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Dehydroepiandrosterone sulphate as a putative protective factor against tardive dyskinesia

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ABSTRACT

Background: Tardive dyskinesia (TD) is a potentially irreversible consequence of long term treatment with antipsychotic drugs which is according to a well-known theory believed to be related to oxidative stress induced neurotoxicity. Dehydroepiandrosterone (DHEA) is an endogenous antioxidant with neuroprotective activity. The biosynthesis of DHEA depends upon the activity of cytochrome P450c17α (CYP17). The gene that encodes for CYP17 has a (T34C) single nucleotide polymorphism which enhances CYP17 transcription and expression.
Objective: To test the hypothesis that carriership of a more active CYP17 variant would result in higher DHEA(S) levels and protect against neurotoxicity which results in orofaciolingual TD (TDof), limb-truncal TD (TDlt) or both (TDsum).
Method: Tardive dyskinesia was assessed cross-sectionally in 146 Caucasian psychiatric inpatients from Siberia.
Results: Patients who are carriers of the Cyp17 genotype CC have less chance of developing TD compared to patients who are carriers of the Cyp17 genotypes TC or TT ($p < 0.05$). However, these carriers have significant lower circulating DHEAS levels compared to carriers of the Cyp17 genotypes TC and TT ($p < 0.05$). Conversely, carriers of the CYP17 T-allele have significant elevated DHEAS levels. After correcting for gender and age no significant relationship between Cyp17 genotype CC, the T-allelle and the C-allele and the DHEAS concentration of patients was observed.
Conclusions: Although an association between the CYP17 CC genotype and TD is indicated, our findings do not support the hypothesis that this is mediated through increased DHEA(S) levels. We believe that the relationship between this polymorphism and neuroprotective effects of steroids is more complex and cannot be elucidated without taking the posttranslational regulation of the enzyme into account.

1. Introduction

Tardive dyskinesia (TD) is a potentially irreversible drug-induced movement disorder with a prevalence of about 20% in psychiatric patients chronically exposed to antipsychotics (Marsalek, 2000; Soares-Weiser and Fernandez, 2007). Phenotypically, TD can be dissected into two distinct subsyndromes: orofaciolingual (TDof) and limb-truncal dyskinesias (TDlt). Accumulating evidence suggest that TDof and TDlt are two distinct clinical entities with different clinical features, different risk factors, different prognosis, and probably a different genetic liability (Al Hadithy et al., 2009, 2010; Ivanova et al., 2012a,b).

The pathophysiology of TD has not yet been fully elucidated (Loonen and Ivanova, 2013). A well-known theory states that TD is related to oxidative stress induced dopaminergic neurotoxicity (Kulkarni and Naidu, 2003; Miyazaki and Asanuma, 2008). According to this theory

Abbreviations: AIC, Akaike's Information Criterion; AIMS, Abnormal Involuntary Movement Scale; CYP17, Cytochrome P450c17α; DHEA(S), Dehydroepiandrosterone (sulphate); HWE, Hardy–Weinberg equilibrium; MI, Multiple imputation; TD, Tardive dyskinesia; TDof, Orofaciolingual tardive dyskinesia; TDlt, Limb-truncal tardive dyskinesia; TDsum, Both types tardive dyskinesia; TPM, Two part model.

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treatment with antipsychotic drugs results in a mismatch between free radical metabolism and the antioxidant defense mechanism (Cho and Lee, 2013; Sayre et al., 2008). Among these antioxidant defense enzymes superoxide dismutase (SOD) plays a critical role in preventing cell damage by free radicals. Several authors report a relatively consistent association between manganese SOD gene Ala-9Val polymorphism with the susceptibility for developing TD (Bakker et al., 2008; Cho and Lee, 2013). However, in their study and meta-analysis Zai et al. (2010) did not replicate these findings. Possibly, this is at least partly related to ethnic differences between the patient samples. In a previous study, we found evidence for an association between Ala9Val (MnSOD) and TDof, but not TDlt in the also currently studied Siberian Caucasian patients (Al Hadithy et al., 2010).

