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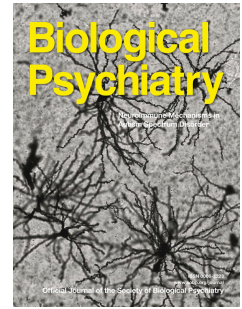
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Heritability of schizophrenia and schizophrenia spectrum based on the nationwide Danish Twin Register

Short title: Heritability of SZ based on the Danish Twin Register

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Abstract

Background: Twin studies have provided evidence that both genetic and environmental factors contribute to schizophrenia risk. Heritability estimates of schizophrenia in twin samples have varied methodologically. This study provides updated heritability estimates based on nationwide twin data and an improved statistical methodology.

Method: Combining two nationwide registers, the Danish Twin Register and the Danish Psychiatric Research Register, we identified a sample of twins born 1951-2000 (N=31,524 twin pairs). Twins were followed up until June 1st 2011. Liability threshold models adjusting for censoring with inverse probability weighting were used to estimate probandwise concordance rates and heritability of the diagnoses of schizophrenia and schizophrenia spectrum disorders.

Results: The probandwise concordance rate of schizophrenia is 33% in monozygotic (MZ) twins and 7% in dizygotic (DZ) twins. We estimated the heritability of schizophrenia to be 79%. When expanding illness outcome to include schizophrenia spectrum disorders, the heritability estimate was almost similar, 73%.

Conclusion: The key strength of this study is the application of a novel statistical method accounting for censoring in the follow-up period to a nationwide twin sample. The estimated 79% heritability of schizophrenia is congruent with previous reports and indicates a substantial genetic risk. The high genetic risk also applies to a broader phenotype of schizophrenia spectrum disorders. The low concordance rate of 33% in MZ twins demonstrates that illness vulnerability is not solely indicated by genetic factors.

Introduction

The risk of developing schizophrenia is influenced by both genetic and environmental factors(1,2). Twin studies have provided important insight with several reports indicating a strong genetic risk. Previous twin studies have varied methodologically, hence there is a need for new studies that utilize current diagnostic practice and escape some of the methodological pitfalls. Twin studies are particularly powerful when estimating the proportion of genetic variance to disease susceptibility by modeling the variance of phenotypic concordance across monozygotic (MZ) and dizygotic (DZ) twin pairs(3). Importantly, twin concordance itself, while expressing the probability that a twin is affected given that the co-twin is affected, does not provide a quantification of the genetic influence(3). Modeling phenotypic concordance in quantitative genetics is based on the concept of disease liability, which is assumed to be a continuous metric that is normally distributed in the population. Individuals with a liability above a certain threshold will develop the disease(4). Heritability modeling includes the coefficient of the additive genetic relation between family members, which for MZ twins is $r=1$ and for DZ twins is $r=0.5$. The closer the relation between family members included in such analysis (highest for MZ twins) the better the quantification of the genetic contribution to the liability of disease(4–6).

Liability threshold models usually define the outcome as having a diagnosis versus no diagnosis at the study endpoint. Thus a large proportion of subjects are still at risk for disease at the end of the observation period. The lack of complete outcome information for these individuals results in what is referred to as censored data, a potential bias that has not been accounted for in previous schizophrenia heritability studies. A more robust way of quantifying genetic influence on schizophrenia can be obtained by extending the classic liability threshold model to include inverse probability weighting. This method takes the censoring of data into account which possibly provides more accurate heritability estimates(7).

As outlined in an overview of the five most recent twin studies, schizophrenia concordance rates are between 41-65% in MZ twins and 0-28% in DZ twins (8). A meta-analysis of 12 twin studies found evidence for large additive genetic effects with heritability estimates of 81% in liability to schizophrenia(9). However, these results stem from distinct methodological differences across studies regarding factors such as diagnostic categories and sample selection procedures, which limit study comparability.

Two studies estimated heritability based on national twin samples. One study from Finland(10) covered same sex twin pairs born 1940-1954 and found that 83% of the variance in liability to

schizophrenia could be attributed to additive genetic effects. Another study from Sweden(11) included both same- and opposite-sex twin pairs born 1926-1958 and estimated the gender-specific heritability for schizophrenia spectrum to be 67% in females and 41% in males. By including older cohorts, the studies may be biased by a truncation mechanism, since case status requires that the individual survive until initiation of the register. The Finnish Psychiatric Case Register was established in 1968 and the Swedish in 1987(12,13). Additionally, a censoring mechanism may have contributed to less accurate estimates, which if unaccounted for could result in downwards biased concordance rates and heritability estimates that are biased in both directions depending on the dependence structure and the censoring distribution(14).

It is important to refine the methodology when estimating the heritability for schizophrenia as the total heritability can be interpreted as an upper limit for the variance in a phenotype which is explained by the variance in genes(6). Genome Wide Association study (GWAS) is one approach estimating Single Nucleotide Polymorphisms (SNP) heritability, which may account for around 30% of the heritability in schizophrenia(15,16). The discrepancy between the SNP-heritability estimated in GWAS studies and the heritability based on diagnostic outcome in population studies is referred to as “missing heritability”, with a large part of the heritability unexplained by estimates from the genetic studies. Rare variants including copy number variants (CNVs), such as deletions and duplications which affect large areas of the genome, can possibly explain a marked part of the missing heritability(17,18). An updated total heritability estimate is important for future GWAS studies, since it reflects a theoretical maximum of the variance explained by the potential genetic factors in schizophrenia.

The present study aims to estimate concordance rates and heritability of schizophrenia in a twin cohort identified in the Danish Twin Register using diagnostic information in the Danish Psychiatric Central Research Register. The study examines two nested phenotypes. First, the narrow definition of schizophrenia as defined in ICD-8 and ICD-10, and second, a broader diagnostic category of schizophrenia spectrum in ICD-8 and ICD-10 (see Methods section for details).

