Schizophrenia Research 134 (2012) 246–252

Contents lists available at SciVerse ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Modelling the contribution of family history and variation in single nucleotide polymorphisms to risk of schizophrenia: A Danish national birth cohort-based study

Esben Agerbo [a,*], Preben B. Mortensen [a], Carsten Wiuf [b], Michael S. Pedersen [a], John McGrath [c,d], Mads V. Hollegaard [e], Bent Nørgaard-Pedersen [e], David M. Hougaard [e], Ole Mors [f], Carsten B. Pedersen [a]

[a] National Centre for Register-Based Research, Aarhus University, Denmark
[b] Bioinformatics Research Centre, Aarhus University, Denmark
[c] Queensland Brain Institute, University of Queensland, Australia
[d] Queensland Centre for Mental Health Research, The Park Centre for Mental Health, Australia
[e] Section of Neonatal Screening and Hormones, Statens Serum Institut, Copenhagen, Denmark
[f] Centre for Psychiatric Research, Aarhus University Hospital, Psychiatric Hospital, Denmark

ARTICLE INFO

Article history:
Received 8 August 2011
Received in revised form 6 October 2011
Accepted 29 October 2011
Available online 22 November 2011

Keywords:
Schizophrenia
Familial history of psychiatric disorder
Epidemiology
Genome-wide association
Single-nucleotide polymorphisms

ABSTRACT

Background: Epidemiological studies indicate that having any family member with schizophrenia increases the risk of schizophrenia in the probands. However, genome-wide association studies (GWAS) have accounted for little of this variation. The aim of this study was to use a population-based sample to explore the influence of single-nucleotide polymorphisms (SNPs) on the excess schizophrenia risk in offspring of parents with a psychotic, bipolar affective or other psychiatric disorder.
Method: A nested case–control study with 739 cases with schizophrenia and 800 controls. Their parents and siblings. Information from national health registers and GWAS data from the national neonatal biobank.
Results: Offspring schizophrenia risk was elevated in those whose mother, father or siblings had been diagnosed with schizophrenia or related psychosis, bipolar affective disorder or any other psychiatric disorder. The rate ratio was 9.31 (3.85; 22.44) in offspring whose 1st degree relative was diagnosed with schizophrenia. This rate ranged between 8.31 and 11.34 when adjusted for each SNP individually and shrank to 8.23 (3.13; 21.64) when adjusted for 25% of the SNP-variation in candidate genes. The percentage of the excess risk associated with a family history of schizophrenia mediated through genome-wide SNP-variation ranged between −6.1% (−17.0%;2.6%) and 4.1%(−3.9%;15.2%). Analogous results were seen for each parent and for histories of bipolar affective and other psychiatric diagnoses.
Conclusions: The excess risk of schizophrenia in offspring of parents who have a psychotic, bipolar affective or other psychiatric disorder is not currently explained by the SNP variation included in this study in accordance with findings from published genetic studies.

1. Introduction

Classical epidemiological family (Mcgue et al., 1983; Mortensen et al., 1999; Byrne et al., 2002), adoption (Kety et al., 1971; Ingraham and Kety, 2000), twin (Cardno and Gottesman, 2000; Sullivan et al., 2003), and sibling (Risch, 1990) studies consistently show that schizophrenia is strongly heritable, which has lead to a concentrated international research agenda designed to identify genetic factors that influence susceptibility to schizophrenia. To date, more than 1600 studies have investigated more than 8000 polymorphisms and 950 candidate genes within the human genome (Human Genome Sequencing Consortium, 2004) (http://www.schizophreniaforum.org, accessed 14 July 2010) (Allen et al., 2008). Furthermore, at least 31 independent genome-wide linkage studies based on 3108 multiplex schizophrenia pedigrees and 8.3 million genotypes have been carried out (Konneker et al., 2008; Sullivan et al., 2008), and the International Schizophrenia Consortium has found that a SNP-based polygenic score captured up to ~3% of the variance in the schizophrenia risk (International Schizophrenia Consortium, 2009).

With few exceptions epidemiological studies have used psychiatric family history as the only measure of genetic liability (van Os et al., 2008). Furthermore register-based studies from Denmark (Mortensen et al., 2010) and Sweden (Lichtenstein et al., 2009) indicate that the risk of schizophrenia is increased in the offspring of parents who suffer from a psychotic, bipolar affective or other psychiatric disorder, and the evidence of an etiologic overlap between schizophrenia and bipolar disorder has been supported by genome-wide association studies

Abbreviations: GWAS, genome-wide association studies; SNP, single-nucleotide polymorphism; PCA, principal component analysis.

* Corresponding author at: National Centre for Register-based Research. Aarhus University, Taasingegade 1, 8000 Aarhus C, Denmark. Tel.: +45 89 42 68 15, +45 51 77 93 59(mobile); fax: +45 89 42 68 13.
E-mail address: ea@ncrr.dk (E. Agerbo).

0920-9964/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.schres.2011.10.025

(International Schizophrenia Consortium, 2009) and population-based studies (Laursen et al., 2009; Lichtenstein et al., 2009).

Studies looking for genetic variants associated with an increased risk of schizophrenia have been derived from genetically informative families (e.g. multiplex families, affected sibling pairs) or from case–control studies (Cichon et al., 2009; O'Donovan et al., 2009; Gejman et al., 2010; Schwartz and Susser, 2010; Gershon et al., 2011). With respect to multiplex families, a register-based study from Sweden found that of all families groups affected by schizophrenia, only 3.81% had more than one affected individual (Lichtenstein et al., 2006). Affected individuals included in case–control studies are usually derived from prevalent cases and estimates derived from genetic studies may not generalize to the general population.

The challenge to recruit representative samples for genetic studies has increased substantially in recent years, as studies have indicated that very large samples are required to detect SNPs associated with schizophrenia (Corvin et al., 2010). For example in a typical genome-wide association study of schizophrenia, a sample with 6000 cases and 6000 controls is required to obtain a statistical power of 80% (Corvin et al., 2010). It is unlikely that population-based samples of this size with both genome-wide data and psychiatric family history will be available in the foreseeable future.

We had the opportunity to examine the impact of SNPs and family history in a representative case–control sample that is nested within the Danish population. Mindful that this study would only have sufficient power to detect large effect sizes, we used the sample to model the influence of SNP on the excess schizophrenia risk in offspring of parents with a psychotic, bipolar affective or other psychiatric disorder, where the SNP-based information is given every chance to explain associations with family history.

2. Materials and methods

2.1. Source of data and population-based registers

Data were obtained by linking Danish population-based registers using the unique personal identification number assigned to all persons living in Denmark and used across all national registration systems (Pedersen et al., 2006). The Danish Civil Registration System (CRS) was established in 1968 and contains information on gender, date of birth, birthplace, nationality and parents' personal identification numbers (Pedersen et al., 2006). The Danish Psychiatric Central Register includes all admission dates and diagnoses according to the World Health Organization eighth and tenth classification and covers all psychiatric inpatient facilities in Denmark since 1969 as well as outpatient contacts from 1994 onwards (Munk-Jorgensen and Mortensen, 1997). There are no private psychiatric hospitals in Denmark and all treatments are free of charge. The Danish Newborn Screening Biobank (DNSB) store dried blood spots at −24 °C taken by heel pricks from infants born in Denmark after May 1, 1981 and cover close to 100% (Norgaard-Pedersen and Simonsen, 1999; Norgaard-Pedersen and Hougaard, 2007). The study was approved by the Danish Data Protection Agency.

