Genetic variability at brain derived neurotrophic factor (BDNF) in opioid dependence

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Introduction

Drug induced adaptations in reward circuits have been described as a form of synaptic plasticity. Brain derived neurotrophic factor (BDNF)-signalling pathway have been shown essential for opiate-induced plasticity. A common single-nucleotide polymorphism (G196A) in the BDNF gene that results in a Val to Met substitution in the prodomain has been shown to affect intracellular trafficking and activity-dependent secretion of BDNF (Chen et al., 2004; Egan et al., 2003). In a recent study (Cheng et al, 2005) the BDNF-gene 66Met allele was less common in the methamphetamine- and heroin-dependent groups than in the control group (Cheng et al., 2005).

We conducted a case-control study in opioid-dependent patients and healthy controls to evaluate general BDNF variability in these subjects and its relation to Methadone Maintenance Therapy (MMT) response.

Material and Methods

Patients

We included 101 consecutive patients starting MMT and compared them to 46 healthy controls, gender and age matched. Sociodemographical data and characteristics of MMT were collected with a questionnaire designed "ad-hoc". Psychiatric comorbidity (DSM-IV) was assessed with the Psychiatric Research Interview for Substance and Mental Disorders (PRISM; Spanish version: Torrens et al., 2004). Personality was evaluated by the Cloninger's Temperament and Character Inventory (TCI; Spanish version Gutierrez et al., 2002). At 7-month follow-up, patients were divided into responders and non-responders to MMT, based on retention in treatment and illicit opioid use (in urine controls).

SNPs and genotyping description

- Gene: BDNF
- Genotyping method: SNPlex, Call rate 97%, conversion rate 63%, and no Mendelian errors
- SNPs: 44 SNPs across BDNF; 1 SNPs/ 6Kb; 86% of informative SNPs with MAF>10%

Genotyped SNPs with a call rate under 80% were not considered for the association analysis. Genotyping quality was cheeked in CEPH pedigrees. We tested deviations from HWE in the healthy control group using the exact test for HWE.

Inter-group comparisons of haplotypes were performed using haplotypes derived from the estimated htSNP from the entire data set. Analysis were undertaken in R software (R Foundation for Statistical Computing) using the gap and haplo.stats package and own written functions, reported P values are two-tailed.
Sample have enough power (1-b=0.8) to detect a highly-moderated RR=3.2 at a=0.01 for risk factors whit a frequencies >0.3 (Power calculator).
Differences in the Temperament and Character Inventory scores and social characteristics among groups were tested with a One-Way ANOVA test.

SNP redundancy reduction strategy

We perform an analysis of disequilibrium patterns in the region in order to reduce the SNP testing redundancy problem. We selected the TAG SNPs in two ways: i) based in structured data and inferred haplotypes (htSNPs), and ii) from non-structured data and based on correlation measures (TagSNPs).

Conclusions

1. Association analysis showed genotype differences for five markers in the BDNF gene, with statistical nominal differences between opiate-addicted-patients and healthy controls after adjusting for gender and TCI. Associated SNPs in genotype test have a high correlation (r^2>0.9) and this make difficult to find out what is the causal variant.
2. Haplotype analysis revealed a common haplotype associated to opiate addiction. This haplotype is the most frequent and contains the same associated allele as observed in genotype analysis (AAC, at *hcv1751795, rs7103873, rs2030324*). In addition the haplotype carrying the *Val66* allele at rs6265 position, similar to a recent report in a Han Chinese population of male opioid addicts (Cheng et al., 2005).
3. BDNF show an association to MMT response. Homozygous for the rare allele were more frequent in responders than in non-responders. This effect was even stronger in male subjects, but data on female group have not enough power to consider a gender effect.

Results

1. Patients characteristics and MMT Response

A total of 71 (70%) patients were responders to MMT and 30 were non-responders. In table 1 are described patients and controls characteristics.

Table 1. Characteristics of patient group (responders and non-responders) and control subjects. χ^2 test and One-way ANOVA.

	Patients Responders N=71 N(%)	Patients Non-Responders N=30 N(%)	Healthy controls N=46 N(%)	P
Males	67 (73.6)	32 (74.4)	34 (74)	NS
Caucasian origin	73 (95)	34 (92)	45 (98)	NS
Single	36 (47)	15 (42)	22 (47)	NS
Employed	25 (33)	12 (40)	38 (83)	<0.001Ψ
HIV Infection	32 (41)	7 (20)	none	0.034*
Psychiatric comorbidity	19 (26)	11 (34)	none	NS
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Age	37.68 ± 7.8	36.14 ± 8.1	33.6 + 7.9	0.021Ψ
School years	9.16 ± 2.5	8.25 ± 3.5	17.8 + 2.6	<0.001Ψ
Length heroin use (months)	134.32 ± 83.06	96.16 ± 52.4	ND	0.018*
Methadone dose (mg/day)	106.58 ± 70.64	73.59 ± 45.17	ND	0.016*

Ψ Significant differences between patients and controls.
**Significant differences between "responder" and "non-responder" patients.*

2. Cloninger's Temperament and Character Inventory

In table 2 are described mean and standar deviations scores of TCI. Differences in most of scales of TCI were found between patients and controls (*). Non-responders showed a higher score in "Cooperativeness" scale than responders (ψ).

Table 2. Temperament and Character Inventory scores. One-way ANOVA.

