Hypnotizability and Catechol-O-Methyltransferase (COMT) polymorphisms in Italians

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INTRODUCTION

The cognitive trait of hypnotizability (Green et al., 2005) – the ability to accept hypnotic suggestions – has been classically attributed to peculiar characteristics of the supervisory attentional system (Norrman and Shallice, 1996; Posner and Fan, 2004) allowing a more flexible attentional control in the subjects scoring high (highs) at hypnotizability scales. In fact, a few neuropsychological studies (Lichtenberg et al., 2000; Raz, 2005; Raz et al., 2006; Szekely et al., 2010) have suggested greater abilities of focused attention in highs with respect to low hypnotizable individuals (lows). The three association studies conducted so far on the relation between hypnotizability and COMT single nucleotide polymorphism (SNP) rs4680 (Val158Met) were inconsistent. Here, we used a selective genotyping approach to re-evaluate the association between hypnotizability and COMT in the context of a two-SNP haplotype analysis, considering not only the Val158Met polymorphism, but also the closely located rs4818 SNP. An Italian sample of 53 highs, 49 low hypnotizable subjects (lows), and 57 controls, were genotyped for a segment of 805 bp of the COMT gene, including Val158Met and the closely located rs4818 SNP. Our selective genotyping approach had 97.1% power to detect the previously reported strongest association at the significance level of 5%. We found no evidence of association at the SNP, haplotype, and diplotype levels. Thus, our results challenge the dopamine-based theory of hypnosis and indirectly support recent neuropsychological and neurophysiological findings reporting the lack of any association between hypnotizability and focused attention abilities.

Higher brain dopamine content depending on lower activity of Catechol-O-Methyltransferase (COMT) in subjects with high hypnotizability scores (highs) has been considered responsible for their attentional characteristics. However, the results of the previous genetic studies on association between hypnotizability and the COMT single nucleotide polymorphism (SNP) rs4680 (Val158Met) were inconsistent. Here, we used a selective genotyping approach to re-evaluate the association between hypnotizability and COMT in the context of a two-SNP haplotype analysis, considering not only the Val158Met polymorphism, but also the closely located rs4818 SNP. An Italian sample of 53 highs, 49 low hypnotizable subjects (lows), and 57 controls, were genotyped for a segment of 805 bp of the COMT gene, including Val158Met and the closely located rs4818 SNP. Our selective genotyping approach had 97.1% power to detect the previously reported strongest association at the significance level of 5%. We found no evidence of association at the SNP, haplotype, and diplotype levels. Thus, our results challenge the dopamine-based theory of hypnosis and indirectly support recent neuropsychological and neurophysiological findings reporting the lack of any association between hypnotizability and focused attention abilities.

Keywords: hypnotizability, attention, COMT, absorption, selective genotyping, haplotype analysis
As a control group representative of the general population, 57 students of the Universities of Pisa and Siena (410 M, 633 F). As a control group representative of the general population, 57 umbilical cords (Controls) from the Immuno-hemathology Unit Bank at the Azienda Ospedaliera–Universitaria Pisana, were genotyped anonymously (Controls). Consensus on the employment of umbilical cords for research had been obtained from mothers at the Azienda Ospedaliera–Universitaria Pisana soon after delivery.

DNA EXTRACTION, AMPLIFICATION AND ANALYSIS

Genomic DNA was isolated by the QIAamp DNA Blood kit (QIAGEN GmbH, Hilden, Germany) according to manufacturer’s instructions from highs and lows peripheral blood leukocytes. The same was done with umbilical cords samples from (Controls). For privacy requirements, blood samples were coded anonymously. The DNA extracted from 200 μl of blood was diluted with 200 μl of H2O, quantified by UV measurement at OD 260 nm and stored at −80°C until further processing.

PCR amplification was carried out using forward and reverse primers (COMT-F: 5′-ATCCAGTCCCTGCTCTCCACCTG-3′ and COMT-Seq-R, 5′-CTTTTTCCAGTCCTGAAACA-3′) and BigDye Terminator v3.1 in accord to protocol (Applied Biosystems, USA). The sequencing were run on the ABI 3130xl Prism Genetic Analyser (Applied Biosystems) and analyzed using the software SeqScape v2.5 (Applied Biosystems).