A second defense mechanism against oxidative stress-induced neurotoxicity is the effect of neuroprotective steroids. One of these substances is dehydroepiandrosterone (DHEA), which is together with its sulfate ester (DHEAS) one of the most abundant steroids in the human body. Both DHEA and DHEAS have antioxidant effects (Camporez et al., 2011; Gao et al., 2005; Maninger et al., 2009). Evidence also indicates that DHEA and DHEAS are synthesized in the brain (Maninger et al., 2009). DHEA is a 19-carbon steroid that is synthesized from cholesterol by two steroid metabolizing enzymes: P450scc and cytochrome P450c17 (Maninger et al., 2009; Miller, 2005). The gene that encodes for this cytochrome P450c17 alpha (17 alpha-hydroxylase; 17/20 lyase) enzyme (CYP17) has a single nucleotide polymorphism, a T (A1) → C (A2) substitution at 34 base pair upstream of the translation initiation site (T34C) (rs 743572) (Carey et al., 1994). The substitution was thought to create an additional SP1-type (CCACC box) binding site in promoter region, enhance CYP17 transcription, and expression. This would result in higher DHEA levels. To our knowledge the relationship between CYP17 (T34C) polymorphism and the TD has only been measured by Segman et al. (2002) without measuring DHEAS levels. We hypothesized that the carriership of C allele would result in higher DHEAS levels (in blood 99% of DHEA is present as its sulphate) and that this would protect against neurotoxicity which results in TDof, TDlt or both.

2. Methods

2.1. Subjects

Informed consent was obtained from each subject after obtaining approval of the study protocol by the institutional bioethics committee. Subjects were included from two psychiatric departments (for permanent and temporal hospitalization) of the Mental Health Research Institute in Tomsk, Siberia (Russia).

We included subjects with clinical diagnosis of schizophrenia or schizotypal disorder (ICD-10: F20 and F21, respectively) and excluded subjects with non-Caucasian physical appearance (e.g., Mongoloid, Buryats, or Khakassians), subjects on clozapine but without TD (clozapine may ameliorate TD), subjects with clinically relevant withdrawal symptoms and those with organic disorders.

2.2. Assessments

Clinical and demographic data were extracted from patients' medical files. TD was assessed cross-sectionally by the use of the Abnormal Involuntary Movement Scale (AIMS). Four trained raters assessed the presence of TD and, when present, the rating of TD was established by consensus with either one of the two senior doctors. The presence of TDof and TDlt was established by a cutoff AIMS score of ≥ 2 (mild but definite) on any of the items 1 through 4 and 5 through 7, respectively. The sum of the first four items and of items 5 through 7 were used as a proxy for the severity of TDof and TDlt, respectively, whereas the sum of items 1 through 7 was used as a proxy for the severity of TDsum (Al Hadithy et al., 2010).

2.3. Medication

On the day of TD assessment, a complete documentation of the medications utilized was compiled by the raters. The dose of the antipsychotic medication was converted into chlorpromazine equivalents (Rijcken et al., 2003).

2.4. DHEA

Since 99% of the circulating DHEA is present in its sulfated form, only DHEAS serum levels (μg/mL) were measured. Blood samples were collected by antecubital venapuncture between eight and nine o'clock in the morning. The patients were instructed to abstain from unusual physical activity or stress, 24 h before blood samples were taken. After separation of serum by centrifugation, the samples were immediately frozen and stored at −80 °C. The DHEAs levels were tested with aid of the DHEA-S ELISA Kit (catalog number EIA1562, DRG International Inc.). The range of analysis is 0.1–10 μg/mL, analytic sensitivity — 0.044 μg/mL. The DHEAS concentration in all samples were measured in one analytic run in order to avoid inter-assay variability.

2.5. Genotyping

DNA extraction and fluorogenic 5′-exonuclease TaqMan genotyping assays were conducted according to standard protocols and blind to the clinical status of the subjects (Al Hadithy et al., 2009, 2010). The CYP17 (T34C) (rs 743572) polymorphism was genotyped by polymerase chain reaction using an amplifier real-time polymerase chain reaction system 7500 (Applied Biosystems).

