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Research article

# Genetic association analysis of serotonin and signal transduction pathways in suicide attempters from an Italian sample of psychiatric patients

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## HIGHLIGHTS

- A polygenic risk score detected for three genes *HTR2A* (A-1438G), *TPH1* and *TPH2* increased the prediction of SA risk.
- A gender stratification evidenced nominal associations with *HTR1A*, *TPH1* and *GNB3* genes.
- A phenotypic dissection evidenced nominal associations with *SLC6A4, HTR1A*, *HTR2A* (-1438A/G) and *GNB3* genes.
- Although some limitations, this could represent a further study for future meta-analyses in larger samples.

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## ABSTRACT

Genetic factors have been reported to contribute to the liability of suicide. We aimed to investigate functional polymorphisms in eight genes (serotonin transporter, *SLC6A4*; receptors, *5HTR1A, 1B, 5HTR2A;* Tryptophan Hydroxylase, *TPH1, TPH2*; Monoamine Oxidase, *MAOA* and G Protein Subunit Beta 3, *GNB3*) to investigate their predictive value for suicide. The possible confounding effects of gender and phenotypic patients dissection were also valued. A sample of 111 consecutive psychiatric inpatients was recruited and assessed using specific psychometric instruments. Genomic DNA was isolated from peripheral white blood cell samples and polymorphisms were genotyped by pyrosequencing technology. Although no differences were observed between allele and genotype frequencies for all polymorphisms and suicide attempt (SA), a polygenic risk score was detected for three genes *HTR2A* (A-1438G), *TPH1* and *TPH2* increasing the prediction of SA risk (Thresh = 0.43, $p = 0.038$, $R^2 = 0.053$). Moreover some nominal associations were obtained after gender and phenotypic dissection stratification (TEMPS-A, TEMPs-H, GSMD, SHSS, GAF, CGI) for *SLC6A4* (5-HTTLPR), *HTR1A* (C-1019G), *HTR2A* (A-1438G), *TPH1* (A799C) and *GNB3* (C825T) genes, that were lost after Bonferroni correction. This is a first evidence that specific

*Abbreviations:* *SLC6A4*, serotonin transporter gene; *5HTR1A, 1B, 5HTR2A*, serotonin receptors; *TPH1, TPH2*, Tryptophan Hydroxylase; *MAOA*, Monoamine Oxidase; *GNB3*, G Protein Subunit Beta 3; SA, suicide attempt; TEMPS-A, temperament evaluation of the Memphis, Pisa, Paris and San Diego-Autoquestionnaire (A = anxious); TEMPS-H, (H = hyperthymic); GSMD, Gotland Scale for Male Depression; SHSS, Suicidal History Self-Rating Screening Scale; GAF, Global Assessment of Functioning Scale; CGI, Clinical Global Impressions.

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additive combinations of genes could increase the prediction of SA risk and that gender and phenotypic dissection could influence the association of the genes with SA. This could represent a further study also for future meta-analyses on larger samples.



## 1. Introduction

Suicide is a serious public health problem worldwide. The prevalence rate of suicide attempts (SA) among patients with different psychiatric disorders varies from 25 to 50% in schizophrenia, 29–51% in bipolar disorder, 16–33% in major depressive disorder [1–3]. Importantly, there is a gender disparity among suicide attempts with females committing twice as many suicide attempts but with males representing 80% of fatal attempts [4], pointing out both social and biological influences in suicidal behavior.

Genetic factors have been suggested to contribute to the liability of SA, accounting for 30–50% of the variance in suicide thoughts and behavior, and are largely independent of the inheritance of psychiatric disorders [5]. However, the mechanism and magnitude of the genetic contribution in the emergence of suicidal behavior is still poorly understood [5].

The serotoninergic pathway has been implicated for several reasons as having a major role in the pathophysiology of suicide behavior (SB), including SA [5,6]. In particular, *common polymorphisms* in serotonin transporter (*SLC6A4*), receptors (*5HTR1A, 1B, 5HTR2A*), and enzymes involved in serotonin metabolism: both synthesis (Tryptophan Hydroxylase, *TPH1* and *TPH2*) and degradation (Monoamine Oxidase, *MAOA*) genes have been linked to SB and SA, but these findings are not consistently supported by existing evidence. For instance, most studies found an association between lower expressing variant of the most investigated 5-HTTLPR upstream promoter region polymorphism in SLC6A4 gene, early life stress, and SB. However, the relationship between 5-HTTLPR and SB was not confirmed by all studies. The rs6295 single nucleotide polymorphism (SNP) in the 5-HTR1A promoter region was associated with SA, psychiatric hospitalizations, and substance use disorder. Both TPH1 and TPH2 polymorphisms were found to be associated with SA by some but not all studies [for reviews 5,6]. These associations include both SA and SB and some of these studies investigated psychiatric patients with different diagnosis whereas other specific diagnosis [5][for review 5]. These findings suggest complexity and heterogeneity of the results.