A DNA fragment of 240 bp of the COMT gene containing the well-known non-synonymous SNP rs4680 (G/A at position 472, or Val158Met) was sequenced in highs, lows, and Controls. In addition to rs4680, a second SNP was identified at position 408, namely the synonymous rs4818 (C/G, or Leu136Leu).

STATISTICAL ANALYSIS

Adherence of genotype frequencies to Hardy–Weinberg equilibrium was assessed by goodness-of-fit tests. Heterogeneity of allele frequencies among population samples was assessed by contingency-table χ2 analysis. Difference of allele frequency between highs and lows was measured by calculating odds ratio and 95% confidence limits. The odds ratio was calculated as follows: OR = (a×d)/(b×c) where a, b, c, and d are the observed cell frequencies of the contingency table. The 95% confidence interval (CI) is obtained by multiplying the square root of the odds ratio by the standard normal deviate (Z) corresponding to the exact level of confidence desired. The frequency of an allele was considered significant if the 95% CI did not overlap with 1. To determine the difference in the allelic frequencies of the two subsamples, the χ2 (chi-square) test was applied. The p-values were corrected for multiple comparisons using the Bonferroni correction.

RESULTS

Table 1 shows the genotypes at the COMT Val158Met polymorphism and the Met allele frequency in highs, lows, and Controls. No heterogeneity of allele frequency was detected (χ2 = 2.7, d.f. = 2, p = 0.2). The odds ratio of the Val allele for highs and lows (2 × 2 table) was 0.72; 95% Confidence Interval: 0.4–1.3.

Table 2 shows the genotype counts of rs4818 (C/G, or Leu136Leu) and rs4680 (G/A, or Val158Met) in the form of two-locus haplotype frequencies, for each of our three samples. Hardy–Weinberg equilibrium tests were performed for each of the two SNPs, separately for males and females. No significant deviation was detected in any of these subsamples.

The EM algorithm produced the haplotype frequency estimates shown in Figure 1. One of the four possible haplotypes (G_A, in the order rs4818–rs4680) was absent from both highs and lows, meaning complete linkage disequilibrium, whereas it was present at low frequency (0.02) in the control sample.

Table 1 | Genotypes at COMT Val158Met polymorphism in highs, lows, and Controls

| Sample type | MetMet | MetVal | ValVal | Total | p(A) |
|-------------|-------|--------|--------|-------|------|
| highs       | 11    | 25     | 17     | 53    | 0.443|
| %           | 20.8  | 472    | 32.1   |       |      |
| lows        | 10    | 16     | 23     | 49    | 0.367|
| %           | 20.4  | 32.7   | 46.9   |       |      |
| Controls    | 12    | 34     | 11     | 57    | 0.569|

| Sample type | p(A) |
|-------------|------|
| Met allele frequency. |

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Table 2 | Joint genotype distribution of rs4818 (C/G, or Leu136Leu) and rs4680 (G/A, or Val158Met), in three population samples.

|          | highs   | lows    | Controls |
|----------|---------|---------|----------|
|          | rs4818^1 | rs4818^1 | rs4818^1 |
|          | AA  | GA  | GG  | Total | AA  | GA  | GG  | Total | AA  | GA  | GG  | Total |
| CC       | 11  | 8   | 3   | 22    | 11  | 12  | 2   | 25    | 11  | 12  | 2   | 25    |
| CG       | 0   | 17  | 4   | 21    | 0   | 12  | 7   | 19    | 1   | 21  | 2   | 24    |
| GG       | 0   | 0   | 10  | 10    | 0   | 0   | 14  | 14    | 0   | 1   | 7   | 8     |
| Total    | 11  | 25  | 17  | 55    | 10  | 16  | 23  | 49    | 12  | 34  | 11  | 57    |

^1 AA, (ValVal); GA, (ValMet); GG, (MetMet).

