Schizophrenia Spectrum Disorders in a Danish 22q11.2 Deletion Syndrome Cohort Compared to the Total Danish Population—A Nationwide Register Study

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Objective: Cross-sectional studies have shown associations between 22q11.2 deletion syndrome and schizophrenia. However, large-scale prospective studies have been lacking. We, therefore, conducted the first large-scale population based study on the risk of being diagnosed with schizophrenia in persons identified with 22q11.2 deletion syndrome. Methods: Danish nationwide registers were linked to establish a cohort consisting of all Danish citizens born during 1955–2004 and the cohort was followed from January 1, 1994 until December 31, 2013. Data were analyzed using survival analyses and adjusted for calendar year, age, sex, and parental mental health history. Results: A total of 156 individuals with 22q11.2 deletion syndrome were identified, out of which 6 individuals were diagnosed with schizophrenia spectrum disorders following identification with 22q11 deletion syndrome. Identified carriers of 22q11.2 deletion had an 8.13 (95\% CI: 3.65–18.09) fold increased risk of schizophrenia spectrum disorder. Conclusions: Carriers of a 22q11.2 deletion who had been clinically identified had a highly increased risk of schizophrenia spectrum disorders.

Key words: 22q11 deletion syndrome/schizophrenia/schizophrenia spectrum disorders/incidence rate/health registry

Introduction

The 22q11.2 deletion syndrome (22q11DS) is caused by hemizygous deletions on the long arm of chromosome 22 and is one of the most common microdeletion syndromes in man affecting approximately 1:2000 to 1:4000 live births.\textsuperscript{1,2} The majority (approximately 90\%) of the microdeletions that are detected at chromosome 22q11.2 are 3 megabases in size but a 1.5 megabases deletion is occasionally observed\textsuperscript{3–5} and rare atypical microdeletions of different sizes nested within the common 3 megabase region have also been described.\textsuperscript{6–8} Most of the deletions arise de novo due to nonallelic homologous recombination\textsuperscript{9–11} which have been reported to occur more often on the maternal chromosome.\textsuperscript{12} The phenotypic spectrum is highly variable ranging from severe life threatening conditions to only minor features.\textsuperscript{13,14} The typical clinical presentation includes dysmorphic facial features, congenital heart defects, recurrent infections, velopharyngeal insufficiency, speech impairment, learning difficulties, and behavioral deficits.\textsuperscript{15–18}

Observational and cross-sectional studies have consistently reported a high prevalence (0.22–0.42) of schizophrenia among individuals carrying the 22q11.2 deletion.\textsuperscript{19–22} Case–control studies of schizophrenia have shown that 22q11.2 deletion carriers account for approximately 0.3\% of schizophrenia patients\textsuperscript{23} and the deletion is associated with high penetrance.\textsuperscript{24,25} Recent genetic studies on schizophrenia found no 22q11.2 deletion carriers among the control subjects\textsuperscript{23,26} whereas other studies have reported odds ratio estimates of schizophrenia that ranges from 16 to 44 among 22q11.2 deletion carriers.\textsuperscript{23,27,29} Higher odds ratios of schizophrenia have been found only in individuals having 2 parents with schizophrenia or in individuals with a monozygotic twin with schizophrenia.\textsuperscript{30}

Large-scale epidemiologic studies have identified a wide range of risk factors for schizophrenia including a positive family history of mental illness,\textsuperscript{30,31} urban birth,\textsuperscript{32,33}
advancing paternal age\textsuperscript{34-37} and immigration status.\textsuperscript{38,39} No studies have evaluated the potential impact of these confounders on the effect of 22q11.2 deletions on the risk of schizophrenia.

While early intervention is often successfully provided to treat the somatic complications the risk for the later onset psychiatric illnesses remain a major concern to carriers of a 22q11.2 deletion and their families. As prior risk estimates of the 22q11.2 deletion are derived from cross-sectional case-control studies there is a lack of knowledge about the population-based risk of schizophrenia in persons with a 22q11.2 deletion who have been diagnosed with 22q11DS prior to onset of schizophrenia. Together with the limited information on the effect of additional risk factors that has previously been implicated in schizophrenia, these caveats impede planning of 22q11DS clinical evaluation programs and accurate genetic counseling of parents.

Utilizing information recorded in the comprehensive and nationwide Danish Psychiatric Central Register and the Danish Cytogenetic Central Register, we undertook a population-based study to examine the sex specific incidence rate ratios (IRR) of schizophrenia in known carriers of a 22q11.2 deletion compared to noncarriers in the entire Danish population. We also assessed the potential influences of a range of confounders (parental history of mental illness, urbanity, parental age, and immigration status).