2.2. Study population and statistical design

All singletons born in Denmark after May 1, 1981 who were registered in the DNSB and who had been diagnosed with an ICD-10F20 code for schizophrenia (World Health Organization, 1992) in the period between 1994 and 2006 were identified in the Danish Psychiatric Central Register. Using a nested case–control design (Clayton and Hills, 1996), each case was matched with a randomly selected control of the same gender and with the same birthday. A control was only eligible provided he was born and resident in Denmark, not diagnosed with schizophrenia before the date the case was diagnosed, and biological material was extractable from the DNSB. Parents and maternal siblings were identified using the CRS. To minimize the risk of confounding by population stratification, the sample was restricted to native Danes, i.e. individuals born in Denmark whose both parents were born in Denmark. Subsequently, cases and controls from broken strata were re-matched into 732 strata conditioning on gender while minimizing the absolute difference between the days of birth.

2.3. Familial history of psychiatric disorder

From the Danish Psychiatric Central Register explanatory variables were extracted indicating whether the subject's parents or siblings had been diagnosed according to the hierarchy: 1) schizophrenia or related psychosis, 2) bipolar affective disorder or 3) any other psychiatric disorder before the matching date (diagnostic details (Mortensen et al., 2010)). Family history was also classified into two exclusive groups: 1) schizophrenia narrowly defined as ICD-8(World Health Organization, 1967) codes 295 and ICD-10(World Health Organization, 1992) codes F20 and 2) other psychiatric disorders. The procedure was applied to each parent and sibling separately as well as all family members jointly.

2.4. DNA-extraction, genotyping and SNP-based risk factors

Genomic DNA was extracted from two dried blood spot punches with a diameter of 3.2 mm (Hollegaard et al., 2007; Hollegaard et al., 2009). To ensure sufficient DNA for genotyping, extracted material was whole-genome amplified in triplicates using the REPLI-g mini kit (Qiagen). Genome-wide scanning was performed using the Illumina Human 610-Quad BeadChip array. The amplification protocol has previously been tested by comparing stored blood samples with contemporary high-quality genomic DNA from the same eight individuals and conflicts were observed in three per 10,000 genotype calls (Hollegaard et al., 2009). To avoid selective genotyping biases, the genotyping was performed consecutively on a matched-pair basis. SNPs on the X, Y and mitochondrial chromosomes were removed. To ensure estimable rate ratios, only SNPs with a minor allele frequency of more than 10 were kept. Missing SNPs were imputed within loci based on the observed allele frequency by assuming missingness at random (Rubin, 1976). Genotypes were coded as 2 (rare allele homozygous), 1 (heterozygote) and 0 (wildtype = most frequent homozygote).

Principal component analysis (PCA) or singular value decomposition analysis is a statistical method commonly used in population genetics to identify genome-wide structure by reducing the dimensionality of a dataset (Patterson et al., 2006; Reich et al., 2008). The PCA technique was used to generate principal components (PC) based on the dataset indexed by individuals and polymorphic markers. PCs capture population ancestry and the underlying genealogical history (McVean, 2009), furthermore, previous studies have shown that PCA performs well (Alter et al., 2000; Holter et al., 2000; Khan et al., 2001; Gauderman et al., 2007; Ballard et al., 2010a; Ballard et al., 2010b). Furthermore, to identify structure within candidate genes for schizophrenia, PCA was applied to a sub-dataset consisting of SNPs located within 50 kilobases from exons in the top 39 candidate genes (PGBD1, NRGN, NOTCH4, PDE4B, TCF4, DAOA, TPH1, HTR2A, RELN, MDGA1, CCKAR, DRD4, DRD1, APOE, DISC1, PLXNA2, GABRB2, AKT1, DRD2, SRR, PRSS16, HIST1H2BJ, ZNF804A, AHI1, MTHFR, RPP21,DTNBP1, NRG1, RGS4, RPGRIP1L, GRIK3, HP, COMT, OPCML, GRIN2B, PPP3CC,DAO, IL1B and SLC18A1) as defined in the SzGene database [http://www.schizophreniaforum.org/res/sczgene/default.asp (accessed 30 April 2010)] (Allen et al., 2008). Linkage disequilibrium is expected to extend over at most 50 kilobases (Clark et al., 2003).

2.5. Data analyses

Data were analysed using conditional logistic regression (King and Zeng, 2002) with each case and its matched control forming a stratum. Since the controls were selected randomly within the risk-sets,

the estimated measures of relative risk are rate ratios (Borgan et al., 1995). Standard Wald confidence intervals were used. All analyses were done using SAS version 9.2.

Initially the risk of schizophrenia in relation to family history of psychiatric disorder was assessed. These relationships were adjusted for the effect of each SNP (additive in the number of copies of the rare allele) separately and, in order to evaluate the modifying impact of each SNP, plotted against the unadjusted effect of the particular SNP.

Subsequently, the associations between the risk of schizophrenia and family history of psychiatric disorder were adjusted for the variation captured by each PC. A change-in-estimate strategy was used to measure the percentage of the excess risk associated with family history that was mediated through each PC (Freedman et al., 1992; Glymour, 2006). Each PC was divided into seven equal groups and entered in the regression as categorical factors. Other categorizations were also assessed. Bootstrap resampling was used to estimate upper and lower confidence intervals and to adjust for overfitting (Davison and Hinkley, 1997; Sauerbrei, 1999). To evaluate the impact of outliers, Z-values (Armitage et al., 2002) exceeding ± 3 were set to zero.

Lastly, the associations between the risk of schizophrenia and family history of psychiatric disorders were adjusted for PCs obtained from SNPs in candidate genes. Each PC was categorized in fifty equal groups and included as continuous regressors. The intention in modelling the PC as continuous regressors was to capture and adjust for a substantial part of the variation in the candidate genes by adjusting for several PC's simultaneously. To increase each PC's ability to reduce the impact of family history, the direction of every categorization was selected, so that the associated schizophrenia log-rate was positive. Other categorizations were also assessed. To determine whether values beyond a certain genetic threshold could modify the relationship with familial psychopathology, a number of PC's were dichotomised five-hundred times. The numbers of PC's were 1, 2, 3, 5, 10, 20, 50, 100, 500 and 1500 corresponding to five-thousand ($=10*500$) dichotomisations. To capture a cumulative polygenic effect, a genetic susceptibility score based on rate ratio associated with SNPs that were significant at the $p<0.5$ level was constructed (International Schizophrenia Consortium, 2009; Speliotes et al., 2010). The references were chosen in homozygotes that ensured a rate ratios >1.