FIGURE 1 | Estimated haplotype frequencies of the two SNPs, rs4818 (C/G) and rs4680 (G/A, Val158Met) in highs (N = 53), lows (N = 49), Controls (N = 57). Marker order: rs4818–rs4680.

The large overlap of the 95% confidence intervals of the three samples makes it clear that there is no association between hypnotizability and these COMT haplotypes. Indeed, there was no evidence of heterogeneity ($\chi^2 = 3.78$, d.f. = 5, $p = 0.582$) in highs and lows also for the absolute frequencies of the two-SNP diplotypes.

DISCUSSION

The present study does not show any association between COMT polymorphisms and hypnotizability at the SNP, haplotype, and diplotype levels.

GENETIC FINDINGS AND NEUROPSYCHOLOGICAL EVIDENCE ON HYPNOTIZABILITY-RELATED ATTENTIONAL ABILITIES

Previous studies (Lichtenberg et al., 2000; Raz, 2005; Raz et al., 2006; Szekely et al., 2010) presented some evidence of association between the Val158Met polymorphism and hypnotizability (Table 3).

The most important discrepancy concerns our results and those reported by Szekely et al. (2010). That work found a significant difference in allele frequencies between highs and lows, but its power was limited by the necessarily small proportion of highs in the samples (N = 19), which is due to the distribution of hypnotizability scores in the general population (Balthazard and Woody, 1989; De Pascalis et al., 2000; Carvalho et al., 2008). Our alternative approach of selective genotyping, in which individuals are sampled from the opposite tails of a quantitative trait, can substantially increase the power of population-based associations studies (Schork et al., 2000; Van Gestel et al., 2000). It should be noted that the odds ratio of the Val allele (recalculated from published data) is 3.0 in the work by Szekely and coll. (Szekely et al., 2010), whereas it is 0.7 in our data, and the 95% CI do not overlap. The power of our study to detect significant heterogeneity of allele frequency between highs and lows, if their frequency were as in (Szekely et al., 2010), was 97.1% at the significance level of 5%, and it was 89.8% at the significance level of 1%.

Theoretically, the different methods of hypnotic assessment between studies might account for the different results, but we consider this unlikely, as the methods used in the present and in other works provide highly correlated results (Sheehan and McConkey, 1982). Another factor possibly accounting for the discrepancy is a different level of association in different populations; this can happen if the association is caused by a nearby locus that shows variable levels of linkage disequilibrium among populations.

The present results are in line with the findings showing the absence of any hypnotizability-related difference in attentional tests (Varga et al., 2011), and also the absence of significant correlation between COMT polymorphism and executive attention performance as measured by Posner Attentional Network Test (Fossella et al., 2002). Moreover, recent neuropsychological studies contrast the classical view of hypnotizability based on high abilities of focused attention and attribute the hypnotizability-related cognitive characteristics to impaired frontal executive functions inducing a lower capacity to disengage attention from its current focus (Jamieson and Sheehan, 2004; Egner et al., 2005). Finally, the assumption that the larger content of the homovanillic acid (HA) found in highs (Spiegel and King, 1992) depends on reduced DOPA catabolism (responsible for high abilities of focused attention) is weak, as HA is a catabolite of both dopamine and norepinephrine and its content in the cerebrospinal fluid derives from their catabolism in several neural circuits (Gu,
Table 3  Genetic association studies between the COMT Val158Met polymorphism and hypnotizability.