Guanine nucleotide binding proteins (G proteins) are key regulators of cellular responses through the cyclin adenosine monophosphate (cAMP) pathways. The abnormal expression and function of G-proteins are closely related to the pathophysiology of a variety of mental illnesses, including major depressive disorder. G-proteins contain multiple subunits, each encoded by several isoforms. A functional polymorphism (C825T, rs5443) of the G-protein beta3 subunit (GNB3) has been associated with increased signal transduction and ion transport activity, the risk of major depressive disorder and antidepressant treatment response [7,8]. To the best of our knowledge, no studies are currently available between this gene and SA in psychiatric samples.

As for genetic studies in psychiatry, a number of variables could have contributed to generate the inconsistency of the available results. Genetic differences associated with ethnic background as well as gender effects and the inherent phenotypic heterogeneity in suicidality are considered among the first-line putative sources of variation that should be adequately checked; however, their influence on the putative associations between *SLC6A4, 5HTR1A, 1B, 5HTR2A, TPH1, TPH2, MAOA* and *GNB3* genes and SA still remains largely undetermined.

Given this background, we planned to replicate the associations of twelve functional well-known polymorphisms in eight genes such as *SLC6A4, 5HTR1A, 1B, 5HTR2A, TPH1, TPH2, MAOA* of the serotoninergic system and *GNB3* in our Italian population and to construct, for the first time, polygenic risk scores to test their predictive value for SA. Furthermore, we analyzed the possible confounding effects of gender and phenotypic dissection of these polymorphisms on this cohort.

## 2. Material and methods

### 2.1. Participants

We recruited 111 (70 men and 41 women) consecutive psychiatric inpatients with a mean age of 42.84 ± 12.81 years, who were admitted to the Psychiatric Unit of Sant'Andrea Hospital in Rome. Considering multiple diagnoses, 5 patients had acute schizophrenia (4.5%), 26 chronic schizophrenia (23.4%), 7 schizoaffective disorder (6.3%), 36 bipolar disorder either type I or II (32.4%), 14 major depressive disorder (12.6%), and 13 a personality disorder (11.7%). Psychiatric diagnoses were assessed by appropriately trained clinicians during the first 48 h after admission with the use of the Mini International Neuropsychiatric Interview (MINI). The Mini is a brief, fully structured diagnostic interview that assesses 17 Axis I disorders, antisocial personality, and suicidality according to *DSM–IV* criteria. Interviews typically consisted of 15–20 min per person. One section of this instrument is dedicated to the assessment of suicidal risk, with questions about past and current suicidality. The suicide risk section of the MINI classifies subjects into four groups: no suicidal risk, low suicidal risk, medium suicidal risk, and high suicidal risk. The MINI has demonstrated good validity, with median kappa coefficients greater than 0.63 against other interviews and interrater reliabilities ranging from kappas of 0.79–1.00. Additionally, participants completed a socio-demographic interview at intake, the Temperament Evaluation of the Memphis, Pisa, Paris and San Diego-Autoquestionnaire (TEMPS-A) [9], the Beck Hopelessness Scale (BHS) [10], the Suicidal History Self-Rating Screening Scale (SHSS) [11] and the Gotland Scale for Male Depression (GSMD) [12], the Global Assessment of Functioning Scale (GAF) [13], and the Clinical Global Impressions (CGI) [14].

Study subjects participated voluntarily and provided written informed consent, following review by the Sant'Andrea Hospital research ethics review board and final approval of the study protocol by Policlinico Umberto I – Sapienza University of Rome ethics review board. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

### 2.2. Measures

SA was defined as a non-fatal, self-directed, potentially injurious behavior with an intent to die as a result of the behavior; it might not result in injury [15–17]. Assessment of current suicide attempts were performed by the MINI module for suicide and answers at SHSS.

As part of a consolidated tradition in the study of suicide risk, authors also assessed temperaments, hopelessness and other vari-