3. Results

In total, 739 cases with schizophrenia and 800 matched controls were identified.

3.1. Schizophrenia risk and family history of psychiatric disorder

Table 1 shows rate ratios associated with family history of psychiatric disorder as well as the number of exposed subjects. In keeping with previous findings (Dean et al., 2010), familial histories of psychiatric disorders were highly predictive of schizophrenia. For instance, the rate ratio of schizophrenia in subjects whose 1st degree relative had been diagnosed with narrow schizophrenia was 9.31 (95% confidence interval, 3.85–22.44). The analogous rate for subjects with a family history of schizophrenia or other psychoses was 3.90 (2.28–6.67) and the rate associated with a family history of bipolar affective disorder was 2.80 (1.80–4.34).

3.2. Family history adjusted for each SNP separately

Following the pruning steps, 547,071 SNPs were left for analyses. A total of 542 SNPs were significant with a false discovery rate set at 90% (Benjamini and Hochberg, 1995). Yet, 4735 SNPs increased the rate ratio associated one allele copy with more than 50% and 29 SNPs conferred an increased rate of at least 500%.

Fig. 1 shows schizophrenia rate ratios associated with familial psychiatric disorder (right column of Table 1) along the twenty-two autosomes when adjusted for each SNP separately. The adjusted rates associated familial schizophrenia or other psychoses range from 3.54 to 4.52. The analogous rates associated with familial bipolar affective disorder and other psychiatric disorders range from 2.61 to 3.10 and from 2.58 to 2.96, respectively. Rates associated with narrow schizophrenia varied within 8.31 to 11.34. Note, by comparing with Table 1, that all SNP-adjusted rate ratios are well within the 95% confidence intervals of the unadjusted rate ratios. Equivalent results were obtained for rates associated with disorders in mother, father and siblings (available on request). In summary, no SNP altered significantly the excess schizophrenia risk associated with any measure of family history of psychiatric disorder.

To evaluate the modifying effect of single SNPs, Fig. 2 shows rate ratios for schizophrenia associated with familial psychiatric disorder adjusted for each SNP plotted against the unadjusted log-rate ratio associated with the particular SNP. A few SNPs with log-rate ratio exceeding ± 3 were not shown (rs1029145, rs11960716, rs12530617, rs17023957, rs2745626, rs4149238, rs4543153 and rs4625350). For these SNPs, the adjusted rate ratios associated familial schizophrenia or other psychoses were 4.09, 3.99, 3.89, 3.97, 3.90, 3.73, 4.24 and 3.87. For SNPs, where a large rate ratio is not a chance finding, the impact on rates associated with familial psychiatric disorder could be expected to be considerable, but this is not seen in Fig. 2. Note that SNPs with significant deviation from Hardy–Weinberg equilibrium in the controls are not excluded; these SNPs are simply a subgroup of the shown SNPs in Figs. 1 and 2.

3.3. Familial history and genome-wide structure

Fig. 3 shows the percentage of the excess risk associated with familial history of psychiatric disorder mediated through or explained by the genomic structure, as separately captured by each of the 1538 PCs. A total of 72 PCs were significant at the 5% level and 364 of the PC-groups conferred a rate increase of at least 50%. The percentages, which range from −6.12 (−22.54; 5.02) to 5.31 (−4.08; 18.01), were ordered by size. Negative percentages can be obtained where an association with schizophrenia is lacking. Essentially the

Table 1
Rate ratios of offspring schizophrenia in relation to family history of psychiatric disorder among 739 cases and 800 matched controls (95% confidence intervals).

Diagnosis[a]	Mother		Father		Siblings[b]		Familial	
	N cases/controls	Rate ratio	N cases/controls	Rate ratio	N cases/controls	Rate ratio	N cases/controls	Rate ratio
Schizophrenia or other psychoses[c]	24/8	3.13 (1.39–7.03)	25/11	2.69 (1.27–5.70)	15/2[d]	9.27 (2.08–41.4)	58/20	3.90 (2.28–6.67)
Bipolar affective disorder	40/19	2.38 (1.35–4.18)	27/9	3.91 (1.78–8.56)	19/8	3.52 (1.42–8.74)	70/35	2.80 (1.80–4.34)
Other psychiatric disorder	59/35	2.02 (1.29–3.14)	79/25	4.19 (2.58–6.81)	80/44	2.20 (1.49–3.23)	153/84	2.71 (1.99–3.71)
No psychiatric disorder	616/738	1	608/755	1	625/746	1	458/661	1

[a] None of the four sets of ratio ratios was statistical distinguishable across the three diagnostic categories (p-values: 0.63, 0.62, 0.15 and 0.48).
[b] Adjusted for being an only child which apply to 88 cases and 61 controls resulting in a rate ratio of 1.83 (1.29–2.60).
[c] Only 6 controls (40 cases) had a first-degree relative who had been diagnosed with a narrowly defined diagnosis of schizophrenia (ICD8 code 295 and ICD10 code F20) which results in a rate ratio of 9.31 (3.85–22.44).
[d] Based on only 2 controls with a sibling who had been diagnosed with schizophrenia or other psychoses i.e. interpreted with caution.

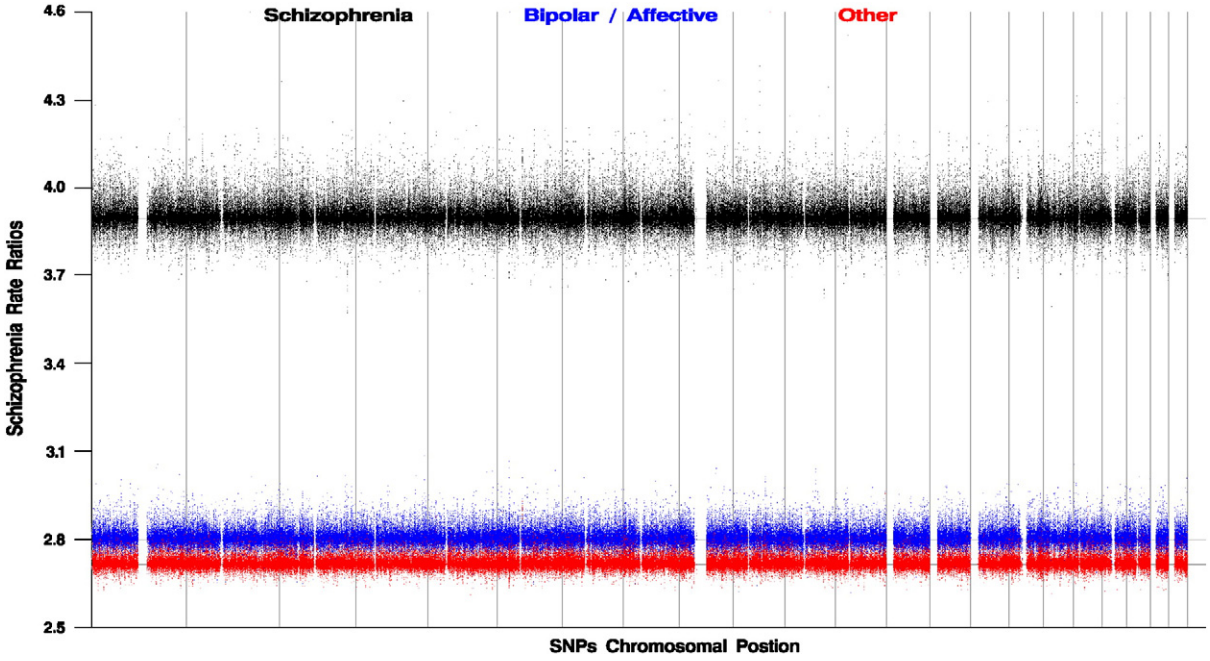

Fig. 1. Rate ratios of schizophrenia in relation to family history of psychiatric disorder sequentially adjusted for each of the 547,071 SNPs separately based on 739 cases and 800 matched controls.