Methods

The study is approved by the Danish Data Protection Agency and the National Board of Health.

National registers

In Denmark each person is assigned a unique identification number at birth and registered in the Danish Central Civil Registration System, making it possible to identify each person in all registers(19). The Danish Twin Register, initiated in 1954, is population-based and includes twins born in Denmark from 1870 onwards. Ascertainment of the Danish Twin Register is complete from 1968, i.e. it covers all twins born in Denmark before the year of 1968, and the ascertainment rate is 72%(20). Information on zygosity is obtained from the register by questionnaire. The Danish Psychiatric Central Research Register was computerized in 1969 and contains information on all psychiatric admissions in Denmark. From 1995 onwards, outpatient contacts were also included. This register differentiates between main and secondary diagnoses(21).

Disease classifications

In Denmark the classification system ICD-8 was used from 1969-1993 while ICD-10 was put into use in 1994(22,23). In this study schizophrenia was defined as a main or secondary lifetime diagnosis in the following ICD versions (ICD-10: F20.xx and ICD-8: 295 (excluding 295.79, schizoaffective disorder)) and schizophrenia spectrum as a main or secondary lifetime diagnosis in (ICD-10: F2x.xx and ICD-8: 295, 297, 29829, 29839, 29889, 29899, 29905, 29909, 30109, 30129). In this study a lifetime diagnosis covers a diagnosis received at any time point during the observation period, i.e. births prior to June 1st 2011. For schizophrenia this is defined as the first date of diagnosis, thus ignoring a possible diagnosis in the schizophrenia spectrum prior to this date. For schizophrenia spectrum it is defined as the first date of diagnosis.

Statistical Analysis

The concordance rate is an estimate of probability that measures the proportion of affected twins given that the co-twin is also affected. It can be calculated in two ways: pairwise and probandwise. Both methods refer to conditional probabilities, however, probandwise rates are applicable to twin individuals, and not twin pairs, thus comparable with the incidence rate and prevalence in a non-twin population(24). In this study we calculate the probandwise concordance rates with 95% confidence intervals (95% CI).

Tetrachoric correlations among twin pairs are calculated to measure the degree of agreement in diagnostic status in the twin pairs. Structural equation and liability threshold models are applied to estimate the heritability of schizophrenia.

The general assumptions for the structural equation modeling are: i) gene-environment interactions are minimal for the trait, ii) twins are comparable to the general population and iii) mating in the

population is random(5,25), while the specific assumptions are: (iv) MZ and DZ pairs share common environmental effects (C) to the same extent (v) the additive genetic effects (A) are shared with correlation equal to 1 between MZ pairs and correlation 0.5 between DZ pairs.

Liability threshold models estimate the heritability as the contribution to variance in liability of additive genetic effects (A), common environmental effects (C) and unique environmental effects (E). Because (A) is considered the most important component for the observable genetic properties in a population(4) and because schizophrenia is a complex disorder assumed to be caused by many genes with small effects(15), we chose to apply ACE models (and sub-models). The full ACE model is fitted to data on the two diagnostic groups and compared with the two nested AE and CE models using delta X2 goodness of fit statistics and Akaike's information Criterion (AIC)(5,25). In the AE model, C is set to 0 having no uncertainty. The likelihood ratio test statistic, with one degree of freedom, is used to compare models to the full (ACE) model resulting in a P-value indicating if the model fit is significantly better according to the data. Traditionally, when C is estimated in the analyses to be equal to zero (0) in the ACE model, the confidence intervals of the uncertainty are achieved and the ACE model is chosen as the best fitting model.

A major issue when estimating heritability of schizophrenia is that a large proportion of study participants are still at risk of developing the illness at the end of the observation period. This induces a bias referred to as censoring (in survival analysis), since there is a lack of complete outcome information. In this study we attempt to overcome this censoring mechanism by using inverse probability weighting (IPW)(26). This means adding a weight to each complete observation (i.e. cases and deaths) based on the data from the censored observations. The IPW model, which estimates the weights, is adjusted for sex and stratified on zygosity. In practice it is only possible to get reliable estimates of the dependence between twins on the part of the time-scale where sufficient information is available. Therefore, to model the correct weights, the follow-up period needs to be the same in both zygosity groups. Consequently, this means we can estimate concordance rates and heritability until the age of the last observed concordant pair in both the MZ and DZ group. This is up to age 40 in the present population, both for schizophrenia and schizophrenia spectrum. Accordingly, heritability estimates in our study are only generalizable to a disease onset before 40 years of age.

Data analyses were carried out using Stata version 12(27) and the Mets package (<https://cran.r-project.org/web/packages/mets/index.html>) in R(28).

Results

All twin pairs born in Denmark from 1951-2000 (N=37,891 pairs) were identified through the Danish Civil Register and coupled with (1) information on psychiatric hospital admissions and corresponding clinical diagnoses from the Danish Psychiatric Central Research Register, and (2) information on zygosity was obtained from the Danish Twin Register (on the majority of twin pairs; N=31,524 pairs). The twin pairs with unknown zygosity (UZ) (N=6,367) were excluded from subsequent analyses, as liability threshold modeling cannot include UZ pairs. A flowchart describing the sample size in the main data and in the supplementary data is pictured in Figure 1. Among UZ twins, 88 twins have schizophrenia and 157 twins have schizophrenia spectrum disorder. The concordance rate of schizophrenia is 0.28 (0.16-0.45) and 0.22 (0.13-0.34) for schizophrenia spectrum in UZ twins.

