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SHORT COMMUNICATION

The Val66Met polymorphism in the BDNF gene is associated with epilepsy in fragile X syndrome

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Summary The Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) gene may modulate the epilepsy phenotype. We investigated the impact of polymorphisms in the *BDNF* gene on clinical features in fragile X syndrome (FXS). In our study sample, the Met66 allele associated with epilepsy of finnish FXS men. Abnormalities in BDNF-mediated plasticity are shown in FXS and the present data suggest that the Met66 allele might predispose FXS males to epilepsy.
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Introduction

Brain-derived neurotrophic factor (BDNF) is a central mediator of neuronal plasticity in brain. Activity-dependent BDNF secretion is required for cellular mechanisms associated with learning and memory, including long-term potentiation (LTP) (Huang and Reichardt, 2001). BDNF can increase excitability of neurons and it is linked to diseases, such as epilepsy (Scharfman, 2005). A single-nucleotide polymorphism (SNP) in the human *BDNF* gene, which leads to a Methionine (Met) substitution for Valine (Val) at amino acid 66 in the prodomain of BDNF (Val66Met or Rs6265), interferes with the intracellular trafficking and the activity-dependent secretion of BDNF in cortical neurons (Egan et al., 2003; Chen et al., 2004). Frequency of the Met66 allele is around 20% in Caucasian population (Gratacòs et al., 2007). The Val66Met polymorphism has been associated with alterations in brain anatomy (Pezawas et al., 2004; Szeszko et al., 2005) and albeit conflicting results, with various neuropsychiatric disorders (Chen et al., 2006; Gratacòs et al., 2007;

Abbreviations: AED, anti-epileptic drug; BDNF, brain-derived neurotropic factor; CGG, triplet repeat, cytocine—guanine—guanine triplet repeat; EEG, electroencephalography; FMR1gene, fragile X mental retardation 1 gene; FMRP, fragile X mental retardation protein; LTP, long-term potentiation; SNP, a single-nucleotide polymorphism.

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Lanktree et al., 2008). Furthermore, the BDNF Met66 allele was recently shown to modulate the epilepsy phenotype in Rett syndrome (Nectoux et al., 2008).

Fragile X syndrome (FXS) [MIM 300624] is a common cause of inherited mental retardation and affects approximately 1 in 4000 males and 1 in 8000 females (see review Garber et al., 2008). The syndrome is characterized by intellectual disabilities, mild facial dysmorphology, macro-orchidism, and a distinct neurobehavioral phenotype, including hyperactivity, hyper-arousal, attention deficit, social anxiety, and autistic features. Epileptic seizures are seen in 13—44% of FXS individuals (Kluger et al., 1996; Musumeci et al., 1999; Sabaratnam et al., 2001; Berry-Kravis, 2002). The syndrome is typically caused by a CGG triplet repeat mutation, which leads to the transcriptional silencing of the *FMR1* gene and a reduction of fragile X mental retardation protein (FMRP) expression (for review see Jin and Warren, 2000). Studies of the mouse model for FXS revealed a role of BDNF in the pathogenesis of FXS (Castrén et al., 2002; Lauterborn et al., 2007) and led us to investigate the impact of the polymorphisms in the BDNF gene on the clinical phenotype of FXS.

Materials and methods

A total of 27 Finnish FXS males of the client register of the Pääjärvi Centre consented to participate in the study which was approved by the local Ethics Committee. The register covers intellectually disabled people living in the Inter-Municipal Association where the FXS diagnosis of 33 men (the prevalence of FXS 1/4100) was ascertained in a previous regional screening study (Arvio et al., 1997). The study protocol comprised clinical examination, collection of clinical data including intellectual and adaptive testing with standardized methods and genotyping analysis.

For genotyping, the DNA was extracted from EDTA-treated blood according to standard procedures (Blin and Stafford, 1976). The functional Val66Met (rs6265) and six other SNPs in the *BDNF* gene (rs2203877, rs11030102, rs11030108, rs6484320, rs1491850, and rs1491851) were utilized. The genotyping was performed using the homogenous Mass Extension (hME) reaction in the Sequenom MassARRAY System (Sequenom®, San Diego, CA) following the manufacturer's guidelines. The automated allele calling from Sequenom MassARRAY Typer software was further verified manually. The quality of the genotyping was controlled by empty water samples and nine duplicate DNA samples that were all congruent. In statistical analysis, independent two population Student's *t*-test and Fisher's exact test were used; the statistical significance was set at level $p \leq 0.05$.

Results

In the study sample of 27 FXS males, 23 (85%) were homozygotes for the *BDNF* Val66 allele (Val/Val), four were heterozygotes for the allele (15%) (Val/Met), and no homozygotes for the Met66 allele were found. Table 1 demonstrates clinical characteristics of the subgroups with the different haplotypes. The subgroups showed similar mental age, but the men bearing the Met66 allele showed poorer adaptive behaviour scores in the Vineland Adaptive Behavioral Scale (VABS) (Sparrow, 1984) than the men with the Val66Val genotype (67% lower scores, $p = 0.002$). All four men bearing the Met66 allele suffered from epilepsy whereas none of the subjects with the Val66Val genotype had experienced epileptic seizures ($p < 0.0001$). An intronic SNP in the *BDNF* gene (rs6484320) was also found to be associated with epilepsy in FXS individuals, while genotyping at SNPs in the *neurotrophic tyrosine kinase receptor type 2* gene did not show any correlations (data not shown).

