

COMT polymorphisms in impulsively violent offenders with antisocial personality disorder

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Abstract

OBJECTIVE: The relationship between catechol-O-methyltransferase (COMT) polymorphisms and violent behaviour was tested in highly selected group of non-psychotic violent offenders.

METHODS: We conducted an association study comparing 47 male repeatedly sentenced for impulsive violent attacks diagnosed with Antisocial Personality Disorder (APD) with 43 healthy male controls matched on education. Three COMT polymorphisms were analysed: COMT Val¹⁵⁸Met and COMT Ala¹⁴⁶Val on exon 4, and untranslated polymorphism on the 6th exon, at the regulatory region of the COMT gene with deletion-insertion character del/C.

RESULTS: Logistic regression analysis revealed that while Val¹⁵⁸Met is not associated with violence in APD, another COMT polymorphism – COMT Ala¹⁴⁶Val is more frequent among violent offenders with APD ($p=0.017$).

CONCLUSIONS: To conclude, our findings provide further support that COMT is a modifying gene that plays a role in determining interindividual variability in the proclivity for violent behaviour in subjects without major mental disorder.

INTRODUCTION

It is hypothesized that modulation of impulsive violence is implemented by equilibrium between serotonin that raises the threshold for overt aggressive response to environmental stimuli, and catecholamines that have an opposite effect (Volavka *et al.*, 2004a). The enzyme catechol-O-methyltransferase (COMT) plays a key role in prefrontal cortical functioning in that it accounts for most of the degradation of dopamine. A common functional polymorphism at COMT codon 158 resulting in differences in enzyme activity has been identified (Lachman *et al.*, 1996) and its impact on correlated

Abbreviations :

Ala - Alanine
APD - Antisocial Personality Disorder
COMT - Catechol-O-methyltransferase
Met - Methionine
Val - Valine

neurophysiology has been repeatedly confirmed (Nolan *et al.*, 2004). Relationship between COMT polymorphism and alcohol dependency (Šerý *et al.*, 2006) and Novelty seeking (Hosak *et al.*, 2006) and possibly other developmental disorders (Ptacek *et al.*, 2009) were previously reported in the Czech population. In patients with schizophrenia, it has been described that the lower activity isoform of COMT encoded by Met allele is associated with antisocial features as perceived dangerousness (Strous *et al.*, 1997), increased hostility (Volavka *et al.*, 2004b) increased history of aggression (Lachman *et al.*, 1998; Strous *et al.*, 2003; Han *et al.*, 2004), homicidal behaviour (Kotler *et al.*, 1999) and dissociative symptoms (Ptacek *et al.*, 2008). It was also demonstrated that Met allele is associated with increased tonic dopamine activity and better cognitive stability, but poorer cognitive flexibility (Nolan *et al.*, 2004). Poorer cognitive flexibility resulting in higher aggression in schizophrenic patients with COMT Met allele was reported recently (Han *et al.*, 2006). However, contrary findings were also reported (Jones *et al.*, 2001; Zammit *et al.*, 2004). Studies in healthy volunteers (Avramopoulos *et al.*, 2002) and patients with axis II personality disorders (Flory *et al.*, 2007) failed to observe an association between the Val¹⁵⁸Met allele and aggression. Other COMT genotype variants have not been fully investigated.

The main aim of our study was to analyse two COMT polymorphisms on exon 4 – COMT Val¹⁵⁸Met and COMT Ala¹⁴⁶Val and one polymorphism on the 6th exon at the regulatory region of the gene COMT del/C (Tab.1) to find whether these polymorphisms differentiate violent offenders with Antisocial Personality Disorder (APD) from controls.

MATERIAL AND METHODS

Subjects and sample collection

Subjects were recruited in high security prison from 410 male convicts. Selected sample pool included 47 impulsive offenders with proven history of repetitive violent assaults, at least two convictions for violent attacks (bodily harm, robbery, murder and attempted murder) and diagnosis of APD. Only subjects scoring 8 or more points on Eysenck IVE Impulsivity scale were included in this study (Zukov *et al.*, 2008; 2009). Because previous report (Nolan *et al.*, 2000) showed association between COMT Val¹⁵⁸Met polymorphism and suicidal behaviour, subjects with history of suicidal attempt were not included in this study. Subjects and controls were further diagnosed according to ICD-10 criteria by using Structured Clinical Interview MINI 5.0 administrated by board-certified psychiatrist (JV).

A total of 43 unrelated men without a current or past history of ICD-10 psychiatric disorders with no criminal records, matched according to education, became our control group. Control subjects were blood-bank donors (36) and patients hospitalised for medical reasons (7). In order to minimize the effects of race and

ethnicity on the analysis of allele frequencies, only Caucasian subjects were recruited. All subjects and controls signed a written informed consent. The study received approval by the Ethical committee of The 1st Psychiatric Clinic, Prague.

