Title: Interaction between Compound Genetic Risk for Schizophrenia and High Birth Weight Contributes to Social Anhedonia and Schizophrenia in Women

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Abstract

Schizophrenia is a highly heritable disease, but despite extensive study, its genetic background remains unresolved. The lack of environmental measures in genetic studies may offer some explanation. In recent Finnish studies, high birth weight was found to increase the risk for familial schizophrenia. We examined the interaction between a polygenic risk score for schizophrenia and high birth weight on social anhedonia and schizophrenia in a general population birth cohort. The study sample included 4223 participants from the 1966 Northern Finland Birth Cohort. As a replication sample we used 256 participants from a systematically collected sample of Finnish schizophrenia families. The polygenic risk score comprised of variants published in the large genome-wide meta-analysis for schizophrenia. We found the association between the polygenic risk score and social anhedonia stronger among those with high birth weight, and the same phenomenon was seen for schizophrenia among women, suggesting a gene-environment interaction. Similar results were found within the replication sample. Our results suggest a role for gene-environment interactions in assessing the risk of schizophrenia. Failure to take environmental effects into account may be one of the reasons why identifying significant SNPs for schizophrenia in genome-wide studies has been challenging.

Keywords: Gene-Environment Interaction, Schizophrenia

1. Introduction

Schizophrenia is a highly heritable mental disorder - its heritability estimate is up to 80% (Sullivan et al., 2003) - and its genetic background has been studied intensively. Nonetheless, the genetic architecture behind schizophrenia is not well understood, as a substantial proportion of its genetic background remains unresolved. It has been shown that thousands of common single nucleotide variants, each with a minor effect, might cumulatively explain one-third of the variance in the disease risk (International Schizophrenia Consortium, 2009).

Several hypotheses have been proposed to explain the unexplained portion of the heritability estimate. One such hypothesis is gene-environment interactions (G x E) (van Dongen & Boomsma, 2013). Environment clearly plays a role in the development of schizophrenia (Tandon et al., 2008), and, like genetic effects, environmental effects have shown wide heterogeneity across individuals as well. Genes do not operate in isolation of the environment and it is highly plausible that we will not fully understand the genetics of schizophrenia if the environment is not also taken into consideration. A proof of principle for gene-environment interactions in schizophrenia has been provided by candidate gene studies (Modinos et al., 2013), and in studies where familial background has been used as a measure of genetic liability (e.g. Clarke et al., 2009). To our knowledge there is one published genome-wide gene-environment interaction study (GEWIS) on schizophrenia (Borglum et al., 2014). In that study Borglum and colleagues (2014) found a significant interaction between a SNP on *CTNNA3* and maternal cytomegalovirus infection on schizophrenia. To date, G x E studies have not yet fully exploited the genetic knowledge gained from large genome-wide meta-analyses. To our knowledge there is one published study that studied G x E with polygenic risk scores on schizophrenia (Trotta et al., 2016).

The prenatal environment is one of the environments most consistently related to schizophrenia. One of the most widely used markers of prenatal environment is birth weight. Both low and high birth weight have been related to the risk of schizophrenia (Moilanen et al, 2010). While the effect of low birth weight seems to be consistent across cohorts (Abel et al., 2010a), high birth weight tends to be a risk factor in older cohorts when, for example, gestational diabetes was neither systematically screened nor treated, induction of delivery was more rare and based on less accurate estimation of fetal weight, and high-quality obstetric care was not always available if a large birth weight caused problems during delivery (Moilanen et al., 2010, Wegelius et al. 2011). The variable associations with *high* birth weight suggest that it is particularly relevant with respect to gene-environment interactions. Accordingly, in two recent Finnish studies, *high* birth weight was found to considerably increase the risk for schizophrenia in already high-risk families (Keskinen et al., 2013; Wegelius et al, 2011). Keskinen and coauthors (2013) observed that high birth weight, not low, increased the risk for schizophrenia if the participant's parents had a history of psychosis. This suggests that genetic liability for schizophrenia, as indicated by parental psychosis, might interact with *high* birth weight in the development of schizophrenia.

Our aim was to examine the interaction between a polygenic risk score for schizophrenia and high birth weight on social anhedonia, an intermediate phenotype reflecting schizophrenia liability (Cohen et al., 2011; Miettunen et al., 2011; Kwapil, 1998), and schizophrenia diagnosis in a general population birth cohort. We hypothesized that having higher genetic risk score together with high birth weight would be associated with higher scores on social anhedonia and with higher risk for schizophrenia.