same results were obtained for disorders in mothers, fathers and siblings, and both for other categorization and after outlier correction. The analogous percentage of the excess risk associated with a family history of narrow schizophrenia that was mediated through the genomic PCs ranged between −6.09 (−16.95; 2.60) and 4.07 (−3.89; 15.23).

3.4. Familial history and structure in candidate genes

Table 2 shows rate ratios for schizophrenia in relation to history of familial psychopathology after adjustments for structure in the top 39 candidate genes. After adjusting for the first twenty PCs, which summarize 24.9% of the genetic variation, the rate ratio associated with a

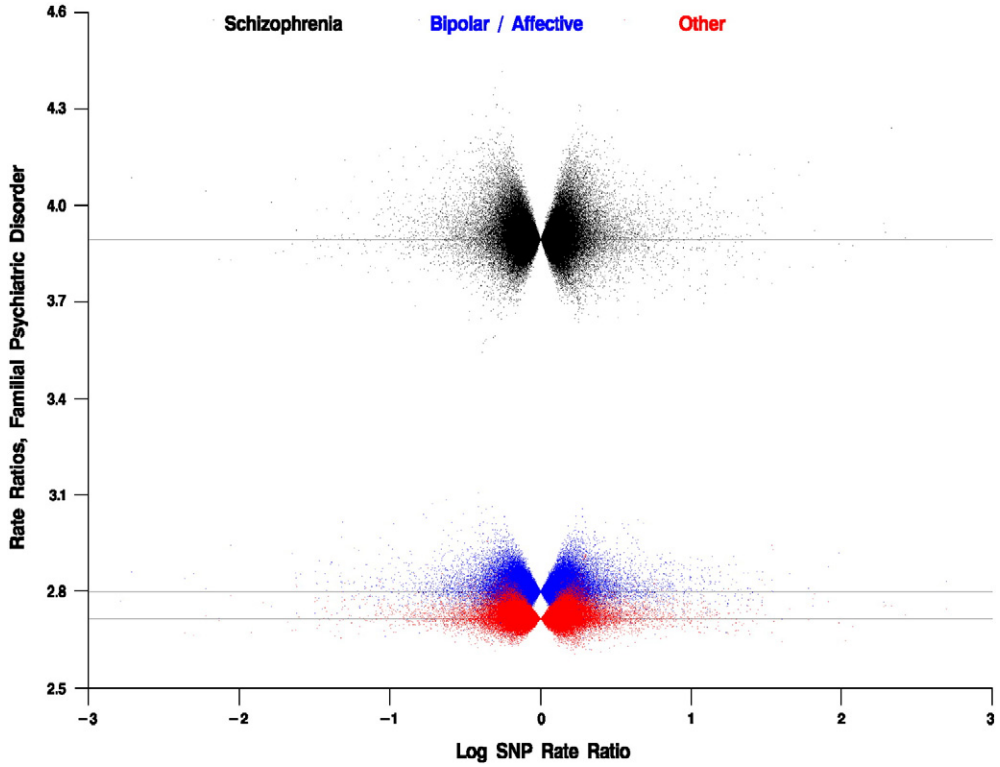

Fig. 2. Rate ratios of schizophrenia in relation to familial history of psychiatric disorder separately adjusted for the 547,071 SNPs plotted against the log-rate ratio estimate associated with the particular SNP in an unadjusted model based on 739 cases and 800 matched controls.

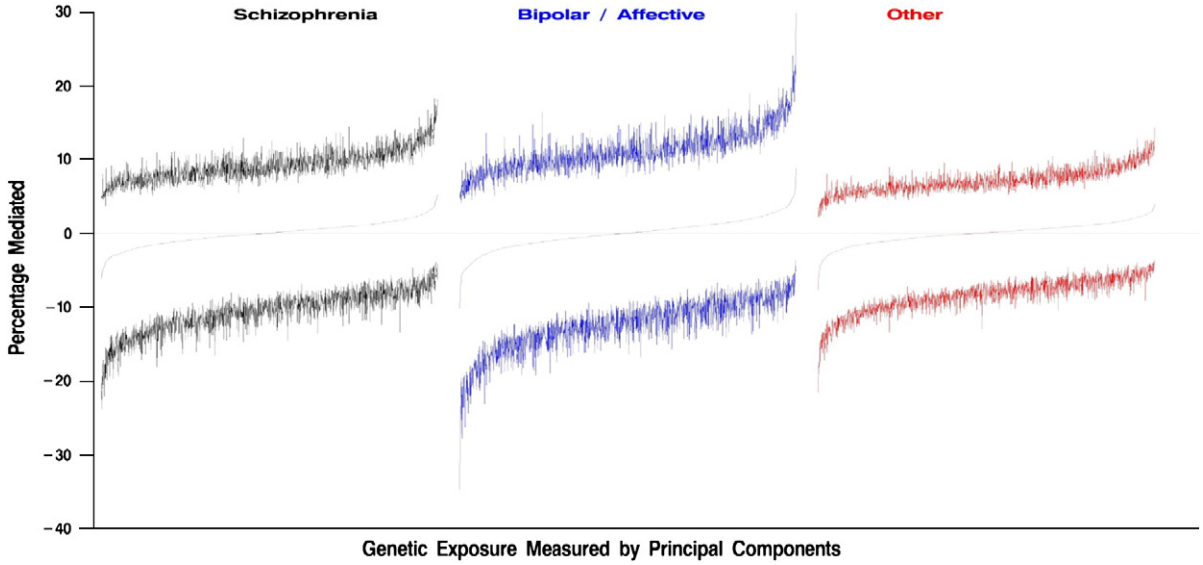

Fig. 3. The percentage of the excess rate ratio associated with familial history of psychiatric disorder mediated through genetic variation as separately captured by each of the 1538 principal components (categorized in seven equal groups) of the genome-wide SNP data with 547,071 markers and 1539 individuals. Upper and lower curves are 95% bootstrap-based confidence intervals.

