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Research report

An association study of suicide and candidate genes in the serotonergic system

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ABSTRACT

Introduction: Strong evidence demonstrates a genetic susceptibility to suicidal behaviour and a relationship between suicide and mental disorders. The aim of this study was to test for association between suicide and five selected genetic variants, which had shown association with suicide in other populations.

Method: We performed a nationwide case-control study on all suicide cases sent for autopsy in Denmark between the years 2000 and 2007. The study comprised 572 cases and 1049 controls and is one of the largest genetic studies in completed suicide to date. The analysed markers were located within the *Serotonin Transporter (SLC6A4), Monoamine Oxidase-A (MAOA)* and the *Tryptophan Hydroxylase I* and *II (TPH1 and TPH2)* genes.

Results: None of the genetic markers within *SLC6A4, MAOA, TPH1* and *TPH2* were significantly associated with completed suicide or suicide method in the basic association tests. Exploratory interaction test showed that the minor allele of rs1800532 in *TPH1* has a protective effect for males younger than 35 years and females older than 50 years, whereas for the oldest male subjects, it tended to be a risk factor. We also observed a significant interaction between age-group and the 5-HTTLPR genotype (with and without rs25531) in *SLC6A4*. The long allele or high expression allele tends to have a protective effect in the middle age-group.

Limitation: We only analysed a limited number of genetic variants.

Conclusion: None of the analysed variants are strong risk factors. To reveal a better understanding of the genes involved in suicide, we suggest future studies should include both genetic and non-genetic factors.

1. Introduction

Suicidal behaviour aggregates in families (Brent et al., 1996; Turecki, 2001), and studies of twins show that monozygotic individuals have a greater concordance for suicide completion and suicide attempts compared to dizygotic individuals (Roy et al., 1991, 1995; Voracek and Loibl, 2007). The heritability is approximately between 30 and 55% (Voracek and Loibl, 2007).

It is well established that psychopathology is an important predictor of suicide completion and that more males than females commit suicide. A meta-analysis comprising 3275 suicides showed, on average, 87% of the subjects who committed suicide had a mental disorder of which affective disorder and any substance disorder were amongst the most common diagnoses (Arsenault-Lapierre et al., 2004). A recent Danish population study, comparing 21,169 suicides over a 17-year period with matched controls, showed that suicide risk is significantly increased for individuals with a hospitalized psychiatric disorder (Qin, 2011).

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Several studies have reported abnormalities in the functioning of the serotonergic system in suicidal behaviour, and genes encoding proteins involved in the regulation of serotonergic neurotransmission have thus been investigated in numerous association studies (for review see Tsai et al. (2011)). One of the major candidate genes for suicide is the *serotonin transporter* (solute carrier family 6 member 4: *SLC6A4*) gene located on chromosome 17q11.2 and involved in the reuptake of serotonin in the synaptic cleft. A common polymorphism (5-HTTLPR, rs4795541), due to a 43 bp deletion located within the promoter region of this gene, has been extensively studied in relation to suicide. The long (*L*) allele of this marker has been associated with a two- to three-fold more efficient transcription of the gene, compared with the short (*S*) allele (Heils et al., 1996). A meta-analysis by Li and He (2007) comprising 39 studies suggests association between the *S*-allele of 5-HTTLPR and suicidal behaviour. We also included a single nucleotide polymorphism (SNP) (rs25531) recently shown to be located 18 bp 5′ to 5-HTTLPR (Perroud et al., 2010) within the *SLC6A4* gene. A functional untranslated variable number tandem repeat (uVNTR) located in the promoter region of the *monoamine oxidase-A* (*MAOA*) gene (referred to as *MAOA*uVNTR) has also been studied in relation to suicide (Courtet et al., 2005; Huang et al., 2004; Hung et al., 2011b; Lung et al., 2011; Ono et al., 2002). *MAOA* is located on chromosome Xp11.3 and is involved in degrading serotonin, noradrenalin, adrenalin and dopamine. The *MAOA*uVNTR has been shown to affect the transcription of the gene (Deckert et al., 1999; Sabol et al., 1998). Alleles of this marker with 3.5 and 4 repeats have a higher activity than the short allele with 3 repeats. The higher activity alleles have been shown to be associated with violent suicide attempts in males (Courtet et al., 2005). Furthermore, the two *tryptophan hydroxylase* genes (*TPH1* on 11p15.1 and *TPH2* on 12q21.1) have been associated with suicide as reviewed by Tsai et al. (2011). *TPH1* and *TPH2* are involved in the initial and rate-limiting step in the synthesis of the neurotransmitter serotonin. Especially, a SNP in intron 7 (rs1800532 also known as A218C) of *TPH1* has been extensively studied (Ohtani et al., 2004; Ono et al., 2000; Saetre et al., 2010; Turecki et al., 2001; Viana et al., 2006). Li and He (2006) have performed a meta-analysis of 34 studies and demonstrated an overall significant association between rs1800532 and suicidal behaviour. Additionally, a SNP located in intron 5 of *TPH2* (rs1386494) was studied by Zill et al. (2004) and found to be significantly associated with completed suicide.