2. Methods

2.1.Study Sample

The study sample (n = 4223) was derived from the Northern Finland Birth Cohort 1966 (NFBC1966), which is an unselected birth cohort consisting of 12 058 live-born children in Northern Finland (Rantakallio, 1969). The cohort represents the general population by covering 96,3% of all births during year 1966 in the provinces of Lapland and Oulu. The cohort has been prospectively followed from the perinatal period to adulthood, and the study sample consists of those participants of the 31-year follow-up study who had DNA and other data used in the study available. Attrition analysis of the 31-year follow-up has been described elsewhere (Haapea et al., 2008). The study has been approved by The Ethics Committee of the Northern Ostrobothnia Hospital District, and all participants gave written consent.

2.2.Replication Sample

The replication sample (n = 256) was derived from a systematically collected sample of Finnish schizophrenia families, the Schizophrenia family sample (FSZ). Families were identified through a search of nationwide health care and population registries. All individuals born in Finland between 1940 and 1976 were screened for hospitalization during the period from 1969 to 1998 (Hospital Discharge Register), for use of free outpatient antipsychotic medication (Medication Reimbursement Register), or disability pension (Pension Register) due to schizophrenia, schizoaffective disorder, or schizophreniform disorder. Pedigrees were constructed by linking the personal identification numbers of the affected individuals to their parents and siblings, derived from the Population Register Centre. Two samples of subjects were contacted: 1) the first sample (All Finland) consisted of families with at least two siblings with schizophrenia and their first-degree relatives from the whole geographical area of Finland; and 2) the second sample (Internal Isolate) comprised patients and their relatives from families with at least one member with

schizophrenia from Kuusamo, a historically isolated region in the north-eastern part of the country with an exceptionally high lifetime risk of schizophrenia (Hovatta et al., 1999, Arajärvi et al., 2005). The study has been approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa, and all participants gave written consent.

The total sample consists of 3335 individuals with DNA samples, but availability of birth weight and social anhedonia data limits the number of participants used for the replication; we had 256 and 133 individuals for the schizophrenia and social anhedonia analysis, respectively.

2.3. Measures

2.3.1. Social anhedonia

Social anhedonia was self-rated using the revised Social Anhedonia Scale (Chapman et al., 1976). The scale includes 40 true/false questions assessing one's interest in social interaction. Sample items of the scale are: "Having close friends is not as important as many people say." and "People sometimes think I am shy when I really just want to be left alone." The distribution of the social anhedonia scale was positively skewed in both samples and we performed a square-root transformation for the scale. *Study Sample*. Social anhedonia was measured as a part of the cohort's 31-year follow-up in 1997. The psychometric properties of the scale in this cohort have been described elsewhere (Miettunen et al., 2010). *Replication Sample*. Social anhedonia was only measured in a subsample derived from the *All Finland Sample* during the data collection in 1999 - 2001. The reliability of the scale in this sample has been reported earlier (Kuha et al., 2011).

2.3.2. Schizophrenia spectrum diagnosis

Schizophrenia spectrum diagnosis in the *Study Sample* was assessed using several national registers (the Finnish Hospital Discharge Register and national registers of the Finnish Social

Insurance Institute) and clinical interviews. The diagnoses were made according to the

criteria of the Diagnostic and Statistical Manual of Mental Disorders, revised 3rd edition

(DSM-III-R). In order to increase the power of our population-based study, we included as

cases all participants who had either schizophrenia diagnosis or a diagnosis belonging to the

schizophrenia spectrum (schizoaffective, schizophreniform, delusional disorder). *Replication*

Sample. Schizophrenia diagnoses were made according to the criteria of the Diagnostic and

Statistical Manual of Mental Disorders, 4th edition (DSM-IV). All available inpatient and

outpatient records were collected from participants and their relatives, if they had any

psychiatric diagnosis according to the registers (Hospital Discharge Register, Medication

Reimbursement Register, and Pension Register). Some of the participants were also

interviewed with the Structured Clinical Interview for DSM-IV diagnosis. Two psychiatrists

blind to the family structure estimated independently the best-estimate lifetime diagnosis

according to the criteria of the DSM-IV. In case of any disagreement, a third reviewer was

used. Consensus diagnoses were made based on these independent estimates.

2.3.3.   Birth weight

Study Sample. Information on birth weight was collected from child welfare clinic registries

and with questionnaires filled in by the mothers during the years 1965-1967. We

dichotomized birth weight as "more than 4 kg" and "4 kg or less" based on earlier Finnish

findings on the association between birth weight and schizophrenia (Wegelius et al., 2011).