family history of schizophrenia or other psychoses is 3.85, which is in close proximity to the crude rate (i.e. 3.90). The analogous rate is 3.72 after adjusting for the twenty PCs with the highest association with the schizophrenia rate, while adjustment for both sets of PCs resulted in a rate of 3.66. Note that each of the PCs is statistically significant with p-values ranging from 0.00027 to 0.011. To reduce the risk of overparameterising the model, no more than forty PC's were considered simultaneously. Across the five-thousand genetic threshold models, the rate ratios in relation to a family history of schizophrenia or other psychoses varied between 3.74 (2.18; 6.40) and 4.17 (2.41; 7.20) and the analogous rates for a family history of narrow schizophrenia spanned between 8.88 (3.68; 21.44) and 10.95 (4.40; 27.23). The polygenic susceptibility score was based on 856 SNPs and the associated rate ratios which range from 2.08 (1.21; 3.56) in rs2919377 to 1.05 (0.91; 1.21) in rs2512713. While, the polygenic susceptibility score was strongly significant with a χ^2_1 value of 140.38, it was no more informative than the PC-derived models. Note that the impact of the score is presumably artificially inflated (Powell and Zietsch, 2011), as the score was not obtained in an independent sample. Thus the score may only explain a negligible fraction of the variation, e.g. up to 3% in a previous study (International Schizophrenia Consortium, 2009). In our study, however, rate ratios of schizophrenia in relation to familial history of psychiatric disorder will tend to be biased downward when adjusted for the score.

4. Discussion

To the best of our knowledge, this study is the first to combine a classical population-based epidemiological design with a genome-wide association approach. The results suggest that the excess risk of offspring schizophrenia in families affected by psychotic, bipolar affective or other psychiatric disorder is essentially unchanged when SNP-based variation is taken into account. The findings are unchanged regardless of the inclusion of SNP-based variation based on genome-wide analyses or within candidate genes. Furthermore no single SNP altered the association between schizophrenia and familial history of psychiatric disorder.

Table 2
Rate ratios of schizophrenia in relation to familial history of psychiatric disorder among 739 cases and 800 matched controls (95% confidence intervals). Based on 1848 SNPs located within 50 kilobases from exons in the top 39 candidate genes, the ratios are adjusted for the twenty principal components that 1) explain most of the genetic variation, 2) the twenty principal components which are closest related to case–control status, 3) the forty principal components combined and 4) adjusted for a polygenic score based on all SNPs with $p<0.5$.

	Crude[a]	1st adjustment[b]	2nd adjustment[c]	3rd adjustment[d]	4th adjustment[e]
Schizophrenia or other psychoses	3.90 (2.28–6.67)	3.85 (2.23–6.64)	3.72 (2.07–6.68)	3.66 (2.00–6.68)	3.85 (2.11–7.02)
Bipolar affective disorder	2.80 (1.80–4.34)	2.76 (1.76–4.33)	3.00 (1.85–4.89)	2.97 (1.79–4.91)	2.42 (1.49–3.94)
Other psychiatric disorder	2.71 (1.99–3.71)	2.74 (1.99–3.78)	2.93 (2.05–4.18)	2.91 (2.02–4.19)	2.58 (1.82–3.65)
No psychiatric disorder	1	1	1	1	1

[a] Crude rate ratios signify adjustment for gender, age and calendar-time by matching. The crude rate ratio associated with a familial history of narrow schizophrenia (ICD8 code 295 and ICD10 code F20) was 9.31 (3.85–22.44).
[b] Adjusted for the twenty first principal components which explain most of the genetic variation. Each principal component was categorized in fifty groups and included in the regression as a continuous variable. The analogous rate ratio associated with a narrow diagnose of schizophrenia was 9.02 (3.69–22.08).
[c] Adjusted for the twenty principal components which are closest related to case–control status (i.e. lowest p-value) when entered as a continuous variable in fifty categories. The analogous rate ratio associated with a narrow diagnose of schizophrenia was 8.54 (3.37–21.59).
[d] The previous adjustments combined. The analogous rate ratio associated with a narrow schizophrenia was 8.23 (3.13–21.64).
[e] Adjusted for a polygenic score based on all SNPs with $p<0.5$. The analogous rate ratio associated with a narrow schizophrenia was 9.17 (3.41–24.68).

It has proven difficult to identify genes that predispose to schizophrenia (Sullivan, 2005; Crow, 2008; Sanders et al., 2008; Sullivan, 2008a; Sullivan, 2008b; Need et al., 2009; Dick et al., 2010; Gejman et al., 2010), and many explanations for the missing heritability have been suggested (Manolio et al., 2009; Gershon et al., 2011). Thus it is not surprising that our SNP-wise analyses did not alter the excess risk associated with familial history of psychiatric disorder — in particular when taking a resolution of only 547,071 SNPs and a sample size of only 739 cases and 800 controls into account. Given the recent interest a polygenic variant model for schizophrenia and bipolar disorder (International Schizophrenia Consortium, 2009), it is of interest to note that variation across the whole genome and across candidate genes was no more informative than single SNPs in this sample. It is noteworthy that this is also the case for family history of narrow schizophrenia.

Our results should not be misinterpreted to suggest that common genetic variants are irrelevant in the etiology of schizophrenia. Modelling exercises (Wray et al., 2011) and expert opinion (Insel, 2010) suggest that very large sample sizes are required to confidently identify sufficient common SNPs to explain a reasonable proportion of the genetic architecture of psychotic disorders. It should also be noted that the SNP arrays do not contain less common SNP variants and that SNP arrays are suboptimal for detection of structural variation such as copy number variants (Bassett et al., 2010). Hence our analyses cannot determine whether the mode of inheritance of schizophrenia is polygenic with common disease variants or heterogeneous with rare private variants (Mitchell and Porteous, 2011). Similarly, our study contributes little information relevant to the conjectured shared genetic liability between schizophrenia and bipolar disorder beyond finding an excess schizophrenia risk in the offspring of parents who suffer from these disorders (Laursen et al., 2009; Lichtenstein et al., 2009). One may conjecture that the lack of correspondence between family history and SNP's reflects the fact that familial aggregation represents a heterogeneous mix of genetic variation, gene–gene and gene–environment interactions, something that is not captured by individual SNP's or their linear combinations.

4.1. Strengths and limitations

The Achilles' heel of our study is that the large number of statistical tests combined with the fact that genetic effects are likely to be subtle may suggest a lack of statistical power (Corvin et al., 2010). Our purpose was to evaluate whether the association between schizophrenia and familial history of psychiatric disorder is mediated through or partly explained by genome-wide SNP variation within this National birth cohort-based sample. Our approach is to make the strong, and perhaps, false assumption that the observed genetic associations are 'true'. Thus, the SNP-based information is given every chance to explain associations with family history, i.e., even when exaggerated by design, the potential influence of our genome-wide data has only minor impact on the familial risk for schizophrenia.