	Patients Responders N=71 Mean ± SD	Patients Non-Responders N=30 Mean ± SD	Healthy controls N=46 Mean ± SD	p
Harm avoidance	59.07 ± 10	55.97 ± 7.4	51.33 ± 7	<0.001*
Novelty seeking	53 ± 8	54.2 ± 9.9	50.39 ± 8	NS
Reward dependence	45.61 ± 9.7	46.2 ± 7.3	54.04 ± 7.43	<0.001*
Persistence	42.87 ± 9	43.57 ± 9.8	43 ± 9.1	NS
Self-directedness	40.82 ± 12	42.73 ± 8.9	57.57 ± 5.5	<0.001*
Cooperativeness	41.23 ± 8.3	45.33 ± 6.9	54.83 ± 5	<0.001*/ 0.02Ψ
Self-transcendence	42.18 ± 10	44.23 ±10.3	33.96 ± 8.8	<0.001*

** Significant differences between patients and controls.*
ψ Significant differences found in responders vs. Non-responders.

3. Genotype analysis

When compared genotype distribution under three models of action in patients and healthy controls we do not observe statistically significant differences (p>0.05). After adjusting the regression model for the observed differences in TCI scales, we observed five alleles that show statistical significance (p<0.05): *rs10767665, hcv1751795 rs2030324, rs7103873* and *rs7934165* (Table 3a). These differences persisted after adjusted for sex. The best model for all five SNPs was the one corresponding to a recessive model, being homozygotes for the rare allele more frequent in controls than in cases. Moreover *hcv1751795* was significant after correction for independent bins (p<0.009 /5).

Table 3a. Analysed alleles and its relation to patient/control status.

SNP	Dominant	Recessive	Overdominant
rs10767665	0.0972	0.0152	0.4318
rs10835211	0.6215	0.21	0.2693
rs11030099	0.5836	0.0515	0.0192
rs11030102	0.3297	0.4223	0.4465
rs11030109	NA	NA	NA
rs11030110	Monomorphic		
rs11030116	Monomorphic		
rs11030119	0.2258	0.5722	0.9037
hcv1751795	0.4033	0.0093	0.0776
hcv1751796	0.913	0.1552	0.1583
hcv1751799	Monomorphic		
hcv1751800	Monomorphic		
hcv1751802	0.9645	0.068	0.0603
hcv26878171	Monomorphic		
hcv9276624	NA	NA	NA
rs1967554	Monomorphic		
rs2030324	0.117	0.017	0.432
rs2049045	0.5808	0.4606	0.4203
rs2353512	Monomorphic		
rs3750934	Monomorphic		
rs6265	0.9248	0.1548	0.1265
rs7100873	0.05	0.0174	0.5789
rs7124442	0.0363	0.5719	0.753
rs7127507	0.0172	0.5437	0.7092
rs7934165	0.0952	0.0154	0.4446
rs7937405	Monomorphic		
rs7940166	NA	NA	NA
rs988748	0.4151	0.1909	0.8128

Specific response to MMT was also analysed. Significant differences were observed for four SNPs when recessive model of action was considered (p<0.01, after correction for 6 independent bins) (Table 3b). **Homozygous for the rare allele was more frequent in responders than in non-responders.**

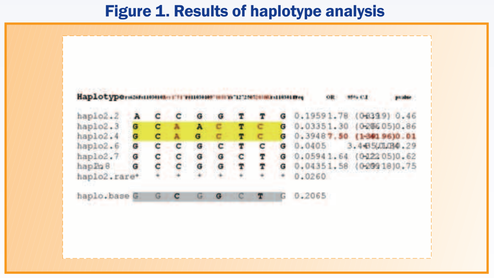
Table 3b. Analysed alleles and its relation to Responder/non-responder status.

SNP	Dominant	Recessive	Overdominant
rs10767665	0.7648	0.6697	0.0665
rs10835211	0.3662	0.2165	0.375
rs11030099	0.1969	0.1304	0.3546
rs11030102	0.26	0.3579	0.4407
rs11030109	NA	NA	NA
rs11030110	Monomorphic		
rs11030116	0.0274	0.3938	0.8992
rs11030119	0.3589	0.1868	0.7407
hcv1751795	0.388	0.1001	0.4189
hcv1751796	0.2144	0.1516	0.3713
hcv1751799	Monomorphic		
hcv1751800	Monomorphic		
hcv1751802	0.1927	0.2551	0.5736
hcv26878171	Monomorphic		
hcv9276624	NA	NA	NA
rs1967554	Monomorphic		
rs2030324	0.6817	0.006	0.0648
rs2049045	0.2954	0.2463	0.4747
rs2353512	Monomorphic		
rs3750934	Monomorphic		
rs6265	0.2573	0.51	0.67
rs7100873	0.939	0.6448	0.0812
rs7124442	0.8113	0.9306	0.9768
rs7127507	0.6515	0.9789	0.8543
rs7934165	0.5859	0.088	0.094
rs7937405	Monomorphic		
rs7940166	NA	NA	NA
rs988748	0.4151	0.1909	0.8128

4. Haplotype analysis

We observed two haplotypes carrying the ACC associated alleles at *hcv1751795*, *rs7100873* and *rs2030324* SNPs. When performed a regression analysis we observed that the common haplotypes was associated to opiate addiction but not the rare one. Both haplotypes carry the *Val66* allele at rs6265 but differs at *rs110030109* SNP. These results persisted after TCI and sex adjusting (Figure 1).

Figure 1. Results of haplotype analysis



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