Of the 31,524 twin pairs included, 448 twin pairs (corresponding to 472 twins) were affected with schizophrenia and 788 twin pairs (842 twins) were affected with schizophrenia spectrum disorder (Table 1). The mean age at onset of schizophrenia and schizophrenia spectrum was 28.92 (SD: 8.52)/29.19 (SD 9.37) and the median age at onset was 27.69/27.85, respectively.

Table 1 shows the number of complete cases in MZ and DZ twins, diagnostic distribution and the number of censored observations in the present analyses. As observed, the majority of the observations are censored, depicted in the category "Censored, e.g. alive without diagnosis", as this category covers the main proportion of the sample (91-95%). The follow-up period in both zygosity groups is specified. The proportion of individuals with a schizophrenia diagnosis is almost similar in the opposite sex and the same-sex DZ groups.

As shown in Table 2, the proband-wise concordance rate and tetrachoric correlations are higher in MZ twins compared to DZ twins. Furthermore, both estimates are nearly identical for schizophrenia and schizophrenia spectrum.

The probandwise concordance rate of schizophrenia and schizophrenia spectrum as a function of years of follow-up (Figure 2a and 2b, respectively) is higher in MZ and DZ twins than the overall cumulative incidence, indicating a familial risk for both conditions. Further, in both conditions, the concordance rate is two-to-three fold higher in MZ compared to DZ twins, with the DZ concordance rates being more stable across years of follow-up.

A liability threshold ACE model was initially fitted to schizophrenia twin data (AIC=14,031.65; Table 3) and compared to a reduced AE model (AIC=14,029.65) resulting in a nearly similar fit ($p=1.0$). Contrary, a CE model did not provide a better fit (AIC=14,062.88; $p<0.001$). We chose the AE model as the best fitting model because it has the lowest AIC. The ACE model is not preferred due to the uncertain C and confidence interval estimates (which are both set to zero). The same model fitting procedure was applied to schizophrenia spectrum data and the AE model was selected as the best fitting model (Table 3). For schizophrenia the dataset included 418 cases, 2,534 deaths and 60,096 censored individuals, of which the IPW weights are based on 34,423 whose follow up time is below 40 years of age. For schizophrenia spectrum the dataset included 721 cases, 2,524 deaths and 59,803 censored individuals, of which the IPW weights are based on 34,298 whose follow up time is below 40 years of age.

The AE models estimated that the additive genetic effects (A) accounted for 78.9% and 73.3% of the variance in liability to schizophrenia and schizophrenia spectrum, respectively, while unique environmental effects (E) accounted for 21.1% and 26.7% of the variance of the two diagnostic categories (Table 3).

For comparison with previous studies, the data for both schizophrenia and schizophrenia spectrum disorder were analyzed without consideration for censoring. When analyzing these data, a reduced twin sample consisting of $N=19,539$ twin pairs born 1951-1981, compared to twin pairs born 1951-2000 in the adjusted analysis, was included to ensure a minimum follow-up of 30 years. Results are placed in the supplementary material (Tables S1 & S2). The analyses resulted in lower proband-wise concordance rates for MZ twins (0,33) and heritability estimates, schizophrenia, 75.5% and schizophrenia spectrum, 69.5%.

Discussion

The present study is based on two nationwide registers in Denmark, which provides the largest twin sample in schizophrenia research to date. The key strengths in this study design are that censoring is adjusted for in the models for heritability estimates and that this improved method is applied to a nationwide twin sample. Our results show a substantial genetic component in liability to schizophrenia, with a heritability estimate of 79%, and that the genetic effects seem to play a similar role in schizophrenia spectrum disorders. The concordance estimates are moderately lower than

generally indicated for MZ twins, 0.33, and even lower with the standard method of concordance estimation, 0.29 (supplement).

We estimated the variance in liability according to additive genetic effects (the heritability) for schizophrenia (79%) and for schizophrenia spectrum (73%). In general, our results are congruent with estimates found in previous twin studies of 83% and 82%¹⁰(29) and in a meta-analysis of 12 twin studies of 81%(9). For both schizophrenia and schizophrenia spectrum the estimate is based on having an illness onset before the age of 40, and large studies confirm that it is reasonable to assume that most cases of schizophrenia will present before this age(30–32). Schizophrenia has been shown to have a higher heritability than most other psychiatric illnesses(2), and the strongest association with schizophrenia risk in the general population is familial aggregation of schizophrenia, although a diagnosis of schizophrenia is associated with a wide range of other psychiatric disorders(33). When expanding the outcome to schizophrenia spectrum disorders, we expected to find a lower rate of genetic influence on the phenotype. We were not able to confirm this in our study since the heritability was similar for schizophrenia and schizophrenia spectrum. This could reflect a robustness of the basic concept of the disorder despite observed heterogeneity in the clinical presentation across the spectrum of schizophrenia. It may also suggest that the genetic risk for disease is not restricted to a narrow illness definition, but includes a broader phenotype, which supports the idea of a continuous illness spectrum.

In our model we estimated C (common environment) to be zero. In general, C may be confounded in the liability threshold models due to assumptions underlying the liability threshold models (methodology section). Another register based study has also estimated C as being 0, while the meta-analysis estimated C as 11% (95% confidence interval, 3%-19%) across studies(9,10). Common environmental factors, such as urbanicity and social disadvantage, have been shown to have important impact on illness risk(34). Thus, these factors are shared by twins but do not necessarily affect both twins in a pair to the same extent. When C is estimated to zero it can be an indication that C is intertwined with genetic factors and that C may be “hidden” in the estimate of A (additive genetic effects), which may contribute to an overestimation of the genetic liability to illness(6,34). Another consideration is that Denmark may be a homogenous society with relatively small variations in C compared to other countries.