Cases were identified through the Danish Psychiatric Central Register which relies on routine clinical diagnoses, and thus, phenotypic misclassification is an issue of concern. Analogously, controls are merely individuals who have not been registered with schizophrenia. On the other hand, the Danish Psychiatric Central Register has high diagnostic validity (Jakobsen et al., 2005), and it is unlikely that an unregistered and randomly chosen person should suffer from schizophrenia. Since familial history is assessed independently of caseness and since the related rates are in keeping with previous findings (Mortensen et al., 2010), it is even less likely that our sample is atypical. Note that the young age of the sample implies that the high risk period has not been passed and that misclassification of family history will reduce the statistical power.

In conclusion, this study finds that the excess risk of schizophrenia in the offspring of parents who suffer from a psychotic, bipolar affective or other psychiatric disorder is not explained by variation across the analysed 547,071 SNPs. Our findings are consistent with the emerging evidence about the genetic architecture of schizophrenia. SNP-based variation in even the largest samples is only able to capture a small proportion of the family-mediated risk of schizophrenia. While our sample was small compared to recent case–control studies, the ability to integrate the SNP-based variation within a population-based sample of family history of several major psychiatric disorders confirms the challenges that lie ahead.

Role of funding source

This study was supported by The Stanley Medical Research Institute, The Danish Council for Strategic research (2101-07-0059) and H. Lundbeck A/S. The sponsors had no role in the design of the study, in analysis and interpretation of the data, or in the writing of the manuscript.

Contributors

EA, PBM and CBP designed the study. EA undertook the statistical analysis. EA wrote all drafts of the manuscript. All authors contributed and have approved the final manuscript

Conflict of interest

The authors have no conflicts of interest to declare

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.schres.2011.10.025.

References

Allen, N.C., Bagade, S., McQueen, M.B., Ioannidis, J.P., Kavvoura, F.K., Khoury, M.J., Tanzi, R.E., Bertram, L., 2008. Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. Nat. Genet. 40, 827–834.
Alter, O., Brown, P.O., Botstein, D., 2000. Singular value decomposition for genome-wide expression data processing and modeling. Proc. Natl. Acad. Sci. U. S. A. 97, 10101–10106.
Armitage, P., Berry, G., Matthews and J.N.S., 2002. Statistical Methods in Medical Research, 4th edition edn. Blackwell Science Ltd., Oxford.
Ballard, D., Abraham, C., Cho, J., Zhao, H., 2010a. Pathway analysis comparison using Crohn's disease genome wide association studies. BMC Med. Genomics 3, 25.
Ballard, D.H., Cho, J., Zhao, H., 2010b. Comparisons of multi-marker association methods to detect association between a candidate region and disease. Genet. Epidemiol. 34, 201–212.
Bassett, A.S., Scherer, S.W., Brzustowicz, L.M., 2010. Copy number variations in schizophrenia: critical review and new perspectives on concepts of genetics and disease. Am. J. Psychiatry 167, 899–914.
Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate — a practical and powerful approach to multiple testing. J. R. Stat. Soc. B Methodol. 57, 289–300.
Borgan, O., Goldstein, L., Langholz, B., 1995. Methods for the analysis of sampled cohort data in the Cox proportional hazards model. Ann. Stat. 23, 1749–1778.
Byrne, M., Agerbo, E., Mortensen, P.B., 2002. Family history of psychiatric disorders and age at first contact in schizophrenia: an epidemiological study. Br. J. Psychiatry Suppl. 43, s19–s25.
Cardno, A.G., Gottesman, I.I., 2000. Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am. J. Med. Genet. 97, 12–17.
Cichon, S., Craddock, N., Daly, M., Faraone, S.V., Gejman, P.V., Kelsoe, J., Lehner, T., Levinson, D.F., Moran, A., Sklar, P., Sullivan, P.F., 2009. Genomewide association studies: history, rationale, and prospects for psychiatric disorders. Am. J. Psychiatry 166, 540–556.
Clark, A.G., Nielsen, R., Signorovitch, J., Matise, T.C., Glanowski, S., Heil, J., Winn-Deen, E. S., Holden, A.L., Lai, E., 2003. Linkage disequilibrium and inference of ancestral recombination in 538 single-nucleotide polymorphism clusters across the human genome. Am. J. Hum. Genet. 73, 285–300.
Clayton, D., Hills, M., 1996. Statistical Models in Epidemiology. Oxford University Press, Oxford.
Corvin, A., Craddock, N., Sullivan, P.F., 2010. Genome-wide association studies: a primer. Psychol. Med. 40, 1063–1077.
Crow, T.J., 2008. The emperors of the schizophrenia polygene have no clothes. Psychol. Med. 38, 1681–1685.
Davison, A.C., Hinkley, D.V., 1997. Bootstrap Methods and Their Application. Cambridge University Press, Cambridge.
Dean, K., Stevens, H., Mortensen, P.B., Murray, R.M., Walsh, E., Pedersen, C.B., 2010. Full spectrum of psychiatric outcomes among offspring with parental history of mental disorder. Arch. Gen. Psychiatry 67, 822–829.
Dick, D.M., Riley, B., Kendler, K.S., 2010. Nature and nurture in neuropsychiatric genetics: where do we stand? Dialogues Clin. Neurosci. 12, 7–23.
Freedman, L.S., Graubard, B.I., Schatzkin, A., 1992. Statistical validation of intermediate end-points for chronic diseases. Stat. Med. 11, 167–178.

