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TRYPTOPHAN HYDROXYLASE 2 (TPH 2) SINGLE NUCLEOTIDE POLYMORPHISMS, SUICIDE, AND ALCOHOL-RELATED SUICIDE

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SUMMARY

Background: *Suicide has been identified as a serious public health problem that is often accompanied by alcohol misuse and dependence. It seems that suicide is a result of an interplay between distal (e.g. genetic loading, family history of suicide) and proximal factors (e.g. existence of psychiatric disorder, events conferring acute stress), as well as their interactions. However, like suicide, alcohol dependence seems to be a multifactorial disorder caused by genetic and environmental factors. Serotonergic dysfunction has been implicated to be involved in the pathophysiology of substance abuse, and has also an important role in suicidal behaviour. Studies investigating suicide, alcohol-related suicide and the rate limiting enzyme of serotonin synthesis, tryptophan hydroxylase 2 (TPH2), remain to date rather limited.*

Results: *Recent studies of TPH2 showed a range of strong, mild or no association with suicide and alcohol-related suicide, depending on a study group and genetic variants tested. Overall, to date the clinical effects seems to be quite modest. Among suicide victims with more impulsive and verbal aggressive behaviour more alcohol misuse or dependency was present.*

Conclusions: *Suicide and alcoholism are often comorbid disorders with a complex nature. They are both strongly linked to serotonin modulation, and therefore association studies of SNPs in genes from the serotonergic system could provide an insight into the genetic background of such disorders. However, based on current results we cannot draw any conclusions, but further research to clarify the interplay between serotonergic system dysfunction, suicide, alcohol dependence, impulsivity and the role of TPH2 enzyme is needed.*

Key words: *TPH2 – SNP - suicidal behaviour - alcoholism*

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INTRODUCTION

Suicide has been identified as a serious public health problem (Bertolote & Felischmann 2005) that is often accompanied by alcohol misuse and dependence (Murphy & Wetzel 1990). In the EU it is estimated that approximately 58 million adults use or abuse alcohol, out of which 36 million people are "heavy drinkers" and around 22 million have alcohol-dependence (5% of men, 1% of women) (Cnossen 2007). The highest prevalence of alcohol-dependence is estimated to be present in Austria, France, Ireland and Slovenia (Cnossen 2007), and among these countries Slovenia is among the countries with the highest suicide rates in the world (World Health Organisation, Health Topics, 2009). Thus, harmful alcohol use can reflect also in suicide, and according to the World Health Organisation (2004) around 10,000 suicides in the EU (1 in 6 of all suicides) were attributable to alcohol each year (Cnossen 2007). Growing evidence from family, twin, and adoption studies substantiates the implication of genetic and environmental factors, as well as their interaction, in suicidal behaviour (Bondy et al. 2006) and alcohol dependence (Dick & Foroud 2003). The genetic background represents a distal risk factor for suicide, which is 'necessary but not sufficient' for suicide (Costanza et al. 2013). Beside that, the presence of an alcohol use disorder has been confirmed as a distal risk factor for completed suicide, as well as for attempted suicide (Vijayakumar et al. 2011). The latest analysis of alcohol and completed suicide, performed by Wilcox et al. (2004), that updated a previous study by Harris & Barraclough (1997), augmented their results where it was shown that alcohol dependent patients had a standardized mortality ratio (SMR) for suicide 14 times higher than expected (Wilcox et al. 2004). Like suicide, alcohol dependence seems to be a multifactorial disorder caused by a combination of genetic and environmental factors (Zill et al. 2007). Strong evidence exists that supports the implication of the serotonergic system in both disorders. Thus, it has been shown that low levels of serotonin metabolite 5-hydroxy indole acetic acid (5-HIAA) are found in the cerebrospinal fluid of alcoholics (Banki 1981) and in subjects with suicidal behaviour (Asberg et al. 1976). The serotonergic system itself is comprised of numerous serotonin receptors, different enzymes involved in serotonin synthesis and degradation, as well as the serotonin transporter. The majority of these genes have been previously studied in association with suicidal behaviour (Costanza et al. 2013).