We report a low concordance rate in MZ and DZ twins, of 0.33 and 0.07, respectively. This may seem to contradict a substantial heritability estimate. However, an explanation can be found in quantitative genetics and the general assumption that liability to disease is continuous and normally distributed

with disease becoming evident after passing a specific threshold(4). Looking at disease liability from this continuous perspective in a disorder like schizophrenia with a low disease prevalence of 1%, it is possible even for MZ twin pairs to appear different clinically, and at the same time be similar in their underlying genetic disease liability(35). The estimation of concordance rates depends on disease prevalence. A low concordance rate does not directly imply a low contribution of genetic factors in disease liability(36). Twin studies have varied regarding methodological factors, e.g. illness severity, ascertainment procedures and whether or not follow-up time is included in the studies. This limits the ability to compare concordance rates across studies. Two publications provide overviews of previous twin studies (8,37), and these include, among others, two early register-based studies reporting a low MZ concordance rate: 0.31 in Kendler and Robinette,1983(38) and 0.36 in Fischer et al., 1973(39). Also a low DZ concordance rate has been reported previously, e.g. 0.07 in Kendler et al., 1983(38), 0.09 (Cannon et al., 1998)(10) and 0.05 (Cardno et al., 1999)(29). One study has addressed to which extent methodological factors may influence the estimate of concordance rates in twin studies, and concluded that, among other factors, register based sample selection procedures significantly lowered concordance rates in twins(40). We argue that our results are in line with findings in some earlier twin studies, which were mainly register-based. We further argue that it may constitute a more accurate estimate, since it is based on register data from a national twin sample, includes a follow-up period of 40 years and an improved methodology. Our rates might be affected by the relatively low prevalence found, around 1% for schizophrenia and 2% for schizophrenia spectrum, reported in our supplement (Table S1). This is lower compared to the prevalence in another register based twin study from Finland, reporting a prevalence of 2% for schizophrenia and 3,4% for schizophrenia spectrum(10). In summary, our findings do indicate that genetic factors have a significant influence on the underlying illness susceptibility, but that an environmental trigger/interaction may be needed to affect illness penetrance, as previously described in a landmark twin study from 1989(41).

Many factors, e.g DNA sequence, epigenetic DNA modifications, differences in gene expression, environmental factors and the complex interaction between these are thought to act in concert to influence the outcome of a complex psychiatric phenotype like schizophrenia(17). In general, heritability estimated from twin studies is precise since the pattern of correlation between family members is higher in twins than in other relatives(36), but on the other hand, estimating heritability in closely related individuals can make it difficult to tease out possible confounding factors, such as non-additive genetic combinations and environmental factors shared in the family(6,36). This may lead to an overestimation of the heritability(42). Furthermore, MZ and DZ twin pairs are assumed to

share their environment to the same extent, but if MZ twins are exposed to more equal environments than DZ twins the heritability can be overestimated(5).

To estimate true concordance rates and heritability, a life long follow period for all individuals would be ideal. By applying IPW methodology we were able to overcome censoring mechanisms, and therefore we expect to observe changes in heritability estimates compared to previous studies and compared to our own data with standard methodology (supplement). To minimize bias when IPW is not applied (i.e. censoring is not taken into account) we have only included twin pairs born 1951-1981 in the supplement dataset in order to secure a minimum follow-up time of 30 years at the end of follow-up in June 2011. The main and supplementary data are not directly comparable because they are based on two differently defined samples and analysis approaches. Despite this limitation, the results are almost identical between the main data and the supplementary data, which indicates that *if* a sufficiently long follow-up period is included in the analyses, the bias may not have influenced the heritability estimates to a marked degree. By applying IPW we were able to include a much larger twin sample (N = 31,524 pairs compared to N=19,539 pairs), thereby contributing to further accuracy of the heritability estimate in the main data of our study. Furthermore, the apparent solidity of heritability estimates across previous studies and in the data presented here could reflect a relative stability in the diagnostic outcome in twin pairs over time making bias less prominent(14). Although data was drawn from national registers, the number of concordant pairs is low, the last pair becoming concordant at age 40. In this study, we estimate the heritability for an onset before age 40. We do capture the majority of the cases, but including a wider age span would also increase the generalizability of our results. Additionally, a large proportion of the sample is censored in 2012; with a longer (e.g. lifelong) follow-up time we could obtain more accurate estimates. The latter applies to all studies of heritability. We stipulate that addressing the censoring mechanism in the estimates as an improvement of methodology.

We estimated the mean age at schizophrenia onset to 28.9. In our study onset is defined as age at first contact to a psychiatric facility which is considered a valid marker of illness onset(31). A Finish twin study reports similar mean age(10), and a recent study examining sex- and age-specific incidence rates and lifetime risks of psychiatric illness in the Danish population found a peak in age at onset of schizophrenia in the early twenties and new cases diagnosed late in life(30).

Strengths and limitations: This study examined a representative twin sample from comprehensive, nationwide registers, which is less liable to ascertainment bias. The Danish Psychiatric Research Register is highly representative of patients with schizophrenia in Denmark, since the number of

privately treated patients is minimal(21). One concern might be that our approach is highly dependent on the consistency and validity of the clinical diagnoses drawn from health registers. However, a recent study demonstrated the high validity of the register diagnosis in schizophrenia(43). Despite this, undiagnosed cases could exist. Due to reasons mentioned in the method section our results cannot be generalized to disease onset after age 40. All confidence intervals were rather wide because the analysis only included complete observations (cases with disease onset before 40 years of age and observations followed until death).

In general it is assumed that twins are representative of the general population and as such results are considered generalizable(5,44). The generalizability of our findings may be affected by the exclusion of UZ twins and by the use of a Danish twin sample.