DNA collection

DNA was extracted non-invasively from buccal smears. A coding system was applied to the DNA samples in order to preserve the confidentiality of all subjects and to blind the samples for genotyping. DNA was isolated by standard protocols and genotyped at the Department of Zoology, Charles University, Prague, Czech Republic.

Genotype analysis

The same set of primers were used to analyse Val¹⁵⁸Met (rs 4680) and Ala¹⁴⁶Val (rs 4986871) polymorphism on exon 4:

F: GCC CGC CTG CTG TCA CC; R: CTG AGG GGC CTG GTG ATA GTG

and a second set of primers were used for amplifying the part of exon 6 where del/C polymorphism (rs 3838146) in untranslated region is found:

F: GAC AAC GTG ATC TGC CCA GG; R: AGG TGT GCT TTG CAT TTA GG

PCR conditions were as follows: after initial incubation at 95 °C for 5 min, amplification (30 cycles) was performed with denaturation at 95 °C for 30s, annealing at 59 °C for 20s and extension at 72 °C for 20s. PCR products were sequenced to ensure correct priming. After amplification, an aliquot of PCR product was incubated overnight with the recommended amount of appropriate restriction enzyme (i.e. NlaIII for Val¹⁵⁸Met, SsiI for Ala¹⁴⁶Val and BglI for del/C polymorphism) according to the protocol (Fermentas®). The digested PCR fragments were subjected to electrophoresis and run in 15% polyacrylamide gel for optimal separation.

RESULTS

Genotype frequencies in violent and control groups are given in Tab. 1. The logistic regression analysis revealed that among the three studied polymorphisms (Val¹⁵⁸Met, Ala¹⁴⁶Val and untranslated del/C polymorphism), only the Ala¹⁴⁶Val polymorphism differentiated violent offenders with APD and controls (i.e. when adjusted for effects of other polymorphisms: $\chi^2_2=8.14$, $p=0.017$, see Figure 1) whilst the other two had no significant effect (i.e. adjusted for effects of other polymorphisms; polymorphism del/C: $\chi^2_2=3.67$, $p=0.159$; Val¹⁵⁸Met: $\chi^2_2=0.92$, $p=0.63$). The minimal adequate model contained only Ala¹⁴⁶Val as the polymorphism that characterised a sub-group of violent offenders with APD ($\chi^2_2=7.30$, $p=0.026$). After pooling the homozygous T/T with subjects bearing heterozygous states C/T at Ala¹⁴⁶Val polymorphism and C/C with C/- at del/C polymorphism, the analysis revealed an even stronger effect of Ala¹⁴⁶Val upon impulsively violent antisocial

Tab. 1. Genotype frequency of analyzed COMT polymorphisms.

Polymorphism	dbSNP id (NCBI)	Restriction Enzyme	Genotype	Violent offenders (n=47)		Control subjects (n=43)	
				Number of cases	Genotype frequency	Number of Cases	Genotype frequency
COMT Ala ¹⁴⁶ Val	rs4986871	Ssi I	C/C (Ala/Ala)	30	63.8%	37	86.0%
			C/T (Ala/Val)	15	31.9%	6	14.0%
			T/T (Val/Val)	2	4.3%	0	0
COMT Val ¹⁵⁸ Met	rs4680	Nla III	G/G (Val/Val)	12	25.5%	15	34.9%
			A/G (Met/Val)	24	51.1%	20	46.5%
			A/A (Met/Met)	11	23.4%	8	18.6%
COMT Del/C untranslated	rs3838146	Bgl I	-/-	23	48.9%	26	60.5%
			-/C	22	46.8%	15	34.9%
			C/C	2	4.3%	2	4.6%

behaviour (adjusted for effects of other polymorphisms: $\chi^2_1 = 6.43, p=0.011$), but only a slight effect of/of/on del/C polymorphism (adjusted for effects of other polymorphisms: $\chi^2_1 = 3.16, p=0.074$) and no effect of/on Val¹⁵⁸Met (adjusted for effects of other polymorphisms: $\chi^2_1 = 0.94, p=0.64$). The minimal adequate model again suggests that Ala¹⁴⁶Val polymorphism characterise a sub-group of violent offenders with APD ($\chi^2_1 = 6.04, p=0.014$).