The mean birth weight for gestational week 40 in the total Northern Finland 1966 birth cohort

was 3460g (Rantakallio, 1973), while the current Finnish reference gives mean values for

birth weight at gestational age of 40 weeks as 3766 g and 3624g for singleton boys and girls,

respectively (Sankilampi et al., 2013). Therefore, using the cut-off of 4 kg is reasonable with

the relatively old cohorts we studied, while a higher cut-off might be better in more recent

cohorts. *Replication Sample.* Data on birth weight was obtained from registries kept by

obstetric and healthcare clinics. Because of the limited number of participants in our replication study, birth weight was not categorized as "low" (i.e. less than 2.5 kg) or "high" (i.e. more than 4 kg), but studied as a continuous variable.

2.3.4. Genotyping

Study Sample. Genotypes were obtained from genome-wide data genotyped with Illumina Infinium 370cnvDuo platform at the Broad Institute, USA. Detailed description of the genotyping and quality control procedure has been described earlier (Sabatti et al., 2009). The genetic risk score was based on 8 SNPs published in the genome-wide meta-analysis in 2011 (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011). Two of these variants were genotyped (rs10503253 and rs7004633) and six were imputed (rs1625579, rs17662626, rs2021722, rs7914558, rs11191580, and rs548181; HapMap2 as the reference). The broader genetic risk score comprised of 128 variants recently published in a large meta-analysis (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) was based on 15 genotyped and 113 imputed variants (1000 Genomes as the reference). *Replication Sample.* Samples were genotyped for the eight SNPs (rs10503253, rs7004633, rs1625579, rs17662626, rs2021722, rs7914558, rs11191580, and rs548181) with homogeneous mass extension using the MassARRAY System (Sequenom, San Diego, California). Genotyping was performed by following the manufacturer's guidelines in 384-well plates using a total reaction volume of 5 µl including 20 ng of genomic DNA. For quality control, duplicate DNA samples, control DNA, and water controls were included in each plate. We did not have genome-wide data available for the replication sample, and accordingly we were not able to calculate the broader genetic risk score of 128 variants in this sample.

2.3.5. Genetic risk score

We calculated the genetic risk score from eight SNPs (rs1625579, rs17662626, rs2021722, rs7914558, rs11191580, rs548181 rs10503253 and rs7004633) found in the large GWAS meta-analysis for schizophrenia (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011) as a weighed sum of the risk allele count at each locus. We used reported odds ratios for each SNP as weights:

$$Genetic\ Risk\ Score = \sum_{i=1}^{8} OR_i x_i \ ,$$

where OR = odds ratio for the risk allele, x = the number of risk alleles (0, 1, 2) at a locus.

2.3.6.   Genetic risk score / 128 variants

We also calculated a broader genetic risk score of 128 variants recently reported in a large GWAS meta-analysis for schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) in the study sample. Of the 128 variants, one imputed variant did not pass the imputation quality threshold (0.7) we had set, and was therefore dropped out of the score (chr1: rs77149735). Therefore, we calculated the genetic risk score from 127 variants using the following formula:

$$Genetic\ Risk\ Score = \sum_{i=1}^{127} [2 \times p(A_i A_i) + p(A_i B)] \ \times \log(OR_i),$$

where OR= odds ratio for the reference allele, p = probability for the genotype, $A_{i\ =}$ the reference allele of the variant, and B = the alternative allele of the variant

2.4. Statistical analysis

Study Sample. We used linear and logistic regression in analyzing the gene-environment interactions on social anhedonia, and schizophrenia spectrum diagnosis, respectively. In fully adjusted interaction models we had the following covariates: sex, gestational age and three first main principal components calculated from the genome-wide genotype data (Price et al,

2006) to control for population stratification. All the participants were of the same age (born in 1966), and accordingly we did not use age as a covariate. We excluded participants who had other psychoses than a schizophrenia spectrum disorder ($n = 63$) from the schizophrenia analyses. *Replication sample*. We analysed the main effects of sex, age, birth weight, and genetic risk on social anhedonia and schizophrenia diagnosis using all available participants in the replication sample. The sample sizes in these analyses were 144 and 282 for analyses including social anhedonia and schizophrenia, respectively. Because of the limited number of participants in our replication study, birth weight was not categorized as "low" (i.e. less than 2.5 kg) or "high" (i.e. more than 4 kg), but studied as a continuous variable. The effect of birth weight on schizophrenia is assumedly U-shaped: both low and high birth weight has been associated with schizophrenia diagnosis (e.g. earlier study using NFBC1966: Moilanen et al., 2010). Accordingly, to avoid the effect of low and high birth weight canceling each other, we excluded all participants who had birth weight less than 2.5 kg from the interaction analyses ($n = 26$) to be able to analyze the effect of high birth weight. After the exclusion, we had 133 and 256 individuals in the analysis of social anhedonia and schizophrenia, respectively. Because the replication sample was derived from a family-based sample, in which the participants within one family cannot be considered as entirely independent observations, we used a General Estimating Equations (GEE) model in the analyses in order to adjust for the within-family correlations (Liang & Zeger, 1986). We used sex, age and information on which subsample the participant belonged to (Internal isolate or All Finland), as covariates in the analyses. Unfortunately we did not have data on mother's BMI, gestational age, or genome-wide data to calculate the main principal components for the sample, and were not able to control for these variables in the replication analyses.