In conclusion, we present schizophrenia heritability estimates, which mirror previous reports and indicate solid findings across differences in diagnostic practice, ascertainment biases and censored data. Our study suggests a robustness of the basic concept of schizophrenia, including both a narrow and broad illness definition.

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Figure legends

Figure 1: A flowchart describing the two samples included in this study, the main sample and the supplementary sample, identified by combining the Danish Twin Register and the Danish Psychiatric Central Research Register.

Figure 2: Probandwise concordance rates of schizophrenia (top) and schizophrenia spectrum (bottom) as a function of years, i.e. time indicate the time from birth until end of study (age). Independence reflects the marginal (i.e. prevalence) of disorder.

References

1. van Os J, Kenis G, Rutten BPF. (2010): The environment and schizophrenia. *Nature*. Nov 11;468(7321):203–12.
2. Sullivan PF, Daly MJ, O'Donovan M. (2012): Genetic architectures of psychiatric disorders: the emerging picture and its implications. *Nat Rev Genet*. Nature Publishing Group 13(8):537–51.
3. Boomsma D, Busjahn A, Peltonen L. (2002): Classical twin studies and beyond. *Nat Rev Genet*. 3(11):872–82.
4. Falconer DS, Mackay TFC. (1996): *Introduction to Quantitative Genetics*. 4th ed. Burnt Mill, England: Longman.
5. Rijdsdijk F V, Sham PC. (2002): Analytic approaches to twin data using structural equation models. *Brief Bioinform* 3(2):119–33.
6. Visscher PM, Hill WG, Wray NR. (2008): Heritability in the genomics era--concepts and misconceptions. *Nat Rev Genet* 9(4):255–66.
7. Holst KK, Scheike TH, Hjelmberg JB. (2016): The liability threshold model for censored twin data. *Comput Stat Data Anal* 93:324–35.
8. Cardno AG, Gottesman II. (2000): Twin studies of schizophrenia: From bow-and-arrow concordances to star wars Mx and functional genomics. *Am. J. of Med. Gen.* Spring;97(1):12-7.
9. Sullivan PF, Kendler KS, Neale MC. (2003): Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry* 60:1187–92.
10. Cannon TD, Kaprio J, Lönqvist J, Huttunen M, Koskenvuo M. (1998): The Genetic Epidemiology of Schizophrenia in a Finnish Twin Cohort. *Arch Gen Psychiatry* 1;55(1):67.
11. Prescott C a, Kuhn JW, Pedersen NL. (2007): Twin pair resemblance for psychiatric hospitalization in the Swedish Twin Registry: a 32-year follow-up study of 29,602 twin pairs. *Behav Genet* 37(4):547–58.
12. Nordentoft M, Wahlbeck K, Hällgren J, Westman J, Ösby U, Alinaghizadeh H, et al. (2013): Excess Mortality, Causes of Death and Life Expectancy in 270,770 Patients with Recent Onset of Mental Disorders in Denmark, Finland and Sweden. *PLoS One* 8(1):e55176
13. Wahlbeck K, Westman J, Nordentoft M, Gissler M, Laursen TM. (2011): Outcomes of Nordic mental health systems: life expectancy of patients with mental disorders. *Br J Psychiatry* 199:453–8.
14. Scheike TH, Holst KK, Hjelmberg JB. (2014): Estimating heritability for cause specific mortality based on twin studies. *Lifetime Data Anal* 20(2):210–33.

15. Ripke S, Neale BM, Corvin A, Walters JTR, Farh K-H, Holmans P a., et al. (2014): Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 24;511(7510):421-7
16. Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kähler AK, Akterin S, et al. (2013): Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nat Genet* 45(10):1150-9.
17. Haque FN, Gottesman II, Wong AHC. (2009): Not really identical: epigenetic differences in monozygotic twins and implications for twin studies in psychiatry. *Am J Med Genet C Semin Med Genet* 15;151C(2):136-41.
18. Eichler EE, Flint J, Gibson G, Kong A, Leal SM, Moore JH, et al. (2010): Missing heritability and strategies for finding the underlying causes of complex disease. *Nat Rev Genet* 11(6):446-50.
19. Pedersen CB, Gøtzsche H, Møller JO, Mortensen PB. (2006): The Danish Civil Registration System. A cohort of eight million persons. *Dan Med Bull* 53(4):441-9.
20. Skytthe A, Kyvik KO, Holm NV, Christensen K. (2011): The Danish Twin Registry. *Scand J Public Health* 39(7 Suppl):75-8.
21. Mors O, Perto GP, Mortensen PB. (2011): The Danish Psychiatric Central Research Register. *Scand J Public Health* 39(7 Suppl):54-7.
22. World Health Organization. (1967): Manual of the International Classification of Diseases (ICD-8). Int Classif..
23. World Health Organization. (1992): The ICD-10 Classification of Mental and Behavioural Disorders. Int Classif.
24. McGue M. (1992): When assessing twin concordance, use the probandwise not the pairwise rate. *Schizophr Bull* 18:171-6.
25. Neale MC., Cardon LR. (1992): *Methodology for genetic studies of twins and families*. Dordrecht : Kluwer; 1992.
26. Holst KK, Scheike TH, Hjelmberg JB. (2016): The Liability Threshold Model for Censored Twin Data. *Comput Stat Data Anal* 93:324-335.
27. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP.; 2011.
28. R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.; 2008.
29. Cardno AG, Marshall EJ, Coid B, Macdonald AM, Ribchester TR, Davies NJ, et al. (1996): Heritability Estimates for Psychotic Disorders. *Arch Gen Psychiatry* 1;56(2):162.
30. Pedersen CB, Mors O, Bertelsen A, Waltoft BL, Agerbo E, McGrath JJ, et al. (2014): A comprehensive nationwide study of the incidence rate and lifetime risk for treated mental disorders. *JAMA psychiatry* 1;71(5):573-81.