Gauderman, W.J., Murcray, C., Gilliland, F., Conti, D.V., 2007. Testing association between disease and multiple SNPs in a candidate gene. Genet. Epidemiol. 31, 383–395.
Gejman, P.V., Sanders, A.R., Duan, J., 2010. The role of genetics in the etiology of schizophrenia. Psychiatr. Clin. North Am. 33, 35–66.
Gershon, E.S., Alliey-Rodriguez, N., Liu, C., 2011. After GWAS: searching for genetic risk for schizophrenia and bipolar disorder. Am. J. Psychiatry 168, 253–256.
Glymour, M.M., 2006. Using causal diagrams to understand common problems in social epidemiology. In: Oakes, J.M., Kaufman, J.S. (Eds.), Methods in Social Epidemiology. Jossey-Bass - John Wiley & Sons, San Francisco, pp. 393–428.
Hollegaard, M.V., Sorensen, K.M., Petersen, H.K., Arnardottir, M.B., Norgaard-Pedersen, B., Thorsen, P., Hougaard, D.M., 2007. Whole genome amplification and genetic analysis after extraction of proteins from dried blood spots. Clin. Chem. 53, 1161–1162.
Hollegaard, M.V., Grauholm, J., Borglum, A., Nyegaard, M., Norgaard-Pedersen, B., Orntoft, T., Mortensen, P.B., Wiuf, C., Mors, O., Didriksen, M., Thorsen, P., Hougaard, D.M., 2009. Genome-wide scans using archived neonatal dried blood spot samples. BMC Genomics 10, 297.
Holter, N.S., Mitra, M., Maritan, A., Cieplak, M., Banavar, J.R., Fedoroff, N.V., 2000. Fundamental patterns underlying gene expression profiles: simplicity from complexity. Proc. Natl. Acad. Sci. U. S. A. 97, 8409–8414.
Human Genome Sequencing Consortium, 2004. Finishing the euchromatic sequence of the human genome. Nature 431, 931–945.
Ingraham, L.J., Kety, S.S., 2000. Adoption studies of schizophrenia. Am. J. Med. Genet. 97, 18–22.
Insel, T.R., 2010. Rethinking schizophrenia. Nature 468, 187–193.
International Schizophrenia Consortium, 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460, 748–752.
Jakobsen, K.D., Frederiksen, J.N., Hansen, T., Jansson, L.B., Parnas, J., Werge, T., 2005. Reliability of clinical ICD-10 schizophrenia diagnoses. Nord. J. Psychiatry 59, 209–212.
Kety, S.S., Rosenthal, D., Wender, P.H., Schulsinger, F., 1971. Mental illness in the biological and adoptive families of adopted schizophrenics. Am. J. Psychiatry 128, 302–306.
Khan, J., Wei, J.S., Ringner, M., Saal, L.H., Ladanyi, M., Westermann, F., Berthold, F., Schwab, M., Antonescu, C.R., Peterson, C., Meltzer, P.S., 2001. Classification and diagnostic prediction of cancers using gene expression profiling and artificial neural networks. Nat. Med. 7, 673–679.
King, G., Zeng, L., 2002. Estimating risk and rate levels, ratios and differences in case–control studies. Stat. Med. 21, 1409–1427.
Konneker, T., Barnes, T., Furberg, H., Losh, M., Bulik, C.M., Sullivan, P.F., 2008. A searchable database of genetic evidence for psychiatric disorders. Am. J. Med. Genet. B Neuropsychiatr. Genet. 147B, 671–675.
Laursen, T.M., Agerbo, E., Pedersen, C.B., 2009. Bipolar disorder, schizoaffective disorder and schizophrenia overlap — a new comorbidity index. J. Clin. Psychiatry 70, 1432–1438.
Lichtenstein, P., Bjork, C., Hultman, C.M., Scolnick, E., Sklar, P., Sullivan, P.F., 2006. Recurrence risks for schizophrenia in a Swedish national cohort. Psychol. Med. 36, 1417–1425.
Lichtenstein, P., Yip, B.H., Bjork, C., Pawitan, Y., Cannon, T.D., Sullivan, P.F., Hultman, C.M., 2009. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet 373, 234–239.
Manolio, T.A., Collins, F.S., Cox, N.J., Goldstein, D.B., Hindorff, L.A., Hunter, D.J., McCarthy, M.I., Ramos, E.M., Cardon, L.R., Chakravarti, A., Cho, J.H., Guttmacher, A.E., Kong, A., Kruglyak, L., Mardis, E., Rotimi, C.N., Slatkin, M., Valle, D., Whittemore, A.S., Boehnke, M., Clark, A.G., Eichler, E.E., Gibson, G., Haines, J.L., Mackay, T.F., McCarroll, S.A., Visscher, P.M., 2009. Finding the missing heritability of complex diseases. Nature 461, 747–753.
Mcgue, M., Gottesman, I.I., Rao, D.C., 1983. The transmission of schizophrenia under a multifactorial threshold model. Am. J. Hum. Genet. 35, 1161–1178.
McVean, G., 2009. A genealogical interpretation of principal components analysis. PLoS Genet. 5, e1000686.
Mitchell, K.J., Porteous, D.J., 2011. Rethinking the genetic architecture of schizophrenia. Psychol. Med. 41, 19–32.
Mortensen, P.B., Pedersen, C.B., Westergaard, T., Wohlfahrt, J., Ewald, H., Mors, O., Andersen, P.K., Melbye, M., 1999. Effects of family history and place and season of birth on the risk of schizophrenia. N. Engl. J. Med. 340, 603–608.
Mortensen, P.B., Pedersen, M.G., Pedersen, C.B., 2010. Psychiatric family history and schizophrenia risk in Denmark: which mental disorders are relevant? Psychol. Med. 40, 201–210.
Munk-Jorgensen, P., Mortensen, P.B., 1997. The Danish Psychiatric Central Register. Dan. Med. Bull. 44, 82–84.
Need, A.C., Ge, D., Weale, M.E., Maia, J., Feng, S., Heinzen, E.L., Shianna, K.V., Yoon, W., Kasperaviciute, D., Gennarelli, M., Strittmatter, W.J., Bonvicini, C., Rossi, G., Jayathilake, K., Cola, P.A., McEvoy, J.P., Keefe, R.S., Fisher, E.M., St Jean, P.L., Giegling, I., Hartmann, A.M., Moller, H.J., Ruppert, A., Fraser, G., Crombie, C., Middleton, L.T., St, C.D., Roses, A.D., Muglia, P., Francks, C., Rujescu, D., Meltzer, H.Y., Goldstein, D.B., 2009. A genome-wide investigation of SNPs and CNVs in schizophrenia. PLoS Genet. 5, e1000373.
Norgaard-Pedersen, B., Hougaard, D.M., 2007. Storage policies and use of the Danish Newborn Screening Biobank. J. Inherit. Metab. Dis. 30, 530–536.
Norgaard-Pedersen, B., Simonsen, H., 1999. Biological specimen banks in neonatal screening. Acta Paediatr. Suppl. 88, 106–109.
O'Donovan, M.C., Craddock, N.J., Owen, M.J., 2009. Genetics of psychosis; insights from views across the genome. Hum. Genet. 126, 3–12.
Patterson, N., Price, A.L., Reich, D., 2006. Population structure and eigenanalysis. PLoS Genet. 2, e190.
Pedersen, C.B., Gotzsche, H., Moller, J.O., Mortensen, P.B., 2006. The Danish Civil Registration System. A cohort of eight million persons. Dan. Med. Bull. 53, 441–449.