3. Results

3.1. Study group

Descriptions of the study sample are presented in Table I. Men had higher birth weight ($p < 0.001$), higher scores on social anhedonia ($p < 0.001$), and more schizophrenia diagnosis than women ($p = 0.02$) as described earlier for this cohort (Miettunen et al., 2010). There was no difference in the genetic risk score, calculated from the 8 variants (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011) between the sexes ($p = 0.40$). Participants with schizophrenia diagnosis had higher scores on social anhedonia than non-schizophrenics ($p < 0.001$) as described earlier for this cohort (Miettunen et al., 2011).

We first examined the main effects of sex, high birth weight, genetic risk, and gestational age on social anhedonia in a linear regression model. Sex was significantly associated with social anhedonia (standardized $\beta = -0.27$, $p < 0.001$), so that men had higher scores, while high birth weight (standardized $\beta = 0.03$, $p = 0.09$), genetic risk (standardized $\beta = -0.01$, $p = 0.34$) or gestational age (standardized $\beta = 0.004$, $p = 0.81$) were not associated. The genetic risk score failed to correlate with the first main principal components (p-values from 0.15 to 0.86).

In a fully adjusted regression model, there was a significant interaction between birth weight and genetic risk on social anhedonia in adulthood (standardized $\beta = 0.20$, $p = 0.05$), so that having more risk alleles together with high birth weight was associated with higher social anhedonia. When analyzing men and women separately, the standardized betas for the interactions were of the same magnitude but not statistically significant. Similarly, when adding mother's body mass index (BMI) as a covariate for the model, the standardized beta for the interaction term was of the same magnitude, but not significant (standardized $\beta = 0.19$, $p = 0.08$).

No association was seen between the genetic risk score and schizophrenia ($p = 0.27$), but the number of participants diagnosed with schizophrenia was small ($n = 81$). Analysis of gene-environment interaction on schizophrenia was not significant ($p = 0.10$). When we analyzed men and women separately, we found the interaction to be non-significant in men ($p = 0.61$), but significant in women ($p = 0.008$). Participants with high genetic risk and high birth weight had an increased risk for schizophrenia when compared to participants with a low or normal birth weight and low genetic risk (for all, $OR = 2.7$, 95% CI: $1.2 - 6.0$, $p = 0.01$; for women $OR = 7.6$, 95% CI: $2.8 - 20.5$, $p = 6.1*10^{-5}$; see Table II).

3.2. Replication in the Schizophrenia Family Sample

Descriptions of the replication sample are presented in Table I. In the analysis of main effects of sex, age, birth weight, and genetic risk on social anhedonia in a linear regression model ($n = 144$), none of the variables had significant association with social anhedonia (sex $p = 0.09$, $OR = 1.40$, 95% CI: 0.95-2.05 for men having higher scores; birth weight $p = 0.30$, genetic risk $p = 0.14$, age $p = 0.87$). We also studied the main effects by excluding participants with a birth weight lower than 2.5 kg ($n = 133$). No significant association was seen between birth weight and social anhedonia ($p = 0.78$, $OR = 1.03$, 95% CI $0.84 - 1.3$). The interaction between birth weight and genetic risk on social anhedonia in a fully adjusted regression model was non-significant ($p = 0.09$; $OR = 1.1$, 95% CI: $1.0 - 1.3$), but in the same direction as in the original sample (participants having both higher genetic risk score and higher birth weight tended to have higher scores on social anhedonia than other participants).

The genetic risk score was not directly associated with schizophrenia diagnosis in the replication sample ($p = 0.36$). In a fully adjusted regression model, the interaction between genetic risk and birth weight was significantly associated with schizophrenia ($p = 0.02$; $OR = 1.5$, 95% CI: $1.1 - 2.1$). Because the association in the primary sample was found

particularly in women, we further performed the analysis separately in men and women. In men, the interaction was not significant ($p = 0.93$), while in women the interaction between genetic risk and birth weight was significantly associated with schizophrenia diagnosis so that having higher birth weight and higher genetic risk was associated with more schizophrenia diagnosis ($OR = 2.0$, 95% CI: $1.4 – 2.9$, $p < 0.001$).