2.6. Statistics

As in the beginning of this project no blood samples for the determination of DHEAS serum levels were collected, this data was absent in a subgroup of 32 (22%) patients. These missing data was substituted by means of a multiple imputation (MI) technique, which assumes that the missing values are not completely missing at random. By this technique, the ranges of the plausible values of the missing DHEAs levels are estimated based on other variables in the database. In this research the variables age and gender were used. Within the determined range, multiple values for DHEAS levels were calculated. This procedure was repeated 20 times, which ultimately resulted in 20 different data sets as suggested by White et al. (2011). Each imputed data set was analyzed using the standard analytical techniques. After these analyses, the determined regression coefficients and standard errors were averaged to get a pooled estimate of the association (Donders et al., 2006; Patrician, 2002).

For this study, a two part model (TPM) approach was used (Al Hadithy et al., 2009) in order to deal with the clumping of zeroes when measuring severity of TD. In the first part of the two part model, logistic regression analysis was used in order to determine whether there is a significant relation between the presence of TD, TDof and/or TDlt and carriership of a certain Cyp17 genotype. Logistic regression was also used in order to determine whether there is a significant relation between the presence of TDsum, TDof and TDlt and carrier ship of a certain Cyp17 allele (T or C). Both of these analyses were carried out before and after applying multiple imputation. In the second part of the TPM approach, linear regression analysis was used to determine whether there is a significant relation between the severity of TDsum, TDof and TDlt and carriership of a certain Cyp17 genotype. For these analyses, only the patients with TD were included. This analysis was carried out after determining whether the distribution of the errors of variable TDsum and/or log TDsum show a normal distribution by means of applying the Shapiro–Wilk test and the Kolmogorov–Smirnov test. Thereafter, linear regression analysis was used to determine whether there is a significant relation between the severity of TDsum, TDof and TDlt and

carriership of a certain Cyp17 allele. Both linear studies described were carried out before and after applying multiple imputation. The variable DHEAs was taken into account in all analyses in order to determine whether a correlation exists between the DHEAs levels of the patients and carriership of a certain Cyp17 genotype or allele. The variable DHEAs and some other variables of interest, like age, gender, type of psychiatric clinic, use of anticholinergic and antipsychotic medication, were selected in every approach by means of Akaike's Information Criterion (AIC). The same explanatory variables were used before and after multiple imputation. Finally, linear regression analysis was used to determine whether there is a significant relation between the level of DHEAS in serum and the presence of TD, TDof or TDlt. Again this analysis was carried out after determination of normal distribution of errors and before and after applying multiple imputation. Other variables of interest in this analysis were age and gender.

The statistical software "R" was used for the calculations. For creating an overview of the clinical and demographic features of the study population, excel and the statistical SPSS software, release 17, for Windows were used. p values less than 0.05 were considered as significant. The deviation from the Hardy–Weinberg equilibrium (HWE) was tested by the chi-squared test for the Cyp17 polymorphisms using an online tool (http://www.tufts.edu/_mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls).

3. Results

3.1. Demographic and clinical features

In total 146 patient were included of which 91 males and 55 females. Demograpic and clinical details have been provided elsewhere (Al Hadithy et al., 2009; Ivanova et al., 2012a). There is no significant difference in average age and gender proportions between persons of which the DHEAs values were available and persons with missing DHEAs values.

3.2. Genotype and gender

An overview of the genotype distribution in the total population and by gender shows that the female patients of this population are more often carrier of the Cyp17 CC genotype compared to male patients (Table 1). In contrast, the male patients are more often carriers of the TT genotype. The genotype distribution of Cyp17 is not in consistency with the Hardy–Weinberg equilibrium ($p < 0.05$).

3.3. CYP17 polymorphisms and the presence of TD variants

With logistic regression we determined whether a significant relation existed between the presence of TDsum, TDof and TDlt and carriership of a certain Cyp17 genotype or allele. The variables DHEAS, age, gender, type of psychiatric center, use of anticholinergic and use of antipsychotics were included in all models.

Logistic regression analysis of the influence of the Cyp17 genotypes and the DHEAs level on the occurrence of TDsum, TDof and TDlt showed that carriers of the Cyp17 genotypes TC and CC have no significant increased or decreased risk of developing TDsum, TDof or TDlt compared to carriers of the Cyp17 genotype TT (data not shown). The same is true for the influence of the serum concentration DHEAs of the patients.

Table 1
Overview of the genotype distribution in the total population and by gender.