Yearly, there are around 700 completed suicides in Denmark, of which 15% are sent for autopsy by the police and confirmed as suicide according to Danish legislation (Health Law no. 546, 2005). In the present study, we performed a nationwide case-control study on all suicide cases sent for autopsy in Denmark between the years 2000 and 2007 and analysed five genetic markers involved in the serotonergic system.

2. Materials and methods

2.1. Study population

In Denmark, all deaths due to suicide or suspected suicide are reported to the police and referred to a coroner's inquest. If a death is not sufficiently clarified, the police will order an autopsy, which will be performed by one of the three Danish forensic centres in Aarhus, Odense or Copenhagen.

Muscle tissue was collected at autopsy from Danish individuals who committed suicide between the years 2000 and 2007. Suicides were classified as violent (including deaths by hanging, drowning, firearms, air guns and explosives, cutting and piercing instruments, jumping from high places, and other and unspecified means, so long as poisoning could be excluded) or non-violent (comprising of all types of poisoning). This classification method has been widely adopted by other studies (Alvarez et al., 2000; Chung et al., 2008; Marcinko et al., 2005).

Control samples were obtained from Danish working and student populations. The controls from the working population were screened for depression and recent suicidal thoughts by questionnaire. The rest of the controls were unscreened medical students, of whom we were unable to access personal data except for gender and ethnicity. At inclusion, they confirmed that both parents and all four grandparents were born in Denmark. Concerning age-group, we assumed that they were less than 35 years old.

For both cases and questionnaire screened controls, we excluded anyone without a valid personal identification number (CPR number) and anyone not born in Denmark (unless both parents were Danish born), to ensure ethnicity to be primarily Danish and Caucasian. Using the CPR number, we linked the study cases and the questionnaire screened controls to the Danish Psychiatric Central Register (Mors et al., 2011) and the Danish Civil Registration System (Pedersen et al., 2006) to extract psychiatric registrations, gender, date and place of birth, citizenship and place of present residence, as well as place of birth of their parents. Questionnaire screened controls with a record in the psychiatric register were also excluded.

After exclusions, the number of cases was reduced to 572 and controls to 1049 (545 questionnaire screened controls and 504 unscreened medical students), making this one of the largest studies on genetic association in completed suicide so far. The characteristics of the cases and controls are available in Table 1 and additional clinical information on suicide cases is available in Table 2.

Approvals were obtained from the Ethical Committees in Denmark and from the Danish Data Protection Agency.

2.2. DNA extraction and genotyping

DNA from suicide victims was extracted from 25 to 50 mg of tissue sample (psoas muscle or heart muscle), using the Qiamp DNA mini Kit (Qiagen, Gmbh Hilden, Germany). Most samples were embedded in Histovax Paraffin (Sakura Finetek, Copenhagen, Denmark) and a slight modification of the protocol was used, replacing the xylene step with briefly spinning while still warm after the proteinase *K* incubation, to separate off the paraffin. All of the paraffin-embedded samples were additionally cleaned up with phenol and chloroform extraction, followed by a standard precipitation with ammonium chloride and ethanol. This cleaning up stage more than doubled the success of PCR with all fragment sizes tested. All paraffin slice DNA samples were thereafter run on alkaline agarose gels to check the quality. Any samples with no visible DNA, or which appeared to be contaminated with bacterial DNA were excluded completely from the study. Samples which looked badly degraded with the smear of DNA clearly under 300 bp in size were excluded from the rs25531 and 5-HTTLPR data, as this required amplification of a 361–405 bp fragment.

For approximately 12% of the suicide samples, it was possible to obtain frozen muscle tissue which ensured a much better DNA quality.

DNA from control individuals was extracted from whole blood using standard procedures.

Genotyping was performed on an ABI 3100 Prism Genetic Analyzer, and the fluorescent peaks were analysed using Genemapper version 3.7 or 4.0 (Applied Biosystems, Fostercity, CA), except for genotyping of rs1386494 on the questionnaire screened controls, which was performed on a Sequenom MassARRAY platform and