3.3. Results for the broader genetic risk score

Finally, we analyzed the broader genetic risk score comprised of 127 variants (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) in the study sample. The correlation between the narrower and broader genetic risk scores was low ($r=0.03$, $p=0.03$). As with the narrower genetic risk score there was no difference between the sexes ($p = 0.13$) and the genetic risk score did not correlate with the first main principal components (p-values from 0.22 to 0.70). In the analysis of main effects, we found no association between the broader genetic risk score and social anhedonia (standardized β $=0.02$, $p=0.17$); but it was associated with schizophrenia diagnosis ($OR=1.8$, 95% CI: 1.2-2.7, $p=0.005$) so that having higher genetic risk score increased the risk for schizophrenia. No change in the result was seen by including sex and three principal components as covariates in the analysis. In a fully adjusted regression model with broader genetic risk score there was no interaction between birth weight and genetic risk on social anhedonia ($p=0.61$), or schizophrenia diagnosis ($p=0.24$).

4. Discussion

We found that the association between high genetic risk (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011) for schizophrenia and social anhedonia was stronger among those who had also perinatal risk factors as

indicated by high birth weight. The same phenomenon was seen for schizophrenia itself, at least among women. These original results were found in a population-based birth cohort, and, we were able to find similar results in a schizophrenia patient sample. Although our replication study was not powered to assess birth weight as a dichotomous variable, the interaction we found between higher birth weight and genetic risk score on social anhedonia and schizophrenia argues for a true finding. However, we did not see the same interaction results when we used a broader genetic risk score (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) as a measure for genetic liability. We did see a direct association, however, between the genetic risk score and schizophrenia diagnosis.

We expected to find the same interaction results with both the narrower and broader risk scores, because it is plausible to think that the broader score would only contain more information on the genetic risk for schizophrenia. It is unclear, however, whether these two genetic scores reflect the same aspects of genetic liability for schizophrenia. Correlation between the narrower and the broader risk scores was very low, and, the narrower risk score, which was based on the results of the large schizophrenia GWAS in 2011, and the broader score, which was based on the results of the mega-GWAS of schizophrenia reported in 2014, do not share any variants in common. Five of the eight variants in the narrower score are within the 108 genomic areas reported in the 2014 GWA study, but three of them are not (although they are close).

A direct association was seen between the broader polygenic risk score (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) and schizophrenia diagnosis, so that participants with a higher genetic risk score carried a higher risk for schizophrenia diagnosis. This is an expected result that is also seen in other studies (e.g. Agerbo et al., 2015).

High birth weight has been associated in the NFBC1966 cohort with, for example, increased risk of perinatal mortality (Rantakallio, 1968), and it is itself likely to be a proxy of other adversities related to gestation and labor, such as prolonged labor, gestational diabetes and maternal hyperglycemia, which were not screened for at that time. The generalizability of the results to newer cohorts needs to be explored. Yet there is the possibility that the risk variants affect both birth weight and schizophrenia liability, but this also requires further exploration.

It is interesting that we saw the interaction result, considering schizophrenia, only in women. There are sex differences related to schizophrenia that are often reported: e.g. schizophrenia is more common in men than women, the onset of the disease is earlier in men than women, and differences in symptom patterns and cognitive functions (Pedersen et al., 2014; Abel et al., 2010b), although not all studies report similar findings (e.g. Saha et al., 2005). So far, though, there have not been many studies that consider possible different developmental pathways in schizophrenia between men and women. However, a hypothesis of sex-specific genetic risk for schizophrenia has been proposed and it has gained some support (Goldstein et al., 2013). In addition, "a female protective model" in neurodevelopmental disorders has been proposed and supported (Jacquemont et al., 2014). For example, Jacquemont and colleagues (2014) showed that women diagnosed with autism carry more deleterious genetic mutations compared to men and, as such, are able to tolerate a higher deleterious mutational load before becoming seriously ill. Whether this protective model is applicable to environmental insults as well is, to our knowledge, currently unknown. In our study, women diagnosed with schizophrenia had more often both high genetic risk and high birth weight compared to men. Furthermore, it must be noted that the environmental risk factor used in this study was somewhat more extreme for women than for men, because the 4

kg cutoff for high birth weight deviates more from girls' mean birth weight than from boys'

mean birth weight (Rantakallio, 1968).

There are limitations that need to be taken into account. As a population-based

birth cohort, our sample suffers from a low number of schizophrenia patients, especially in

the high birth weight group. Because of limited availability of information on birth weight,

our replication sample is quite small. In addition, we lacked data on all the same covariates as

in the original sample.

