasmosis Gondii (Mortensen et al., 2007; Pedersen et al., 2011), HSV2 (Mortensen et al., 2010) and CMV (Borglum et al., 2013).

Environmental factors identified in neonatal DBSS are in many cases hypothesized to be a measure of the intrauterine milieu. However, if the DBSS is taken late after birth, the content will to a higher degree reflect the neonatal milieu of the child. The timing of the blood testing can therefore indicate to which degree the content of the sample reflects intrauterine or neonatal milieu.

Until 2009, it was recommended that the DBSS for newborn screening in Denmark was collected 4–6 days after birth, but the actual collection times varied from the day of birth to more than 10 days after birth. There is no registration of reasons for early or late sample collection, and it can be hypothesized that the physical condition of the newborn in some cases is responsible for early or late blood collection. The timing of the sampling of heel blood can thus be a confounder in analyses of environmental factors. In previous analyses, we have found that early and late blood collection was associated with increased risk of schizophrenia (McGrath et al., 2010; Mortensen et al., 2010, 2011). As delay in sampling of blood for neonatal screening cannot in itself influence the risk of schizophrenia, it must be seen as a proxy for unknown underlying causes responsible for this association. Therefore, we want to investigate whether the increased risk can be explained or partly explained by other risk factors for schizophrenia.

We planned to investigate the following hypotheses regarding factors that could possibly explain the association between time of blood sampling and later schizophrenia:

1. Late and early blood sampling could be associated with the newborn child being in a critical condition, and thus explained by possible brain damage in pre-, peri-, and neonatal period. This can be investigated in analyses including covariates such as Apgar score, gestational age, birth weight, birth complications, and child's admission to a neonatal department shortly after birth.
2. Late and early blood sampling could be associated with parents' mental illness and thus explained by genetic risk of schizophrenia being transferred to the child. This can be investigated in analyses including covariates such as parents' psychiatric diagnoses.
3. Late and early blood sampling could be explained by parents' situation. Disadvantaged parents might be less able to ensure blood sampling at the stipulated date. In some cases, the hospital would expedite the blood sampling in order to ensure it before discharge. Growing up with disadvantaged parents has been hypothesized to be associated with increased risk of schizophrenia. This can be investigated in analyses including very crude proxy variables for difficulties in parenting, such as psychiatric diagnosis of parents, foreign origin of parents, parent's income and educational level, parental ages at child's birth, and urban versus rural place of birth.

2. Methods

2.1. Data sources

The study was based upon data from the Danish Psychiatric Central Research Register (Mors et al., 2011), the Civil Registration System (Pedersen et al., 2006), the Danish National Patient Register (Lynge et al., 2011), the Danish Medical Birth Registry (Knudsen and Olsen, 1998), Statistics Denmark (Danmarks Statistik (The Danish National Bureau of Statistics), 1991), and the Danish Neonatal Screening Biobank (DNSB) (Norgaard-Pedersen and Hougaard, 2007), which has stored DBSS from practically all children born in Denmark since May 1, 1981.

Information extracted from these sources was merged using the unique personal indication number allocated to all citizens in Denmark.

3. Selection of cases and controls

We identified all singletons who had been diagnosed with schizophrenia (ICD-10: F20) during the period from 1994 to 2006 and who were born in Denmark on 1 May 1981 or later. From these singletons, the sample was restricted to cases who had biological material located in the Danish Neonatal Screening Biobank. For each case, we randomly selected one control based on the following matching criteria: (a) sex; (b) exact date of birth; (c) born in Denmark; (d) alive and with no history of schizophrenia on the date of first diagnosis of schizophrenia of the matched case. The sample is identical to that described previously (Pedersen et al., 2012). Case–control pairs without exact date for the neonatal screening were excluded. We had two different samples, the first including 469 cases and 469 controls and the second including 377 cases and 377 controls. The crude analyses were carried out in the samples independently, and thereafter the two samples were merged. A total of 846 cases were identified. Thus, 846 cases and its 846 individually matched controls were identified.

4. Statistical analysis

Incidence rate ratios were estimated using conditional logistic regression (Andersen et al., 1997), and due to the matching scheme where each case was compared individually to its matched control, all incidence rate ratios were controlled for age, sex, and date of birth. Confidence intervals and p values were two-sided and based on likelihood ratio tests (Clayton and Hills, 1993). The nested time-matched design has the advantage of estimating incidence rate ratios as opposed to odds ratios (Andersen et al., 1997; King and Zeng, 2002;

Table 1
Risk of schizophrenia associated with age at blood sampling for first ($N = 938$) and second ($N = 754$) study samples and for the two samples pooled ($N = 1,692$). Cases and controls are matched 1:1 for gender and exact date of birth.

	Age at test	IRR [95% CI]	N	Cases (%)	Controls (%)
First sample (2005)	0–4 days	1.44 [1.04; 2.00]	319	167 (52%)	152 (48%)
	5 days	1.00 [ref]	418	184 (44%)	234 (56%)
	6–9 days	1.59 [1.09; 2.31]	174	95 (55%)	79 (45%)
	10–53 days	7.11 [2.43; 20.87]	27	23 (85%)	4 (15%)
Second sample (2006)	0–4 days	1.47 [1.02; 2.12]	246	132 (54%)	114 (46%)
	5 days	1.00 [ref]	320	146 (46%)	174 (54%)
	6–9 days	1.40 [0.92; 2.13]	163	86 (53%)	77 (47%)
	10–53 days	1.36 [0.56; 3.28]	25	13 (52)	12 (48%)
Pooled	0–4 days	1.46 [1.15; 1.87]	565	299 (53%)	266 (47%)
	5 days	1.00 [ref]	738	330 (45%)	408 (55%)
	6–9 days	1.50 [1.13; 1.98]	337	181 (53%)	156 (46%)
	10–53 days	3.00 [1.59; 5.67]	52	36 (69%)	16 (31%)