Genotype	Total (146)	Male (91)	Female (55)	HWE χ^2 (p)
TT (A1A1)	63 (43.2%)	42 (46.2%)	21 (38.2%)	5.877 (0.015)
TC (A1A2)	55 (37.7%)	36 (39.6%)	19 (34.6%)	
CC (A2A2)	28 (19. %)	13 (14.3%)	15 (27.3%)	

These effects are absent both before and after applying multiple imputation.

However, the results of the logistic regression analysis of the influence of the Cyp17 T-allele and the level of DHEAs on the occurrence of TDsum, TDof and TDlt indicate that carriers of the Cyp17 genotype CC have a significant lower risk of developing TDsum compared to carriers of the Cyp17 T-allele (p: 0.027). This effect is only observed before adjusting for the level of DHEA and not for TDof and TDlt. Again, the serum concentration of DHEAS has no influence on the development of TDsum, TDof or TDlt, both before and after applying MI (Table 2).

Logistic regression analysis of the influence of the level of DHEAS and the Cyp17 C-allele on the occurrence of TDsum, TDof and TDlt was again without any significant results. As a matter of fact, before multiple imputation, the factor "genotype TT" is most times even identified by Akaike's criterion as not explanatory for the model (data not shown).

3.4. Normality of the distribution of TD scores

To determine whether there is a significant relation between the severity of TDsum, TDof and TDlt and carriership of a certain Cyp17 genotype or allele, linear regression analysis was used. Before these analyses could be carried out, first had to be determined whether the dependent variable conforms to the assumption of normality for the errors. The results indicated that neither the errors of the TDsum scores nor their log versions are normally distributed. However, the Shapiro–Wilk test gave a better result with the variable log TDsum score than with the variable TDsum score. In order to obtain accurate results, the following analyses were performed with both the normal values and the log values of TDsum scores.

3.5. Cyp17 polymorphism and the severity of TD variants

Linear regression analyses investigating the influence of DHEAS concentration and Cyp17 C-allele on the severity of TDsum, TDof and TDlt showed that carriers of the genotypes TC, and CC do not experience a significantly more or less severe form of TDsum, TDof or TDlt compared to carriers of the TT genotype. This effect is observed both before and after applying MI and even when counted with the log variations of TDsum, TDof and TDlt. The same is true for the relationship between the serum concentration of DHEAS and the severity of TDsum, TDof or TDlt. The influence of the level DHEAS on the severity of TD before MI is however almost significant (p: 0.054).

Linear regression analyses investigating the influence of DHEAS concentration and Cyp17 C-allele on the severity of TDsum, TDof and TDlt shows that carriers of the genotype CC do not experience a significant more or less severe form of TDsum, TDof or TDlt compared to carriers of the Cyp17 T-allele. This effect is observed both before and after applying MI and also when counted with the log variations of TDsum, TDof and TDlt. Again, there is no significant relation between the serum concentration of DHEAS and the severity. The influence of DHEAS on the severity of TD before multiple imputation is as stated before almost significant (p: 0.054).

Linear regression analyses investigating the influence of DHEAS concentration and Cyp17 C-allele on the severity of TDsum, TDof and TDlt shows that carriers of the Cyp17 genotype TT do not experience a significantly more or less severe form of TDsum, TDof or TDlt compared to carriers of the Cyp17 C-allele. Also in this analysis, there existed no significant relation between the serum concentration of DHEAS and the severity of TDsum, TDof or TDlt. This effect is observed both before and after applying MI and also when estimated using the transformed values of TDsum, TDof and TDlt scores.

3.6. Cyp17 polymorphisms and DHEAS levels

Linear regression analyses investigating the influence of the Cyp17 genotypes and allele types on serum DHEAS levels show that there is

Table 2
Relationship between CYP17 genotypes/allele types and presence of TD and subtypes. Carriers T allele (TT + TC)/Non-carriers T allele (CC).