Methods

Data Sources

All live-born children and new residents in Denmark are assigned a unique personal identifier, which can be used to link information within and across the nationwide computerized Danish registers. The personal identifier is registered in The Danish Civil Registration System\textsuperscript{40,41} which holds continuously updated information on all Danish citizens who have resided at least one day in Denmark since April 2, 1968, including date of birth, sex, identity of parents and siblings, place of birth and residence, and vital status (death and emigration). The Danish Psychiatric Central Register\textsuperscript{42} contains information about all admissions to Danish psychiatric inpatient facilities since April 1, 1969 and outpatients since 1995. Psychiatric diagnoses are based on the International Classification of Diseases—8th Revision\textsuperscript{43} between 1969 and 1993 and the International Classification of Diseases—10th Revision\textsuperscript{44} from 1994 onward. There are no private psychiatric inpatient facilities in Denmark and all treatment is free of charge. This ensures that all psychiatric admissions, outpatient contacts and emergency clinic visits are included in the register. The Danish Cytogenetic Central Registry\textsuperscript{45} was established in 1968 and contains data on clinical genetic test performed in Denmark since 1960.\textsuperscript{46} The reporting of data to the Danish Cytogenetic Central Registry is self-imposed by all departments performing genetic tests, and the registry is administered by representatives from these departments. Clinical genetic testing for 22q11.2 deletions was implemented in Denmark in 1994 and is performed on clinical indication. Although the Danish Cytogenetic Central Registry does not provide information about the reason for testing, it reflects usual clinical practice where parents are offered genetic testing upon identification of a 22q11.2 deletion in their child. In total the Danish Cytogenetic Central Registry holds information on 264 Danish subjects with 22q11DS.

Study Population

Persons were designated as being at risk of schizophrenia spectrum disorder from the 10th birthday onwards.\textsuperscript{45} From the Danish Civil Registration System we established a population-based cohort that included all individuals born in Denmark between January 1, 1955 to December 31, 2003, who were alive at their 10th birthday and who had reference to both parents in the register ($N = 3\ 107\ 281$). This selection reduced the number of eligible subjects with 22q11DS to 156.

Psychiatric Diagnoses

The study population was linked to the Danish Psychiatric Central Register using the unique personal identifier to obtain the date of the first psychiatric contact ($D_{PC}$) and the study population was linked to the Danish Psychiatric Central Register using the unique personal identifier to obtain the date of the first diagnosis of schizophrenia (ICD-10 code F20 or ICD-8 code 295.x9 (excl 295.79), ie, 295.09, 295.19, 295.29, 295.39, 295.49, 295.59, 295.69, 295.89, and 295.99) and schizophrenia spectrum disorder (ICD-10 codes F20-F29 and ICD-8 codes 295.x9, 296.89, 297.x9, 298.29–298.99, 299.04, 299.05, 299.09, and 301.83) affected individuals. The time of disease onset was defined as the date of the first psychiatric contact leading to the diagnosis of interest.

Genetic Diagnoses

The study population was linked to the Danish Central Cytogenetic Register using the unique personal identifier to identify individuals diagnosed as carriers of a 22q11.2 deletion and to obtain the date of the clinical genetic test result. The time of 22q11 diagnosis was defined as the date of a positive clinical genetic test result. The presence of a 22q11.2 deletion was based on a standard clinical cytogenetic examination, ie, fluorescence in situ hybridization, multiplex ligation-dependent probe amplification, or comparative genomic hybridization arrays. The 22q11.2 deletion is defined as the typically deleted region of 3 megabases, and minor deletions nested within the typical 3 megabase region. We did not discriminate between these 22q11.2 deletions as several genetic techniques with different resolution have been used at the clinical genetic departments over time.
Family History and Other Measures

Age, birth period, sex, parental age, and urbanity at birth were obtained from the Danish Civil Registration System. We defined the variables sex, age in 1 year bands (10, 11, 12, ..., 60), calendar time in 1 year bands (1994, 1995, 1996, ..., 2013), place of birth (capital (Copenhagen), suburban of capital, provincial city (more than 100,000 inhabitants), provincial town (10,000–100,000 inhabitants) and rural areas, maternal and paternal age at birth (<20, 20–24, 25–29, 30–34, and 35+), and maternal and paternal country of origin (Denmark, abroad + Greenland, unknown). Maternal and paternal history of mental illness was constructed as hierarchical variables (no mental illness, any mental illness (ICD10 codes F00-F99 and ICD8 code 290–315), and schizophrenia spectrum disorders (ICD10 codes F20-F29 and ICD8 codes 295.x9, 296.89, 297.x9, 298.29–298.99, 299.04, 299.05, 299.09, 301.83) on information was retrieved from the Danish Psychiatric Central Register.