Our study has several strengths. As our discovery sample is a population-based

birth cohort, it lacks the problems present in selected clinical samples. We were able to

replicate our findings in a different type of sample, a patient sample, which is also not a

selected clinical sample, but a systematically collected sample of Finnish schizophrenia

families. Our measures of genetic risk, environment and outcomes were highly reliable.

Schizophrenia diagnoses in both samples were based on validated register data and

interviews. Our discovery sample is a prospectively followed cohort and, for example, birth

weight was reported by the mothers very close to the actual birth. In the replication sample

birth weights were acquired from health care registries, which are highly reliable in Finland.

In conclusion, our results highlight the role of the interplay between

environmental and genetic risk factors in assessing the risk of schizophrenia. Failure to take

environmental effects into account may be one of the reasons why identifying significant

SNPs for schizophrenia in genome-wide analyses has been challenging. These results suggest

a role for gene-environment interactions in schizophrenia and provide encouragement to

pursue further G x E studies in schizophrenia.

Acknowledgment

References

Abel, K.M., Wicks, S., Susser, E.S., Dalman, C., Pedersen, M.G., Mortensen, P.B., Webb, R.T., 2010a. Birth weight, schizophrenia, and adult mental disorder: is risk confined to the smallest babies? Arch. Gen. Psychiatry 67, 923-930.

Abel, K.M., Drake, R., Goldstein, J.M., 2010b. Sex differences in schizophrenia. Int. Rev. Psychiatry 22, 417-428.

Agerbo, E., Sullivan, P.F., Vilhjalmsson, B.J., Pedersen, C.B., Mors, O., Borglum, A.D., Hougaard, D.M., Hollegaard, M.V., Meier, S., Mattheisen, M., Ripke, S., Wray, N.R., Mortensen, P.B., 2015. Polygenic Risk Score, Parental Socioeconomic Status, Family History of Psychiatric Disorders, and the Risk for Schizophrenia: A Danish Population-Based Study and Meta-analysis. JAMA Psychiatry 72, 635-641.

Arajärvi, R., Suvisaari, J., Suokas, J., Schreck, M., Haukka, J., Hintikka, J., Partonen, T., Lönnqvist, J., 2005. Prevalence and diagnosis of schizophrenia based on register, case record and interview data in an isolated Finnish birth cohort born 1940-1969. Soc. Psychiatry Psychiatr. Epidemiol. 40, 808-816.

Børglum, A.D., Demontis, D., Grove, J., Pallesen, J., Hollegaard, M.V., Pedersen, C.B., Hedemand, A., Mattheisen, M., GROUP investigators10, Uitterlinden, A., Nyegaard, M., Ørntoft, T., Wiuf, C., Didriksen, M., Nordentoft, M., Nöthen, M.M., Rietschel, M., Ophoff, R.A., Cichon, S., Yolken, R.H., Hougaard, D.M., Mortensen, P.B., Mors, O., 2014. Genome-wide study of association and interaction with maternal cytomegalovirus infection suggests new schizophrenia loci. Mol. Psychiatry 19, 325-333.

Chapman, L.J., Chapman, J.P., Raulin, M.L., 1976. Scales for physical and social anhedonia. J. Abnorm. Psychol. 85, 374-382.

Clarke, M.C., Tanskanen, A., Huttunen, M., Whittaker, J.C., Cannon, M., 2009. Evidence for an interaction between familial liability and prenatal exposure to infection in the causation of schizophrenia. Am. J. Psychiatry 166, 1025-1030.

Cohen, A.S., Najolia, G.M., Brown, L.A., Minor, K.S., 2011. The state-trait disjunction of anhedonia in schizophrenia: potential affective, cognitive and social-based mechanisms. Clin Psychol Rev. 31(3), 440-448.

van Dongen, J., Boomsma, D.I., 2013. The evolutionary paradox and the missing heritability of schizophrenia. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 162B, 122-136.

Goldstein, J.M., Cherkerzian, S., Tsuang, M.T., Petryshen, T.L., 2013. Sex differences in the genetic risk for schizophrenia: history of the evidence for sex-specific and sex-dependent effects. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 162B, 698-710.

Haapea, M., Miettunen, J., Läärä, E., Joukamaa, M.I., Järvelin, M.-R., Isohanni, M.K., Veijola, J., 2008. Non-participation in a field survey with respect to psychiatric disorders. Scand. J. Public Health 36, 728-736.