	$Nvar_{(n)}/Ncom_{(n)}$	TDsum estimate	p value	OR	CI	TDof estimate	p value	OR	CI	TDlt estimate	p value	OR	CI
Before MI													
DHEAS level excluded													
CYP17 CC	$N_{CC}(24)/N_T(90)$	−1447	**0.027**	0.235	0.065–0.849	−1151	0.080	0.316	0.087–1.148	−1.513	0.159	0.220	0.027–1.810
Age	–	–	–	–	–	–	–	–	–	−0.048	**0.008**	1.049	0.988–0.920
DHEAS level included													
CYP17 CC	$N_{CC}(24)/N_T(90)$	−3.230	0.094	0.040	<0.01–1.723	−2.931	0.122	0.053	<0.01–2.340	−7.373	0.230	<0.01	<0.01–104.9
DHEAS		−0.059	0.738	0.943	0.669–1.329	−0.058	0.755	0.943	0.659–1.355	−0.066	0.758	0.936	0.702–1.625
After MI													
CYP17 CC	$N_{CC}(28)/N_T(118)$	−3.067	0.106	0.047	<0.01–1.925	−2.829	0.137	0.059	<0.01–2.457	−6.783	0.261	<0.01	<0.01–156.6
DHEAS		−0.048	0.776	0.953	0.683–1.330	−0.048	0.786	0.953	0.672–1.351	−0.075	0.717	0.928	0.719–1.614

a significant negative relation (c: −0.466) between the Cyp17 genotype CC and the DHEAS concentrations of patients (p: 0.008) (Table 3). This indicates that carriers of the Cyp17 genotype CC have significant lower DHEAS levels than carriers of the Cyp17 genotype TT. Also a significant positive relation (c: 0.364) was observed between the carriership of the Cyp17 T-allele and the serum DHEAS levels (p: 0.024). Moreover, a significant negative relation (−0.299) is observed between carriers of the Cyp17 C-allele and serum concentration of DHEAS (p: 0.026) (Table 3).

After adjusting the linear regression model for the effects of gender and age on serum DHEAS levels no significant relationship was observed between the CYP17 genotype CC and the DHEAs concentrations of patients. Also no significant relationship was observed between carriership of the Cyp17 genotype TC and the DHEAs levels of the patients. Neither a significant relationship was present between carriership of the Cyp17 T-allele nor of the Cyp17 C-allele and the serum concentration of DHEAs.

4. Discussion

In the present study we investigated a possible association between the occurrence of TDsum, TDof and TDlt, a T34C polymorphism in the Cyp17 gene and circulating DHEAS levels in Slavonic Caucasians from Siberia. The same patient population was studied in three other investigations (Al Hadithy et al., 2009, 2010; Ivanova et al., 2012a). In the present study we observed that patients who are carriers of the Cyp17 genotype CC have less chance of developing TD compared to patients who are carriers of the Cyp17 genotypes TC or TT. This is in line with the expectation based on our hypothesis. This reduced risk of developing tardive dyskinesia in carriers of the CC genotype is not observable when the two forms of tardive dyskinesia, TDof and TDlt, are considered separately (Table 2). In addition, we observed that patients who are carriers of the Cyp17 genotype CC have significant lower circulating DHEAS levels compared to carriers of the Cyp17 genotype TT (Table 3). Conversely, carriers of the CYP17 T-allele have significant elevated DHEAS levels. When the variables age and gender were included into the calculations, the above described findings were no longer significant. However, the result was still nearly significant, so a strong indication about a negative association between the Cyp17 genotype CC and the DHEAs levels of these patients remains.

Our study has several limitations. Firstly, when we determined the distribution of the Cyp17 genotypes in the population, this distribution is not in consistency with the Hardy–Weinberg equilibrium (HWE). This could possibly be caused by the small sample size. However, it is also possible that there are other disturbing influences involved, like selection bias. In that case, one of the Cyp17 genotypes provides a reproductive advantage over the other genotypes. Deviations from the HWE

Table 3
Relationship between Cyp17 genotypes/allele types and DHEAs levels before and after correcting for variables age and gender.

DHEAS level	$Nvar_{(n)}/Ncom_{(n)}$	Estimate	p value	OR	CI
Genotypes					
CYP17 TC	$N_{TC}(44)/N_{TT}(46)$	−0.208	0.156	0.812	0.611–1.080
CYP17 CC	$N_{CC}(24)/N_{TT}(46)$	−0.466	**0.008**	0.628	0.447–0.882
T allele					
Cyp17 T	$N_T(90)/N_{CC}(24)$	0.364	**0.024**	1.439	1.054–1.966
C allele					
CYP17 C	$N_C(68)/N_{TT}(46)$	−0.299	**0.026**	0.742	0.572–0.962
Variables age and gender included					
CYP17A1 TC		−0.095	0.469	0.909	0.703–1.175
CYP17A1 CC		−0.304	0.056	0.737	0.541–1.005
Age		−0.017	<**0.001**	0.983	0.976–0.989
Gender		0.185	0.127	1.203	0.950–1.523
T allele					
Cyp17A1 T		0.255	0.076	1.290	0.976–1.707
Age		−0.018	<**0.001**	0.982	0.976–0.989
Gender		0.191	0.114	1.210	0.957–1.530
C allele					
CYP17A1 C		−0.166	0.168	0.847	0.669–1.071
Age		−0.016	<**0.001**	0.984	0.976–0.989
Gender		0.199	0.101	1.220	0.964–1.543