Study Design and Statistical Analyses

Data were analyzed prospectively and the cohort was followed from their 10th birthday or January 1, 1994, whichever came later, until the date of their first schizophrenia spectrum diagnosis, the date of death, the date of emigration/disappearance or January 1, 2014, whichever came first. As a consequence also subjects who received their 22q11.2 deletion test result after the diagnosis of schizophrenia were subjected to right censoring to avoid immortal time bias.48 We conducted survival analyses using Poisson regressions, with the logarithm of person-years as an offset variable. This method is equivalent to the Cox regression under the assumption of piecewise constant incident rates49,50 and the method account for uneven distribution of follow-up time among included individuals (ie, some persons were followed for 20 years or more, while others were followed for only 1 day). Therefore, the adjustment for age imposed in survival analyses ensures that the time point from which follow-up was initiated have no impact on the results.

Analyses were conducted using the GENMOD procedure in SAS software version 9.2 (SAS Institute Inc). P-values and 95% confidence intervals (CIs) were based on likelihood ratio test statistic (P < 0.05 was considered statistically significant). Age, calendar year, 22q11.2 deletion and history of mental illness in a parent were treated independent of time. Incidence rates (IRs) from the survival analyses are valid to the entire age-span under the assumption that the age-at-onset distribution of schizophrenia among 22q11.2 deletion carriers is similar to that of idiopathic schizophrenia per-se. The study was approved by the Danish Data protection Agency (project id: 2012-41-0321).

Results

Among the 3,107,281 people born in Denmark from 1955 to 2003, 31,650 developed schizophrenia during the 48,237,813 person-years of follow-up from 1994 to 2014 and 156 persons received a clinical genetic test indicating presence of a 22q11.2 deletion. Among these 156 subjects with 22q11DS the positive clinical genetic test result for the 22q11.2 deletion was obtained before the mid-40s and 75% was identified before the age of 16 years. Figure 1 shows the distribution of age at censoring among these 156 persons with a positive test for a 22q11.2 deletion, which shows that the majority of the 22q11.2 deletion carriers were age 10–25 years old at the time of censoring. Among the 156 22q11.2DS subjects 6 persons were diagnosed with schizophrenia after they obtained the positive genetic test result for a 22q11.2 deletion and 3 persons were diagnosed with schizophrenia before they were referred for genetic testing. The latter 3 subjects were right censored to avoid immortal time bias that would distort the IRR estimates (see “Methods” section).

Table 1 shows the distribution of persons in whom schizophrenia spectrum disorders developed during follow-up, the person-years of risk in the study cohort and crude IR of schizophrenia-spectrum disorder for individuals with or without a 22q11.2 deletion, between men and women, different age groups, place at birth, parental age, and mental health history. Furthermore, table 1 shows the age at first diagnosis of schizophrenia among all schizophrenia cases in our cohort and the amount of person-years at risk in each age category.

Overall, individuals with 22q11.2DS had an IR of schizophrenia spectrum diagnosis of 57.8 per 10,000
person-years compared to 6.56 among persons without the 22q11.2 deletion. Besides 22q11.2DS status, also age (15–25 years), having parents with a psychiatric diagnosis or foreign-born parent’s impact on the estimated IRs (Table 1). The sex specific IRR of schizophrenia spectrum diagnosis for 22q11.2 deletion carriers are significantly increased for both males (IRR: 7.64; 95% CI: 1.90–19.81) and females (IRR: 8.68, 95% CI: 2.16–22.51) compared to nondeletion carriers but the effect of the 22q11.2 deletion was not significantly modified by sex (P = 0.88).

The IRRs associated with the 22q11.2 deletion in different adjustment models are shown in Table 2. In the basic adjusted model, 22q11.2 deletion carriers per se had an overall 8.13-fold increased risk of schizophrenia spectrum disorder compared to persons without 22q11.2DS. Considering only persons who were the first in their family to get a positive 22q11.2 deletion test result the IRR estimate was 7.66 (95% CI: 2.75–16.46). Further adjustment for potential risk factors (ie, maternal and paternal age, country of origin, and mental illness) resulted in nonsignificant reductions of the IRR.