31. van der Werf M, Hanssen M, Köhler S, Verkaaik M, Verhey FR, van Winkel R, et al. (2014): Systematic review and collaborative recalculation of 133,693 incident cases of schizophrenia. *Psychol Med* 44(1):9–16.
32. Thorup A, Waltoft BL, Pedersen CB, Mortensen PB, Nordentoft M. (2007): Young males have a higher risk of developing schizophrenia: a Danish register study. *Psychol Med*. 37(4):479–84.
33. Mortensen PB, Pedersen MG, Pedersen CB. (2010): Psychiatric family history and schizophrenia risk in Denmark: which mental disorders are relevant? *Psychol Med*. 40(2):201–10.
34. Uher R. (2014): Gene-environment interactions in severe mental illness. *Front psychiatry* 15;5:48
35. van Dongen J, Slagboom PE, Draisma HHM, Martin NG, Boomsma DI. (2012): The continuing value of twin studies in the omics era. *Nat Rev Genet* 13(9):640–53.
36. Tenesa A, Haley CS. The heritability of human disease: estimation, uses and abuses. (2013): *Nat Rev Genet*. Nature Publishing Group14(2):139–49.
37. Kendler KS. (1983): Overview: A current perspective on twin studies of schizophrenia. *Am J Psychiatry* 140(11):1413-25.
38. Kendler KS, Robinette CD. (1983): Schizophrenia in the National Academy of Sciences-National Research Council Twin Registry: A 16-year update. *Am J Psychiatry* 140:1551–63.
39. Fischer M. (1973): Genetic and environmental factors in schizophrenia. A study of schizophrenic twins and their families. *Acta Psychiatr Scand Suppl*. 238:9–142.
40. Walker E, Downey G, Caspi A. (1991): Twin studies of psychopathology: why do the concordance rates vary? *Schizophr Res*. 5:211–21.
41. Gottesman II, Bertelsen A. (1989): Confirming unexpressed genotypes for schizophrenia. Risks in the offspring of Fischer’s Danish identical and fraternal discordant twins. *Arch Gen Psychiatry* 46(10):867-72
42. Wray NR, Lee SH, Mehta D, Vinkhuyzen A a. E, Dudbridge F, Middeldorp CM. (2014): Research Review: Polygenic methods and their application to psychiatric traits. *J Child Psychol Psychiatry* 55(10):1068-87.
43. Uggerby P, Østergaard SD, Røge R, Correll CU, Nielsen J. (2013): The validity of the schizophrenia diagnosis in the Danish Psychiatric Central Research Register is good. *Dan Med J*. 60(2):A4578.
44. Christensen K, McGue M. (2012): Commentary: Twins , worms and life course epidemiology. *Int J Epidemiol*. 41(4):1010-1

Tables and figures:

Table 1: Number of cases, observations followed until death and censored observations in MZ and DZ twins

	Schizophrenia			Schizophrenia spectrum		
	MZ	DZ		MZ	DZ	
		Same sex	Opposite sex		Same sex	Opposite sex
Diagnosis	93 (0.64%)	173 (0.76%)	206 (0.80%)	181 (1.25%)	317 (1.39%)	344 (1.34%)
Dead without diagnosis	627 (4.32%)	991 (4.33%)	1,839 (7.17%)	617 (4.25%)	974 (4.26%)	1,821 (7.09%)
Censored e.g. alive without diagnosis	13,794 (95.04%)	21704 (94.91%)	23621 (92.03%)	13716 (94.50%)	21577 (94.35%)	23501 (91.56%)

Table 2: Probandwise concordance rates (CR) and tetrachoric correlations (TCR) for schizophrenia (SCZ) and schizophrenia spectrum (SCZ+) in MZ and DZ twin

	Zyg	Concor.	Discor.	Healthy	Probandwise		TCR	
		pairs	pairs	pairs	CR	95% CI	95% CI	95% CI
SCZ	MZ	12	69	7,176	0.33	0.20-0.49	0.78	0.62-0.87
	DZ	12	355	23,900	0.07	0.04-0.13	0.35	0.21-0.48
SCZ+	MZ	23	135	7,099	0.32	0.22-0.44	0.73	0.61-0.82
	DZ	31	599	23,637	0.09	0.06-0.13	0.34	0.23-0.44