Statistics

We used the logistic regression analysis to detect the probability with which three COMT polymorphisms differentiate violent offenders and controls. There is evidence that the Met and Val alleles are co-dominant, with heterozygotes exhibiting intermediate enzyme activity (Spielman & Weinshilboum, 1981), but there is a lack of information available for the other two COMT polymorphisms. To be conservative, we considered all COMT polymorphisms as three-level categorical variables, i.e., each combination of alleles (A/A, A/G,

G/G for Val¹⁵⁸Met; C/C, C/T, T/T for Ala¹⁴⁶Val; and C/C, C/-, -/- for del/C polymorphism on the 6th exon) was considered as one level of categorical explanatory variable per each of the three polymorphic sites, whilst the violent antisocial behaviour was a binary response variable. However, there were only few T/T (n = 2) at Ala¹⁴⁶Val and C/C (n = 4) at del/C polymorphisms in our sample. Hence we conducted another analysis where levels C/- and C/C in del/C polymorphism on the 6th exon, and C/T and T/T in Ala¹⁴⁶Val polymorphism, were pooled. The best models were chosen using backward elimination of non-significant terms. The significance of a particular term (particular COMT polymorphism) adjusted for the effects of other terms was based on the change in deviance between the full and reduced models, distributed as χ^2 with degrees of freedom equal to the difference in the degrees of freedom between the models with and without the term in question. Presented are minimal adequate models, i.e. models with all terms significant. All analyses were performed using S-Plus 6.0.

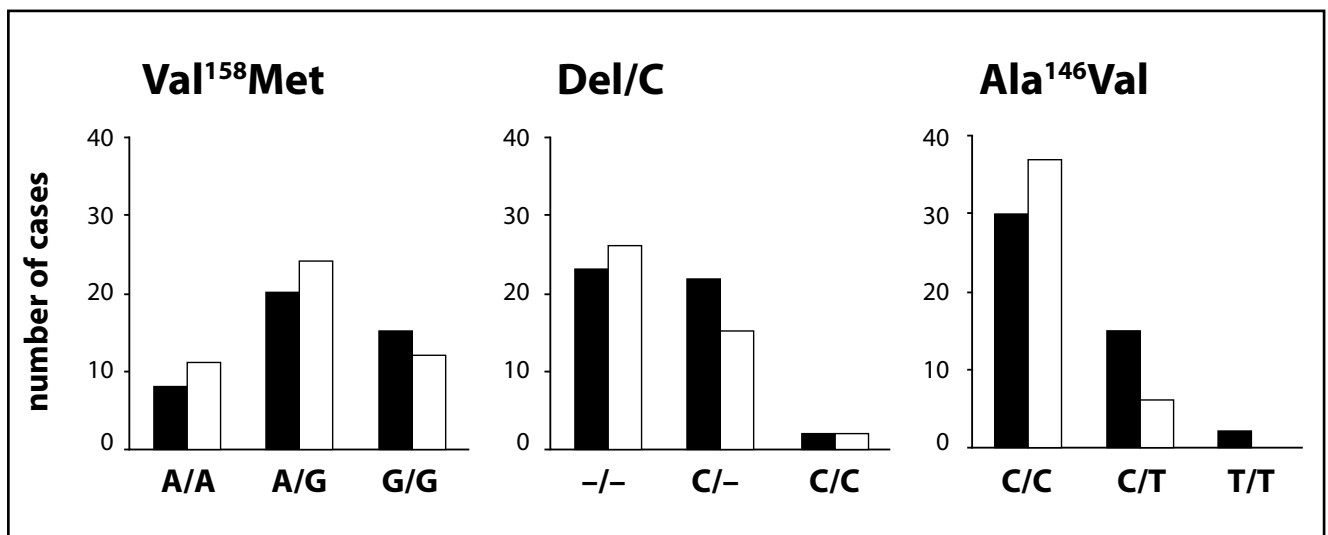


Fig. 1. Genotype frequency of the three studied polymorphisms in violent offenders (black) and controls (white). Only in Ala¹⁴⁶Val polymorphisms was allele T found more often in offenders compared with controls ($p=0.017$).

DISCUSSION

This is the first study of COMT polymorphisms in violent subjects without major mental disorders. Contrary to previous published findings in psychiatric patients we did not find any association of Val¹⁵⁸Met polymorphism with violent antisocial behaviour. Furthermore, there are speculations that association between Met allele and aggression may be an epiphenomenon and the Met allele is associated with a certain form of schizophrenia accompanied with aggressive behaviour (Volavka *et al.*, 2004a). However, a significantly higher frequency of allele T of rarely studied polymorphism Ala¹⁴⁶Val in violent offenders was identified.

According to public databases (e.g. NCBI) the T allele of this polymorphism is rarely found in general population. In recent publications no variability was found in this polymorphism, (Oberacher *et al.*, 2006; Lee *et al.*, 2005). Surprisingly, in our study the T allele was quite common among violent offenders where its frequency was 20% as opposed to 7% detected in controls. However, this polymorphism has not been appropriately studied yet and there is no information on its role upon the activity of the COMT enzyme.

The major limitation of this analysis, as well as of the majority of previous reports, is its association design. The association studies are vulnerable to the confounding effects of population stratification. To at least mitigate this problem, our subjects were members of well-defined Caucasian sample with proven history of repetitive impulsive aggression, without major mental disorder. Patients with suicide attempts (Nolan *et al.*, 2000) that were previously associated with COMT Val¹⁵⁸Met polymorphism were not included in the study.

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