may also reflect genotyping errors. However, we have no reasons to assume that this is the case. A second limitation is related to the cross-sectional character of our study. We observed an association between the genotype CC and the prevalence of TD on one hand and lower DHEAS levels on the other hand. In both cases age and gender appears to influence the findings. Age and gender are important variables during multiple imputation and have an important influence on the results. Prospective stratified studies are needed to assess a possible causal relationship between CYP17 (T34C) genotypes and the development of TD. A third possible limitation would be the limited sample size for a genetic study. However, this is certainly not a common genetic study. What we tried to do in the present study is to develop a hypothesis and trying to falsify it by measuring the genetic make-up of a limited number of patients with respect to a level-determining enzymatic activity. Therefore, our study is not aimed at finding a gene that is associated with a certain disease, but to test a hypothesis concerning the potential role of a pharmacological mechanism in the development of dyskinesia. We hope that our work will inspire other groups to use the tool of genetic association study to test and try to falsify pharmacological hypotheses. It is a far more efficient than common association studies and a sound scientific procedure.

Another limitation is related to nature of the enzyme studied. Cytochrome P450c17 is a single enzyme with two activities: 17α-hydroxylase and 17,20-lyase (Miller, 2005). The first activity catalyzes the 17α-hydroxylation of pregnenolone and the second converts 17-OH-pregnenolone to DHEA. Initially, the observation that these two activities were regulated independently led to the mistaken belief that these were independent enzymes. However, the results of specific biochemical and genetic studies showed that there exists only a single P450c17 gene. In spite of this, the ratio of the 17,20 lyase to 17α-hydroxylase activities varies and determines the amount of C21 to C19 steroids produced. This leads to the conclusion that this ratio is regulated posttranslational and at least three mechanisms have been suggested to mediate these regulatory changes (Miller, 2005). This posttranslational regulation may at least partly explain the controversy about the clinical significance of the CYP17 (T34C) polymorphism. Considerable research efforts have for example been undertaken to demonstrate an association between this polymorphism and the risk of breast cancer (Chen and Pei, 2010). In this field the polymorphism is believed to enhance CYP17 transcription, and expression, which would then be associated with the increased estrogen levels and breast cancer risk. However, the results of mutual investigations into this hypothesis are conflicting. Several population characteristics have to be taken into account in order to be able to demonstrate an association between the CYP17 (T34C) polymorphism and the risk of breast cancer (Chen and Pei, 2010). Also in 259 women with polycystic ovary syndrome the CYP17 (T34C) polymorphism was not found to play a significant role in determining the circulating DHEAS levels in comparison to 161 control patients (Kahsar-Miller et al., 2004).

The possibility of posttranslational regulation may also disturb the relationship between the CYP17 (T34C) genotypes and DHEAS levels on one hand and between these genotypes and TD on the other. The measured DHEAS levels are too dependent upon the actual ratio of the 17,20 lyase to 17α-hydroxylase activities, and do not reflect the effective levels on the long term. Moreover, both pregnenolone (before) and DHEA (after CYP17 biotransformation) are also metabolized to other neuroactive steroids (progesterone, corticosterone, estradiol, testosterone), which could play an independent role.

In conclusion, we were not able to confirm our hypothesis that the CYP17 (T34C) polymorphism is contributing to the vulnerability to develop TD by increasing the neuroprotective effects of DHEA. However, we nevertheless observed an association between the CC carriership and TD, which was also independent upon age and gender. Probably the relationship between this polymorphism and neuroprotective effects of steroids is complex and cannot be elucidated without taking the posttranslational regulation of the enzyme into account.

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