To evaluate the specificity of this finding we performed additional analyses considering narrowly defined schizophrenia (ICD10 code F20) as the outcome of interest. A total of 4 identified 22q11.2 deletion carriers were diagnosed with narrowly defined schizophrenia. Persons with 22q11.2DS had an overall 11.11 (95% CI: 3.45–25.81) fold increased risk of schizophrenia compared to persons without a 22q11.2 deletion under the basic adjustment. Considering persons who were the first in their family to get a 22q11.2DS diagnose the estimate was 11.28 (95% CI: 3.50–26.22).

To further evaluate the consistency the effect of a 22q11.2 deletion was stratified by age and birth year. There was no significant interaction with age for persons younger or older than age 25 (P = 0.91), nor was there a significant interaction with birth year (P = 0.83). The IRR for schizophrenia among persons younger and older than 25 years of age was 6.71 (95% CI: 2.41–14.42) and 7.57 (95% CI: 0.43–33.33), respectively. The IRRs of schizophrenia associated with a 22q11.2 deletion was 7.48 (95% CI: 2.32–17.39) for those born 1955–1993 and 9.00 (95% CI: 1.50–27.83) for those born 1994–2003. These estimates are based on few persons in some of the categories resulting in unstable estimates and very wide confidence intervals.

### Discussion

This is the first nationwide, long-term, prospective cohort study of subjects who have been clinically identified with 22q11DS to estimate the risk of developing schizophrenia later on in life while correcting for a series of additional factors known to predispose to the schizophrenia and reducing the impact of immortal time, recall and ascertainment biases. The lower than expected IRR of schizophrenia compared to previous reports prompted us to consider a number of possible biases that might lower risk estimates in this 22q11DS study cohort.

First, we cannot verify complete registration of all clinically identified 22q11.2 deletion carriers in the Danish Cytogenetic Central Register. In fact, the number of subjects per birth year is lower before the introduction of the 22q11 testing in 1994. After 1994, the rate of 22q11.2DS rapidly approaches the expected number of cases born each year (ie, 1:4000 newborns). However, incomplete registration may reduce the power of the study but only affect IRR estimates in survival analysis if biased toward the outcome (ie, schizophrenia), which there is no reason to suspect. Consistently, we find that stratification on age over/under 25 years as well as individual born before/after 1994 does not affect the IRR estimates.

Second, the validity of IRRs across the entire age-span depends on the assumption that the distribution of age at onset of schizophrenia among 22q11.2 carriers is similar to that of idiopathic schizophrenia. The assumption is supported by the observation that mean age at first schizophrenia diagnosis is 28 years (SD = 9.6) among the schizophrenia cases in our study cohort (data not shown) is consistent with the report by the 22q11 consortium, that the majority of the known 22q11 deletion carriers who develops schizophrenia have been diagnosed when they reach early adulthood (age 26–35 years).

Third, the 22q11.2 deletion, while mostly occurring de novo, is also segregating in families in approximately 10% of the cases. We speculate that the deletion is most often segregating from a seemingly healthy parent, ie, typically identified secondarily to the examination of the child. Inclusion of otherwise healthy parents into the study would both collide with the focus of the study on 22q11.2 deletion carriers that were identified on clinical suspicion and bias lower IRR estimate. However,
including only the first identified 22q11.2 deletion carrier in each family, and thus excluding healthy parents identified secondarily to their children, did not increase the estimate of IRR of schizophrenia.

Fourth, the lower than expected risk of schizophrenia in this 22q11DS study cohort with early somatic and developmental signs may suggest that the risk of schizophrenia is higher among somatically unaffected 22q11DS individuals, either due to pathogenic differences between the 22q11DS subgroups, or because the rate of schizophrenia is reduced among 22q11.2 deletion carriers with early developmental signs because improved clinical care...
and environment. However, without a sufficiently genotyped large and random population sample to compare with this cytogenetic register sample, we are not able to evaluate this hypothesis, nor can we estimate the prevalence of the 22q11.2 deletions in schizophrenia or the general population as such.

The study finds that the well-known risk factors of schizophrenia, both individually and combined, reduced the estimated IRR. However, the study may not have sufficient power to draw firm conclusions regarding the effect of risk factors it is unlikely that the risk of schizophrenia spectrum disorder in identified 22q11.2 deletion carriers exceeds the upper limit in the confidence interval, ie, estimated for the overall IRR of 8.13 (95% CI:3.65–18.09). Our study cohort had a slightly higher IR of schizophrenia among men than women, consistent with the known male predominance of psychotic disorders in the general population. However, we did not observe an interaction between sex and 22q11.2 deletion status on IRRs. This may result from lack of statistical power to detect gender differences or indicate 22q11DS-specific pathogenesis of schizophrenia, ie, independent of gender. Overall our findings stress the importance evaluating additional risk factors and take confounders into consideration when examining the impact of genetic contributions to disease risk. Although our risk estimates are somewhat lower than previous reports, they still provide strong support the conclusion that the 22q11.2 deletion confers high risk of schizophrenia.