TRYPTOPHAN HYDROXYLASE 2 LOCALISATION AND GENE STRUCTURE

The enzyme that plays a pivotal role in serotonin synthesis is tryptophan-hydroxylase (TPH) (Arango et al. 2003). TPH has two isoforms, TPH1 and TPH2, with 71% identity in amino acid sequence. Localization of TPH2 only in the central nervous system (CNS) was determined by Walther and Bader (2003), who showed that serotonin was still synthesized in the brain of THP1 knockout mice, while it was absent or minimized in the peripheral tissues (Walther & Bader 2003) where the TPH1 is expressed. In a post-mortem study it was demonstrated that TPH2 is expressed in several human brain regions: frontal cortex, thalamus, hippocampus, hypothalamus, amygdala, cerebellum and raphe nuclei (Zill et al. 2007). The gene for human TPH2 is located on chromosome 12q21.1 (GeneCards, 27th July 2013), has 11 exons, and covers a region of about 93.5 kilobases (Zill et al. 2004b). The sequence of TPH2 has currently 1675 identified SNPs (GeneCards, 27th July 2013), which gives it a great variability and except for a few examples of functional characterization (eg. R441H), the majority of human TPH2 genetic variants that have been found in the introns, untranslated regions and promoter regions, remain to be physiologically characterized (Zhang et al. 2011). Recently Grohmann et al. (2010) showed that TPH2 transcripts exist in at least two alternatively spliced variants in the coding region designated as TPH2a and TPH2b. It seems also that extensive RNA editing of both TPH2 isoforms leads to protein variants with distinct catalytic properties (Grohmann et al. 2010).

TRYPTOPHAN HYDROXYLASE 2 SINGLE NUCLEOTIDE POLYMORPHISMS AND SUICIDALITY

The first study of TPH2 polymorphisms and suicidal behaviour was performed by De Luca et al. (2004) who studied them on a group of bipolar patients and found no association (De Luca et al. 2004). The first and the most extensive study of TPH2 and completed suicide was done by Zill and co-workers (2004) in which they screened ten single nucleotide polymorphisms (SNPs) in the fifth and sixth intron. They determined that SNP rs1386494 and also three different haplotypes formed from the SNPs tested could be an important risk factor for suicidal behaviour (Zill et al. 2004b). These results could not be replicated by Lopez de Lara et al. (2007) who studied 14 SNPs on patients with depression, amid which half died because of suicide (Lopez de Lara et al. 2007). However, they found an association for two SNPs rs4448731 and rs4641527 when they controlled for suicide risk factors, and they significantly predicted suicide (Lopez de Lara et al. 2007). In the Estonian population, Must et al. (2009) studied 14 SNPs and completed suicide but found no association on allele or haplotype level (Must el al. 2009). In the Korean population of depressed patients Yoon & Kim (2009) found an association for promoter polymorphism -703G/T (rs4570625) that may have an important effect on susceptibility to suicidal behaviour, and is probably not associated with the diagnosis of major depression (Yoon & Kim 2009). However, the association of this particular polymorphism in several other populations did not reveal an association with suicide (Mouri et al. 2009, Stefulj et al. 2011, Zhou et al. 2005). Campos et al. (2010) performed an SNPs analysis using 8 different markers covering the whole human TPH2 gene. However, they failed to show any impact of either alleles, genotypes or haplotypes on suicidal behaviour (Campos et al. 2010). The SNP rs7305115, located at the position 40237 relative to 5′-end of TPH2 gene, was studied on two samples of patients with major depressive disorder with and without suicidal behaviour. Results of both studies suggested that this polymorphism could be a predictive factor for suicidal behaviour (Ke et al. 2006, Zhang et al. 2010). A polymorphism that is proposed to be involved in alternative splicing of TPH2, rs4290270, has been shown to have significantly higher frequency of A/A genotype in the group of suicide victims compared to subjects with major depression or controls (Grohmann et al. 2010). Results for polymorphism rs11178997, position -473A>T at the TPH2 5'-end, are similar in two studies, first by De Luca et al. (2006) and the second by Zupanc et al. (2011). They both found only a trend for association with suicide and a very low frequency of genotype AA (De Luca et al. 2006; Zupanc et al. 2011). In the study of suicide attempt and bipolar affective disorder no association was determined for this polymorphism (Lopez et al. 2007). Furthermore, no association was confirmed also in a larger study of suicide attempt and depression on four different populations (Americans, Finns, African Americans and Indians) (Zhou et al. 2005). A possible association for polymorphisms in intron 5 Rs1843809 and Rs1386493 was determined by Zupanc et al. (2011), and similar genotype frequencies to the Slovene population were determined by Zill et al. (2004a). The functional single-nucleotide polymorphism R*s1386493*, which decreases efficiency of normal RNA splicing, resulting in a truncated TPH2 protein (TPH2-TR) that lacks enzyme activity causing reduced 5-HT production may exhibit important epistasis (Zhang et al. 2011). Single nucleotide polymorphism in intron 8 rs1386483 was associated with completed suicide, and might also be particularly important in understanding the risk of multiple suicide attempts (Fudalej et al. 2010).

The DNA sequence for TPH2 harbours several non-synonymous polymorphisms, among which is a very rare functional polymorphism that changes amino acid arginine to histidine (R441H; in the DNA 1463G>A;

rs1200074175). Zhang et al. (2005) determined that this polymorphism causes 80% lower function of the enzyme TPH2. In the study of Zupanc et al. (2011) a cohort of suicide victims and controls was not found polymorphic for it.