| Sample type | MetMet AA | MetVal AG | ValVal GG | Total | p(A) | Remarks |
|-------------|-----------|-----------|-----------|-------|------|---------|
| ANOVA based approaches | | | | | | |
| Lichtenberg et al. (2000) Unstratified | 77 | 41 | 19 | 137 | 0.712 | Association significant in females only; highest hypnotizability score in heterozygotes |
| | % | 56.2 | 29.9 | 13.9 | | |
| | Mean HS | 5.2 | 6.6 | 4.5 | | |
| Raz (2005) Unstratified | 18 | 33 | 25 | 76 | 0.454 | Highest hypnotizability score in heterozygotes; no significance test provided |
| | % | 23.7 | 43.4 | 32.9 | | |
| | Mean HS | 6.1 | 7.6 | 5.9 | | |
| Szekely et al. (2010) Unstratified | 30 | 66 | 31 | 127 | 0.496 | ANOVA significant for genotype effect (p = 0.016); medium score in heterozygotes |
| | % | 23.6 | 52.0 | 24.4 | | |
| | Mean HS | 4.1 | 4.7 | 5.9 | | |
| Categorical data analysis | | | | | | |
| Szekely et al. (2010) highs (mean HS 9.3 ± 1.0) | 1 | 9 | 9 | 19 | 0.289 | χ² significant for heterogeneity (p = 0.009); Odds Ratio for the Val allele (2 × 2 table) = 3.0; 95% CI: 1.4–6.7 (calculated from published data)

1 Met allele frequency; 2 Hypnotizability Score; 3 Confidence Interval; 4 The corresponding value of our data was 0.7; 95% CI: 0.4–1.3.

MECHANISMS INDEPENDENT OF COMT POLYMORPHISMS POTENTIALLY INVOLVED IN HYPNOTIZABILITY-RELATED ATTENTIONAL CHARACTERISTICS

The highs’ attention seems to be stable rather than flexible. A few authors suggest that the carriers of the Met allele might be comparatively high in cognitive stability, but low in cognitive flexibility (Cools, 2008; Cools and D’Esposito, 2009; Colzato et al., 2010). High flexibility would be associated with greater distractibility, while high stability may be related to scarce distractibility (Goschke, 2000), as suggested for highs (Tellegen and Atkinson, 1974; Lichtenberg et al., 2000, 2004; Zachariae et al., 2000; Raz, 2005; Raz et al., 2006). The balance between cognitive flexibility and stability (Cools and D’Esposito, 2009; Durvas and Palmiter, 2011) could depend on the interaction between the dopaminergic circuits of the prefrontal cortex (where the catecholamines metabolism relies mainly on the activity of the COMT) and of the striatum, where the catecholamines metabolism depends mostly on the mono amino oxidase (MAO) enzymatic system (Durvas and Palmiter, 2011). Actually, polymorphisms in MAO have also been found associated with executive attention and with alerting efficiency (Fossella et al., 2002). Thus, different attentional performance could be accounted for by a peculiar balance between the catecholamines degradation occurring in different brain structures.

However, the existence of multiple subtypes of highs and lows (Balthazard and Woody, 1989; Pekala and Forbes, 1997; Green and Lynn, 2011; Terhune et al., 2011) suggests that it is unlikely that one biological determinant may account for such a complex trait like the susceptibility to hypnosis, and we may expect that several neurotransmitters and neuromodulators influence hypnotizability (Ott et al., 2005; Klinkenberg et al., 2011). Recent evidence suggests a role for nitric oxide (NO) because the hypnotizability-related vascular responses to cognitive and physical stimulation indicate greater NO availability in the highs’ vessels (Jambrik et al., 2004; Jambrik et al., 2005). In the brain, endothelial NO is responsible for basal vascular tone, interacts with other mediators in its modulation, and acts as a neurotransmitter after diffusion to the extracellular compartment (Andresen et al., 2006). Using an in vivo brain microdialysis technique, it has been demonstrated that NO significantly increases the release of acetylcholine and decreases the...
release of dopamine in the rat striatum (Guarana-Guzman et al., 1994), while increasing its metabolism (Nabeshima et al., 1987; Loscher et al., 1994; Egner, T., Jamieson, G., and Gruzelier, J. (2005). Hypnosis decouples cognitive control from conflict monitoring processes of the frontal lobe. Neuron 27, 906–919. doi: 10.1016/j.neuron.2000.05.002). 

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AUTHOR CONTRIBUTIONS

Silvano Presciuttini, Giancarlo Carli, and Enrica L. Santarcangelo have designed the study and written the paper; Serena Barbuti, Michele Curcio, and Fabrizio Scatena have performed the DNA analysis; Alessandro Galluisi and Silvano Presciuttini have performed the statistical analysis.

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