The findings of the study gain their strength from the use of nationwide health registers that collect clinical diagnoses based on contacts with inpatient and outpatient psychiatric departments and visits to psychiatric emergency departments units in a country where treatment is provided through the government health care system without charge to the individual and without any private psychiatric hospitals. Financial factors are thus less likely to influence pathways to care in this study compared to many other nations. The population studied is representative of the Danish population, because all residents are included, independent of demographic, social, and health related issues. The clinical diagnoses of schizophrenia registered in the Danish Psychiatric Central Research Register has shown high validity and therefore limits information bias relating to the psycho-pathological assessment and allowing generalization of findings to clinical practice.

The strength of the study also stems from the population-based survival analysis, ie, unaffected by the types of bias that typically affect case–control studies. Importantly, survival analysis is resistant to incomplete recruitment of cases as long as the recruitment itself is not biased toward the outcome. To ensure this, we carefully apply right censoring at the time of the outcome (schizophrenia), eliminating the risk of immortal time bias and contemporarily allowing us to inform clinicians on schizophrenia risk specifically in subjects identified with 22q11DS following clinical suspicion due to somatic or developmental complications. Furthermore, case–controls studies are generally affected by ascertainment bias with cases being ascertained in subspeciality hospital departments and control subjects not being representative of the background population. In addition, case–control studies do usually not take observation period into consideration, and therefore implicitly assume that control subjects remain unaffected throughout life. In the current study, we are also able to examine the effect of epidemiological factors affect the risk of the outcome as well as to correct for confounders such as age.

Importantly, survival analysis is resistant to incomplete recruitment of cases as long as the recruitment itself is not biased toward the outcome. To ensure this, we carefully apply right censoring at the time of the outcome (schizophrenia), eliminating the risk of immortal time bias and contemporarily allowing us to inform clinicians on schizophrenia risk specifically in subjects identified with 22q11DS following clinical suspicion due to somatic or developmental complications. Furthermore, case–controls studies are generally affected by ascertainment bias with cases being ascertained in subspeciality hospital departments and control subjects not being representative of the background population. In addition, case–control studies do usually not take observation period into consideration, and therefore implicitly assume that control subjects remain unaffected throughout life. In the current study, we are also able to examine the effect of epidemiological factors affect the risk of the outcome as well as to correct for confounders such as age.

In conclusion, individuals diagnosed with 22q11DS based on a clinical suspicion due to developmental signs are significantly more likely to develop a schizophrenia spectrum disorder compared to nondeletion carriers, although it was somewhat lower than expected from previous studies. The epidemiological, population-based design adopted in this study may offer a new standard in

### Table 2. Adjusted IRRs for Schizophrenia Spectrum Disorders Associated with 22q11.2 Deletion

<table>
<thead>
<tr>
<th>Adjustment</th>
<th>22q11.2 Deletion Person Who Were Registered with a 22q11.2 Deletion</th>
<th>IRR (95% CI)</th>
<th>22q11.2 Deletion Person Who Were the First in Their Family to Be Registered with a 22q11.2 Deletion</th>
<th>IRR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic</td>
<td>8.13 (3.65–18.09)</td>
<td></td>
<td>7.66 (2.75–16.46)</td>
<td></td>
</tr>
<tr>
<td>Basic + place of birth</td>
<td>7.74 (3.48–17.24)</td>
<td></td>
<td>7.27 (2.61–15.64)</td>
<td></td>
</tr>
<tr>
<td>Basic + maternal and paternal age</td>
<td>7.94 (3.57–17.68)</td>
<td></td>
<td>7.44 (2.67–16.00)</td>
<td></td>
</tr>
<tr>
<td>Basic + mother and fathers origin</td>
<td>8.08 (3.63–18.00)</td>
<td></td>
<td>7.64 (2.74–16.42)</td>
<td></td>
</tr>
<tr>
<td>Basic + mother and fathers psychiatric history</td>
<td>6.85 (3.08–15.26)</td>
<td></td>
<td>6.22 (2.23–13.38)</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>6.55 (2.94–14.59)</td>
<td></td>
<td>5.94 (2.13–12.77)</td>
<td></td>
</tr>
</tbody>
</table>

Note: IRR, incidence rate ratio. The basic adjustment is sex, age, and calendar time. The reference group is persons without known 22q11.2 deletion.
the analysis of genotype–phenotype interaction providing IRRs corrected for known epidemiological risk factors rather than classical odds ratios.

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