Hovatta, I., Varilo, T., Suvisaari, J., Terwilliger, J.D., Ollikainen, V., Arajärvi, R., Juvonen, H., Kokko-Sahin, M.L., Väisänen, L., Mannila, H., Lönnqvist, J., Peltonen, L.,1999. A genomewide screen for schizophrenia genes in an isolated Finnish subpopulation, suggesting multiple susceptibility loci. Am. J. Hum. Genet. 65, 1114-1124.

International Schizophrenia Consortium, Purcell, S.M., Wray, N.R., Stone, J.L., Visscher, P.M., O'Donovan, M.C., Sullivan, P.F., Sklar, P., 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460, 748-752.

Jacquemont, S., Coe, B.P., Hersch, M., Duyzend, M.H., Krumm, N., Bergmann, S., Beckmann, J.S., Rosenfeld, J.A., Eichler, E.E., 2014. A higher mutational burden in females supports a "female protective model" in neurodevelopmental disorders. Am. J. Hum. Genet. 94, 415-425.

Keskinen, E., Miettunen, J., Koivumaa-Honkanen, H., Mäki, P., Isohanni, M., Jääskeläinen, E., 2013. Interaction between parental psychosis and risk factors during pregnancy and birth for schizophrenia – The Northern Finland 1966 Birth Cohort Study. Schizophr. Res. 145, 56-62.

Kuha, A., Suvisaari, J., Perälä, J., Eerola, M., Saarni, S.S., Partonen, T., Lönnqvist, J., Tuulio-Henriksson, A., 2011. Associations of anhedonia and cognition in persons with schizophrenia spectrum disorders, their siblings, and controls. J. Nerv. Ment. Dis. 199, 30-37.

Kwapil, T.R., 1998. Social anhedonia as a predictor of the development of schizophrenia-spectrum disorders. J. Abnorm. Psychol. 107, 558–565.

Liang, K.-Y., Zeger, S.L., 1986. Longitudinal data analysis using generalized linear models. Biometrika 73, 13-22.

Miettunen, J., Veijola, J., Freimer, N., Lichtermann, D., Peltonen, L., Paunio, T., Isohanni, M., Joukamaa, M., Ekelund, J., 2010. Data on schizotypy and affective scales are gender and education dependent – study in the Northern Finland 1966 Birth Cohort. Psychiatry Res. 178, 408-413.

Miettunen, J., Veijola, J., Isohanni, M., Paunio, T., Freimer, N., Jääskeläinen, E., Taanila, A., Ekelund, J., Järvelin, M.-R., Peltonen, L., Joukamaa, M., Lichtermann, D., 2011. Identifying schizophrenia and other psychoses with psychological scales in the general population. J. Nerv. Ment. Dis. 199, 230-238.

Modinos, G., Iyegbe, C., Prata, D., Rivera, M., Kempton, M.J., Valmaggia, L.R., Sham, P.C., van Os, J., McGuire, P., 2013. Molecular genetic gene-environment studies using candidate genes in schizophrenia: a systematic review. Schizophr. Res. 150, 356-365.

Moilanen, K., Jokelainen, J., Jones, P.B., Hartikainen, A.-L., Järvelin, M.-R., Isohanni, M., 2010. Deviant intrauterine growth and risk of schizophrenia: A 34-year follow-up of the Northern Finland 1966 Birth Cohort. Schizophr. Res. 124, 223-230.

Pedersen, C.B., Mors, O., Bertelsen, A., Waltoft, B.L., Agerbo, E., McGrath, J.J., Mortensen, P.B., Eaton, W.W., 2014. A comprehensive nationwide study of the incidence rate and lifetime risk for treated mental disorders. JAMA Psychiatry 71, 573-581.

Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A., Reich, D., 2006. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 38, 904–909.

Rantakallio P., 1968. The optimum birth weight. Ann Paediatr Fenn 14, 66-70.

Rantakallio P., 1969. Groups at risk in low birth weight infants and perinatal mortality. Acta Paediatr Scand. Suppl. 193, 1-71.

Rantakallio P., 1973. The assessment of small-for-dates infants and associated sociobiological factors. Ann. Chir. Gynaecol. Fenn. Suppl. 184, 3-47.

Sabatti, C., Service, S.K., Hartikainen, A.L., Pouta, A., Ripatti, S., Brodsky, J., Jones, C.G., Zaitlen, N.A., Varilo, T., Kaakinen, M., Sovio, U., Ruokonen, A., Laitinen, J., Jakkula, E., Coin, L., Hoggart, C., Collins, A., Turunen, H., Gabriel, S., Elliot, P., McCarthy, M.I., Daly, M.J., Järvelin, M.R., Freimer, N.B., Peltonen, L., 2009. Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. Nat. Genet. 41, 35-46.