TRYPTOPHAN HYDROXYLASE 2 SINGLE NUCLEOTIDE POLYMORPHISMS AND ALCOHOL-RELATED SUICIDE

Suicide risk in alcohol dependent-subjects is 60 to 120 times higher than in the normal population (Sher 2006c). Since it has been proposed that genes of the serotonergic system might play an important role in both psychopathologies, TPH2 gene gives the opportunity for research. Despite this, so far there are only a few studies covering this topic. (Feudalej et al. 2009, Holmgren & Jones 2010, Zupanc et al. 2011). In three studies of suicide victims blood alcohol was measured and blood alcohol concentration (BAC) above 0.2 g ethanol/kg blood were designated as positive. Across all three studies the frequencies of BAC positive victims were comparable: study from Poland 39.5 % (76.6 % male; 23.4 % female) (Fudalej et al. 2009), from Slovenia 30.9 % (84.2 % male; 15.8 % female) (Zupanc et al. 2011), and from Sweden 34.0 % (74.0 % male, 26.0 % female) (Holmgren & Jones 2010). In the quoted studies male suicide victims were more plausible to have a positive BAC at the time of the act than females. The gender frequency distribution might be simply a reflection of higher frequency of males among alcohol misusers and alcohol dependent subjects, or differential levels of impulsivity could explain the gender differences in acute inebriation before the final suicidal act (Fudalej et al. 2009, Zupanc et al. 2011). In 2007 Zill and co-workers published a study in which they genotyped 20 SNPs covering the entire region of the gene on 305 healthy controls and 353 alcohol-dependent patients of whom 102 had a history of at least one suicide attempt. They found one major haploblock with strong linkage disequilibrium between inton 5 and 8 in alcohol-dependent patients and healthy controls; however the results did not show association with the alcohol-related suicidal behaviour and no data on BAC were available (Zill et al. 2007). One of the polymorphisms that Zill et al. 2007 studied, rs1386483, was studied on a Polish sample of suicide victims. Data on blood alcohol levels (BAC) and common psychiatric diagnosis (eg. alcohol dependence) were available. Results showed that T/T genotype could be associated with suicide in sober victims, and furthermore it remained significantly associated with lower likelyhood of suicide under the influence of alcohol (Fudalej et al. 2009). Another Polish study by Wrzosek et al. (2011) performed on alcohol-dependent patients did not associate polymorphism rs1386494 with suicide attempt (Wrzosek et al. 2011) which is a parallel to the results of Zill et al. (2007). In a Slovenian population of suicide victims five polymorphisms were studied. Results of genotype distributions of subjects with concentration of ethanol above 0.2 g per kg of blood were statistically significant for intronic polymorphism Rs1843809 and a tendency was determined for intronic polymorphism Rs1386493 (Zupanc et al 2011). These two polymorphisms were studied also by Zill et al. 2007, but gave no association. More impulsive and verbal aggressive behavior was also present in the subgroup of suicide victims with alcohol misuse or dependency (Zupanc et al. 2011). Some studies suggest that impulsive aggression is related to lower levels of CNS 5-HT (Oquendo & Mann 2000), and according to the data of Zupanc et al. (2011) differences in TPH2 polymorphisms in suicide victims with alcohol misuse or dependency in comparison to other suicide victims actually exist (Zupanc et al. 2011). However, the determined differences could be associated to alcohol dependency and/or impulsivity/aggression alone (Zupanc et al. 2011). Aggression/impulsivity and severity of alcoholism affect the risk for suicide among individuals with alcoholism (Sher 2006a) and several other studies of suicide victims with alcohol misuse or dependency show impulsive and verbal aggressive behaviour to be more frequently present in this subgroup of suicide victims than in a subgroup of suicide victims without observed alcohol misuse or dependency (Sher 2006b).

CONCLUSIONS

Suicidal behaviour is often associated with alcohol abuse. Neurobiological bases of alcohol abuse on the one side and suicidal behaviour on the other are complex, influenced by multiple genes, epigenetic mechanisms and environmental factors. They are both strongly linked to serotonin modulation, and therefore association studies of SNPs could provide an insight into the genetic background of such behaviours. Recent results on the TPH2 gene are still rather scarce in regard to its role in serotonergic dysfunction. But because of its central role in serotonin synthesis it may be an important factor in the pathophysiology of suicidal behaviour and/in alcohol abuse, but further studies are needed to clarify the interplay among serotonergic system dysfunction, suicidal behaviour, alcohol dependence, impulsivity and the role of TPH2 enzyme.

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