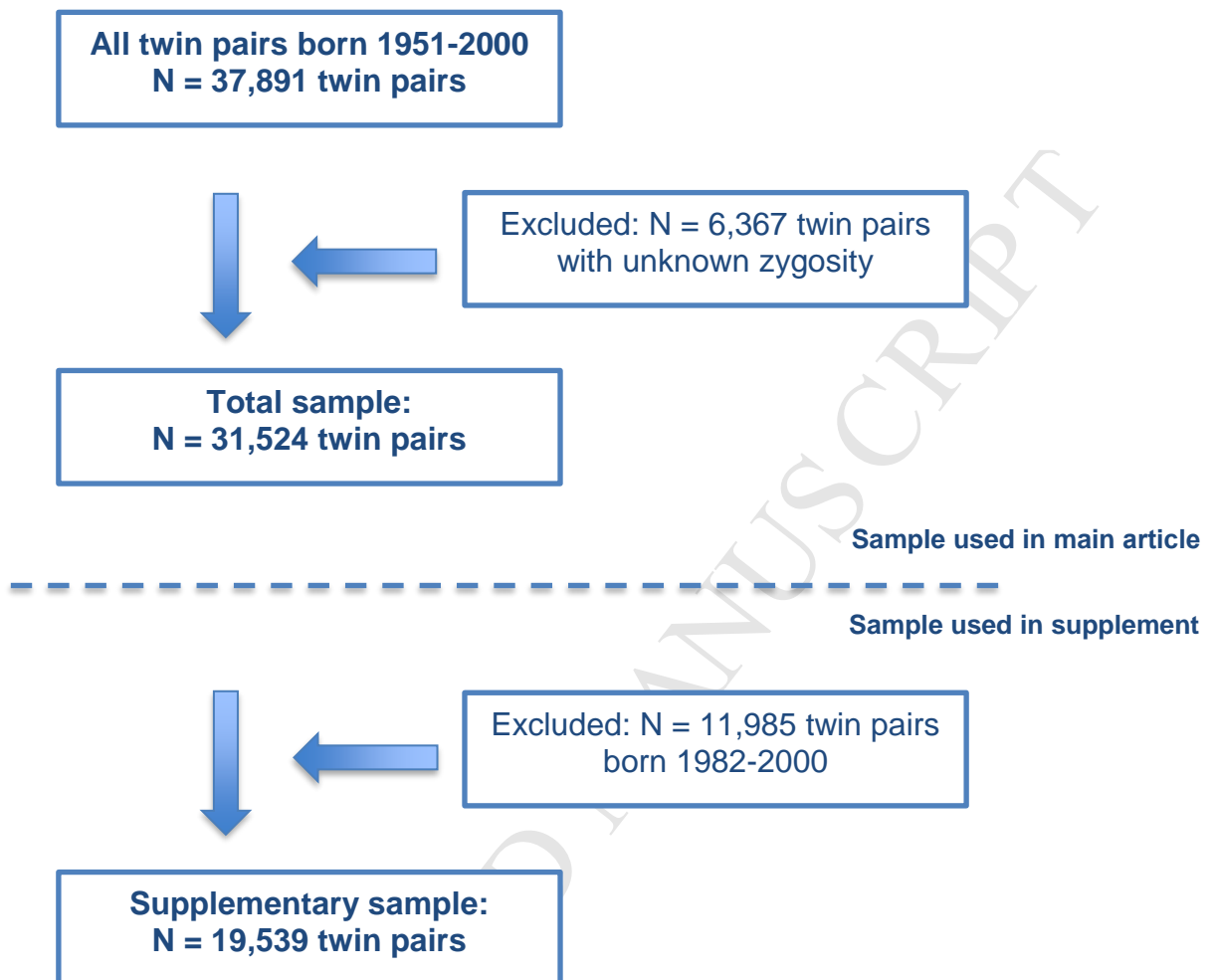
Probandwise concordance rates and tetrachoric correlations are calculated for schizophrenia and schizophrenia spectrum using inverse probability weighting (IPW) up till 40 years of age. CI denotes the 95% confidence intervals.

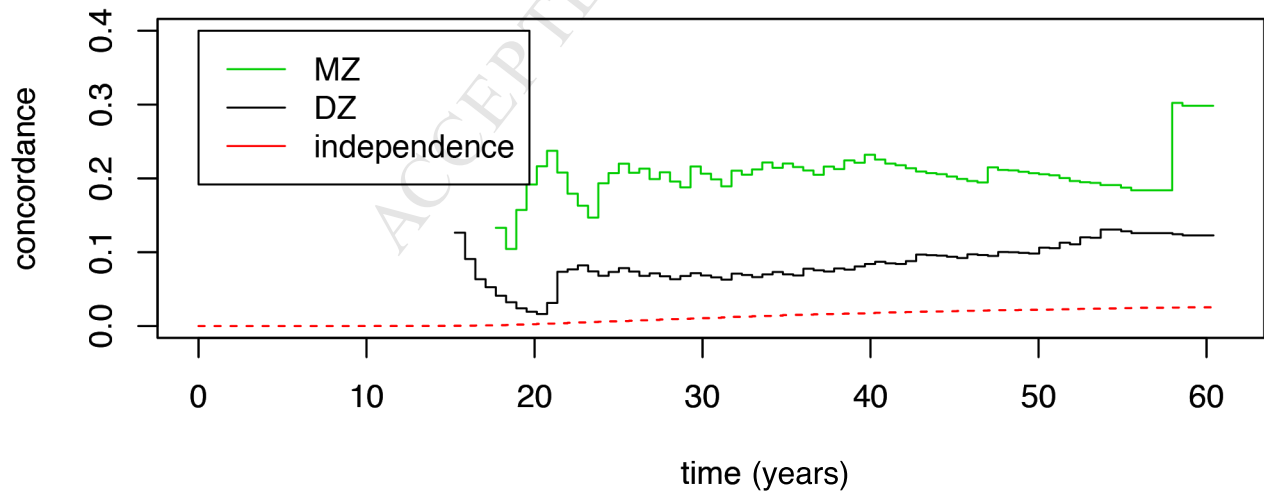
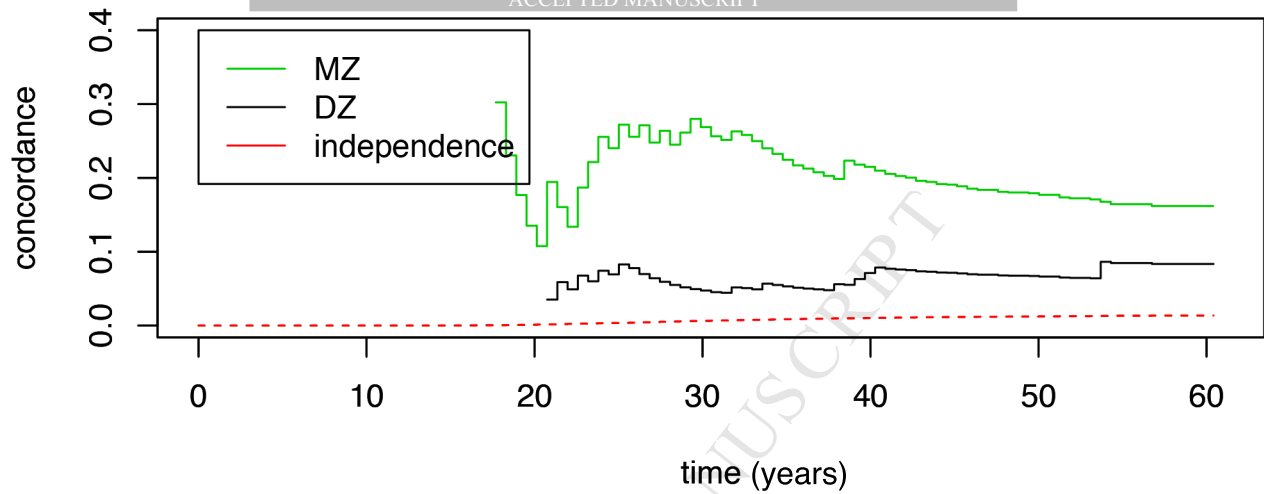
Table 3: Heritability estimates for schizophrenia and schizophrenia spectrum in a national cohort of twin pairs (N=31, 524 twin pairs).