Saha, S., Chant, D., Welham, J., McGrath, J., 2005. A systematic review of the prevalence of schizophrenia. PLoS Med. 2, e141.

Sankilampi, U., Hannila, M.L., Saari, A., Gissler, M., Dunkel, L., 2013. New population-based references for birth weight, length, and head circumference in singletons and twins from 23 to 43 gestation weeks. Ann. Med. 45, 446-454.

Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium., 2011. Genome-wide association study identifies five new schizophrenia loci. Nat. Genet. 43, 969-976.

Schizophrenia Working Group of the Psychiatric Genomics Consortium., 2014. Biological Insights from 108 Schizophrenia-Associated Genetic Loci. Nature 511, 421-427.

Sullivan, P.F., Kendler, K.S., Neale, M.C., 2003. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch. Gen. Psychiatry 60, 1187-1192.

Tandon, R., Keshavan, M.S., Nasrallah, H.A., 2008. Schizophrenia, "just the facts" what we know in 2008. 2. Epidemiology and etiology. Schizophr. Res. 102, 1-18.

Trotta, A., Iyegbe, C., Di Forti, M., Sham, P.C., Campbell, D.D., Cherny, S.S., Mondelli, V., Aitchison, K.J., Murray, R.M., Vassos, E., Fisher, H.L., 2016. Interplay between Schizophrenia Polygenic Risk Score and Childhood Adversity in First-Presentation Psychotic Disorder: A Pilot Study. PLoS One 11 (9), e0163319.

Wegelius, A., Tuulio-Henriksson, A., Pankakoski, M., Haukka, J., Lehto, U., Paunio, T., Lönnqvist, J., Suvisaari, J., 2011. An association between high birth weight and schizophrenia in a Finnish schizophrenia family study sample. Psychiatry Res. 190, 181-186.

Table 1. Description of the study sample (N = 4223) and the replication sample (N = 282).

Variable	Study sample (NFBC1966) Mean (SD) or % (N)	Replication sample (FSZ) Mean (SD) or % (N)
Sex (% men)	45.0 (*1902*)	46.8 (*132*)
Age	31.0 (0)	45.6 (9.2)
Birth weight (kg)	3.47 (0.5)	3.40 (0.8)
High birth weight	11.5 (*487*)	16.7 (*47*)
Gestational age (weeks)	40.1 (1.9)	no data
Social anhedonia	9.50 (5.6)	11.6 (7.1)[b]
Schizophrenia diagnosis	1.6 (*81*)[c]	31.2 (*88*)
Genetic risk score[d]	11.4 (1.8)	12.8 (1.6)
Broader genetic risk score[e]	-0.50 (0.54)	no data

[a]Descriptives are presented for the total replication sample. N for the analyses was 256 (after exclusion of participants with birth weight < 2.5 kg)

[b]Only from 144 participants

[c]Includes also schizophrenia spectrum diagnoses (schizoaffective, schizophreniform, delusional disorder).

n for the schizophrenia analysis was 5033

[d]calcluated with 8 SNPs (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011)

[e]calculated with 127 variants (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014)

Table 2. Results of logistic regression analyses on schizophrenia diagnosis for all individuals, and men and women separately.

Dataset	Genetic Risk	Birth weight ≤ 4 kg				Birth weight > 4 kg			
		OR	95% CI	p	n cases / n total	OR	95% CI	p	n cases / n total
ALL	Low genetic risk[a]	1.0	ref	-	42 / 2757	0.7	[0.3 - 1.9]	0.73	5 / 382
	High genetic risk[b]	1.1	[0.7 - 1.9]	0.60	23 / 1323	**2.7**	**[1.2 - 6.0]**	**0.013**	8 / 170
MEN	Low genetic risk	1.0	ref	-	24 / 1269	0.8	[0.3 - 2.5]	0.76	4 / 239
	High genetic risk	0.9	[0.5 - 1.9]	0.87	11 / 622	0.9	[0.2 - 4.0]	0.91	2 / 109
WOMEN	Low genetic risk	1.0	ref	-	18 / 1488	0.4	[0.1 - 3.4]	0.44	1 / 143
	High genetic risk	1.4	[0.7 - 2.9]	0.37	12 / 701	**7.6**	**[2.8 - 20.5]**	**$6.1 * 10^{-5}$**	6 / 61

The group with low genetic risk and birth weight ≤ 4 kg was used as the reference group.
Sex, gestational age, mother's BMI, and 3 PCAs were used as covariates in the analyses.
[a]Low genetic risk group includes the two lowest tertiles of the genetic risk score
[b]High genetic risk group is defined as the highest tertile of the genetic risk score