Table 1 Clinical characteristics of the patients.

	Val66Val, $N = 23$	Val66Met, $N = 4$
Age range, years (median)	16—71 (32)	26, 36, 47, and 58
Weight (mean ± SD)	84 ± 27.9	94 ± 18.7
Height (mean ± SD)	177 ± 6.8	174.5 ± 4.6
Mental age, months (mean ± SD)		
Leiter (Roid and Miller, 1997)	55 ± 14.2	50 ± 20.0
Merril-Palmer (Stutsman, 1948)	53 ± 7.3	58 ± 12.0
Adaptive skills, months (mean ± SD)		
Vineland Adaptive Behavioral Scale (Sparrow et al., 1984)	98 ± 19.3	66 ± 15.6***
Epilepsy	0	4 (100%)

*** $p = 0.002$.

Epilepsy was diagnosed at the age of seven years in two men and at the age of 20—30 years in the other two Met66 allele carriers. One had anti-epileptic drug (AED) treatment continuously from the age of seven. The AED treatment of the three other men had been withdrawn after several seizure free years but restarted after relapse. At the time of the study, three men were seizure free on carbamazepine monotherapy and the seizures of one man were controlled by a combination therapy of oxcarbatsepine, topiramate and levetirasetame. All FXS individuals with epilepsy exhibited mild or moderate general slowing in electroencephalography (EEG) recordings. The FXS man with multiple AEDs had unilateral centrotemporal epileptiform changes in sleep EEG recording.

Discussion

In the present population based study, epilepsy was found in 15% of FXS individuals in accordance with the previous estimations for the prevalence of epilepsy and seizures in FXS at 13—44% (Kluger et al., 1996; Musumeci et al., 1999; Sabaratnam et al., 2001; Berry-Kravis, 2002). As seen in the present study, epileptic seizures in FXS show an age-related appearance in the childhood or young adulthood. Seizures are frequently of the complex partial type and involve the temporal and frontal lobes. Furthermore, epilepsy of FXS individuals responds usually well to carbamazepine treatment.

A Met66 allele of the *BDNF* gene was found in all FXS individuals with epilepsy at one of their chromosomes suggesting that this functional human polymorphism in combination with a mutation in the *FMR1* gene promotes epilepsy. Given that the frequency of the Met66 allele is about 20% in Caucasian population (Gratacòs et al., 2007) this polymorphism may account for a significant proportion of FXS individuals

with epilepsy. A low frequency of the Met66 allele (8%) in the present study in comparison to the allele frequency in general Finnish population (15%) might be a simple stochastic incident due to the small number of the examined individuals of the current study ($n = 27$). Alternatively, combination of FXS and the Met66Met genotype could be detrimental to health and affect survival. This hypothesis remains to be investigated in larger populations.

The Val66Met polymorphism is one of the most frequent polymorphisms of the BDNF gene. A Met substitution for Val at amino acid 66 in the prodomain of BDNF alters the protein function by interfering with the intracellular trafficking and the activity-dependent secretion of BDNF in cortical neurons (Egan et al., 2003; Chen et al., 2004). The second SNP (rs6484320) of the *BDNF* gene was also shown to be associated with epilepsy of FXS. This SNP is intronic and has not been shown to affect BDNF function. Its association with epilepsy may reflect strong linkage disequilibrium with functional Val66Met.

A recent study found no association between Val66Met polymorphism and serum BDNF levels in males (Zhang et al., 2008). Platelets are the primary storage place of BDNF in blood and similar mechanisms regulate vesicle trafficking in platelets and neurons (Lemons et al., 1997). However, molecular mechanisms of BDNF release from platelets are not yet well understood and it is currently unknown whether platelet BDNF is of mature or proform.

Various studies support the role of BDNF in epileptogenesis and epilepsy (Scharfman, 2005). Heterozygous *Bdnf* knockout mice display decreased seizure susceptibility whereas BDNF overexpression may predispose to seizures. In transgenic mice with a *Mecp2* gene deletion, a mouse model of Rett syndrome, BDNF overexpression enhances the electrophysiological activity of mutant neurons and alleviates progression of the disease (Chang et al., 2006). The Met66 allele of the *BDNF* gene, was recently shown to protect against early seizures of Rett patients with a missense mutation in the *MECP2* gene (Nectoux et al., 2008). BDNF can restore LTP impairment of the mouse model for FXS implicating BDNF also in the pathophysiology of plasticity changes in FXS (Castrén et al., 2002; Lauterborn et al., 2007). The present study suggests that interference with the activity-dependent intracellular trafficking of BDNF-containing vesicles to neuronal dendrites and spines by Met66 polymorphism of the *BDNF* gene can be of critical importance in the clinical appearance of epileptic seizures in the phenotype of FXS. The relationship between FMRP and BDNF, however, is complex and the cellular mechanisms by which the combined action of these molecules might influence susceptibility to epilepsy remains to be studied further.

Conflict of interest

None of the authors had competing interests.

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