Definition/ Model	a2		c2		e2		AIC	vs	df	X2	P-value
	Heritability	95% CI	95% CI	95% CI	95% CI						
Schizophrenia											
ACE	78.9%	65.1-99.3%	0.0%	0.0-0.0%	21.1%	11.8-34.9	14031.65				
AE*	78.9%	65.1-88.2%			21.1%	11.8-34.9	14029.65	ACE	1	0.00	1.00
CE			47.0%	37.3-57.0%	53.0%	43.0-62.7	14062.88	ACE	1	33.23	<0.001
Schizophrenia spectrum											
ACE	73.3%	62.5-81.9%	0.0%	0.0-0.0%	26.7%	18.1-37.5%	21545.74				

AE*	73.3%	62.5-81.9%	26.7%	18.1-37.5%	21543.19	ACE	1	0.00	1.00		
CE			44.9%	37.2-52.9%	55.1%	47.1-62.8%	21585.72	ACE	1	42.53	<0.001

Liability threshold models, adjusted for inverse probability weighting (IPW). These models estimate the following contributors to the variance in liability. A (additive genetic effects). C (common environmental effects) and E (unique environmental effects). A star (*) indicates the best fitting model. CI denotes confidence intervals of 95%; a2, c2 and e2 is the variance of liability due to either A, C or E; AIC denotes Akaike's information criterion while X2 is the chi square statistic. We used a cut off at age 40.





Heritability of Schizophrenia and Schizophrenia Spectrum Based on the Nationwide Danish Twin Register

Supplementary Information

Table S1. Probandwise concordance rates (CR) and tetrachoric correlations (TC) for schizophrenia and schizophrenia spectrum in MZ and DZ twin pairs in a sample of 19,539 twin pairs born 1951-1981, compared to twin pairs born 1951-2000 in the analysis presented in the paper (results section, Table 2), identified by combining the Danish Twin Register and the Danish Psychiatric Central Research Register. This insures a minimal follow-up time of 30 years.

	Zyg	Prevalence	Concor	Discor	Healthy	Probandwise		TC	
			pairs	pairs	pairs	CR	95% CI		95% CI
SCZ	MZ	0.008	12	60	5,257	0.29	0.18-0.43	0.73	0.59-0.83
	DZ	0.012	12	304	13,894	0.07	0.04-0.12	0.34	0.22-0.46
SCZ+	MZ	0.016	22	120	5,187	0.27	0.19-0.37	0.67	0.56-0.76
	DZ	0.020	28	503	13,679	0.10	0.07-0.14	0.35	0.26-0.44

Probandwise CR and TC are calculated with standard methodology (without inverse probability weighting (IPW)).

Table S2. Heritability estimates for schizophrenia and schizophrenia spectrum in a national cohort of 19,539 twin pairs born 1951-1981, compared to twin pairs born 1951-2000 in the analysis presented in the paper (results section, Table 3), identified by combining the Danish Twin Register and the Danish Psychiatric Central Research Register. This insures a minimal follow-up time of 30 years.

Definition/	a2		c2		e2						
Model	Heritability	95% CI		95% CI		95% CI	AIC	vs	df	X2	P-value
Schizophrenia											
ACE	75.5%	63.4-84.62%	0.0%	0.0-0.0%	24.5%	15.4-36.6%	4,479.60				
AE*	75.5%	63.4-84.6%			24.5%	15.4-36.6%	4,477.60	ACE	1	0.00	1.00
CE			46.7%	37.8-55.9%	53.3%	44.1-62.2%	4,498.19	ACE	1	20.60	<0.001
Schiz. spectrum											
ACE	69.5%	60.3-77.3%	0.0%	0.0-100.0%	30.6%	22.7-39.7%	7,060.36				
AE*	69.5%	60.3-77.3%			30.6%	22.7-39.7%	7,058.36	ACE	1	0.00	1.00
CE			44.9%	38.1-52.0%	55.1%	48.0-61.9%	7,080.27	ACE	1	21.91	<0.001

Classical liability threshold models calculated with standard methodology (without inverse probability weighting (IPW)). These models estimate the following contributors to the variance in liability A (additive genetic effects), C (common environmental effects) and E (unique environmental effects). A star (*) indicates the best fitting model.