Powell, J.E., Zietsch, B.P., 2011. Predicting sensation seeking from dopamine genes. Psychol. Sci. 22, 413–415.
Reich, D., Price, A.L., Patterson, N., 2008. Principal component analysis of genetic data. Nat. Genet. 40, 491–492.
Risch, N., 1990. Linkage strategies for genetically complex traits. I. Multilocus models. Am. J. Hum. Genet. 46, 222–228.
Rubin, D.B., 1976. Inference and missing data. Biometrika 63, 581–592.
Sanders, A.R., Duan, J., Levinson, D.F., Shi, J., He, D., Hou, C., Burrell, G.J., Rice, J.P., Nertney, D.A., Olincy, A., Rozic, P., Vinogradov, S., Buccola, N.G., Mowry, B.J., Freedman, R., Amin, F., Black, D.W., Silverman, J.M., Byerley, W.F., Crowe, R.R., Cloninger, C.R., Martinez, M., Gejman, P.V., 2008. No significant association of 14 candidate genes with schizophrenia in a large European ancestry sample: implications for psychiatric genetics. Am. J. Psychiatry 165, 497–506.
Sauerbrei, W., 1999. The use of resampling methods to simplify regression models in medical statistics. J. R. Stat. Soc. Ser. C Appl. Stat. 48, 313–329.
Schwartz, S., Susser, E., 2010. Genome-wide association studies: does only size matter? Am. J. Psychiatry 167, 741–744.
Speliotes, E.K., Willer, C.J., Berndt, S.I., Monda, K.L., Thorleifsson, G., Jackson, A.U., Allen, H.L., Lindgren, C.M., Luan, J., Magi, R., Randall, J.C., Vedantam, S., Winkler, T.W., Qi, L., Workalemahu, T., Heid, I.M., Steinthorsdottir, V., Stringham, H.M., Weedon, M.N., Wheeler, E., Wood, A.R., Ferreira, T., Weyant, R.J., Segre, A.V., Estrada, K., Liang, L., Nemesh, J., Park, J.H., Gustafsson, S., Kilpelainen, T.O., Yang, J., Bouatia-Naji, N., Esko, T., Feitosa, M.F., Kutalik, Z., Mangino, M., Raychaudhuri, S., Scherag, A., Smith, A.V., Welch, R., Zhao, J.H., Aben, K.K., Absher, D.M., Amin, N., Dixon, A.L., Fisher, E., Glazer, N.L., Goddard, M.E., Heard-Costa, N.L., Hoesel, V., Hottenga, J.J., Johansson, A., Johnson, T., Ketkar, S., Lamina, C., Li, S., Moffatt, M.F., Myers, R.H., Narisu, N., Perry, J.R., Peters, M.J., Preuss, M., Ripatti, S., Rivadeneira, F., Sandholt, C., Scott, L.J., Timpson, N.J., Tyrer, J.P., van, W.S., Watanabe, R.M., White, C.C., Wiklund, F., Barlassina, C., Chasman, D.I., Cooper, M.N., Jansson, J.O., Lawrence, R.W., Pellikka, N., Prokopenko, I., Shi, J., Thiering, E., Alavere, H., Alibrandi, M.T., Almgren, P., Arnold, A.M., Aspelund, T., Atwood, L.D., Balkau, B., Balmforth, A.J., Bennett, A.J., Ben-Shlomo, Y., Bergman, R.N., Bergmann, S., Biebermann, H., Blakemore, A.I., Boes, T., Bonnycastle, L.L., Bornstein, S.R., Brown, M.J., Buchanan, T.A., Busonero, F., Campbell, H., Cappuccio, F.P., Cavalcanti-Proenca, C., Chen, Y.D., Chen, C.M., Chines, P.S., Clarke, R., Coin, L., Connell, J., Day, I.N., Heijer, M., Duan, J., Ebrahim, S., Elliott, P., Elosua, R., Eiriksdottir, G., Erdos, M.R., Eriksson, J.G., Facheris, M.F., Felix, S.B., Fischer-Posovszky, P., Folsom, A.R., Friedrich, N., Freimer, N.B., Fu, M., Gaget, S., Gejman, P.V., Geus, E.J., Gieger, C., Gjesing, A.P., Goel, A., Goyette, P., Grallert, H., Grassler, J., Greenawalt, D.M., Groves, C.J., Gudnason, V., Guiducci, C., Hartikainen, A.L., Hassanali, N., Hall, A.S., Havulinna, A.S., Hayward, C., Heath, A.C., Hengstenberg, C., Hicks, A.A., Hinney, A., Hofman, A., Homuth, G., Hui, J., Igl, W., Iribarren, C., Isomaa, B., Jacobs, K.B., Jarick, I., Jewell, E., John, U., Jorgensen, T., Jousilahti, P., Jula, A., Kaakinen, M., Kajantie, E., Kaplan, L.M., Kathiresan, S., Kettunen, J., Kinnunen, L., Knowles, J.W., Kolcic, I., Konig, I.R., Koskinen, S., Kovacs, P., Kuusisto, J., Kraft, P., Kvaloy, K., Laitinen, J., Lantieri, O., Lanzani, C., Launer, L.J., Lecoeur, C., Lehtimaki, T., Lettre, G., Liu, J., Lokki, M.L., Lorentzon, M., Luben, R.N., Ludwig, B., Manunta, P., Marek, D., Marre, M., Martin, N.G., McArdle, W.L., McCarthy, A., McKnight, B., Meitinger, T., Melander, O., Meyre, D., Midthjell, K., Montgomery, G.W., Morken, M.A., Morris, A.P., Mulic, R., Ngwa, J.S., Nelis, M., Neville, M.J., Nyholt, D.R., O'Donnell, C.J., O'Rahilly, S., Ong, K.K., Oostra, B., Pare, G., Parker, A.N., Perola, M., Pichler, I., Pietilainen, K.H., Platou, C.G., Polasek, O., Pouta, A., Rafelt, S., Raitakari, O., Rayner, N.W., Ridderstrale, M., Rief, W., Ruokonen, A., Robertson, N.R., Rzehak, P., Salomaa, V., Sanders, A.R., Sandhu, M.S., Sanna, S., Saramies, J., Savolainen, M.J., Scherag, S., Schipf, S., Schreiber, S., Schunkert, H., Silander, K., Sinisalo, J., Siscovick, D.S., Smit, J.H., Soranzo, N., Sovio, U., Stephens, J., Surakka, I., Swift, A.J., Tammesoo, M.L., Tardif, J.C., Teder-Laving, M., Teslovich, T.M., Thompson, J.R., Thomson, B., Tonjes, A., Tuomi, T., van Meurs, J.B., van Ommen, G.J., 2010. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat. Genet. 42, 937–948.
Sullivan, P.F., 2005. The genetics of schizophrenia. PLoS Med. 2, e212.
Sullivan, P.F., 2008a. Schizophrenia genetics: the search for a hard lead. Curr. Opin. Psychiatry 21, 157–160.
Sullivan, P.F., 2008b. The dice are rolling for schizophrenia genetics. Psychol. Med. 38, 1693–1696.
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.
Sullivan, P.F., Lin, D., Tzeng, J.Y., van den Oord, E., Perkins, D., Stroup, T.S., Wagner, M., Lee, S., Wright, F.A., Zou, F., Liu, W., Downing, A.M., Lieberman, J., Close, S.L., 2008. Genomewide association for schizophrenia in the CATIE study: results of stage 1. Mol. Psychiatry 13, 570–584.
van Os, J., Rutten, B.P.F., Poulton, R., 2008. Gene–environment interactions in schizophrenia: review of epidemiological findings and future directions. Schizophr. Bull. 34, 1066–1082.
World Health Organization, 1967. Manual of the International Classification of Diseases (ICD-8). WHO, Geneva.
World Health Organization, 1992. The ICD-10 Classification of Mental and Behavioural Disorders. WHO, Geneva.
Wray, N.R., Purcell, S.M., Visscher, P.M., 2011. Synthetic associations created by rare variants do not explain most GWAS results. PLoS Biol. 9, e1000579.