The relationship between a common catechol-O-methyltransferase (COMT) polymorphism *val^{158}met* and fibromyalgia

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ABSTRACT

Objectives. *Fibromyalgia syndrome (FM) is an idiopathic chronic pain syndrome characterised by widespread nonarticular musculoskeletal pain, generalised tender points, in the absence of inflammatory or structural musculoskeletal abnormalities, accompanied by a constellation of symptoms that include fatigue and disturbances of sleep and mood. Catechol-O-methyltransferase (COMT) is the major catecholamine-clearing pathway and involved in the mediation of pain perception in humans, and the hypothesized role of pain perception in FM. The association between* Val/Met *polymorphism at the COMT gene was evaluated in FM disorder.*

Methods. *209 FM female patients were compared with 152 of their non-affected relatives. DNA was obtained from all family members and extracted. We used the logistic based variant of the transmission disequilibrium test to assess association (and linkage) without confounding effect of population stratification.*

Results. *We observed an association between FM and the COMT* val^{158}met *polymorphism in a dose response effect of the COMT genotype and the number of pressure points reported. We also observed that non-affected relatives of FM patients had a reduced percentage of the COMT met allele.*

Conclusions. *Our results are consistent with carriers of the COMT met/met genotype showing increased sensitivity to pain as one mechanism for the role of this gene in conferring risk for FM. We suggest that the reduced frequency of the met allele in the non-affected relatives acts as a 'protective' allele in this group and prevents the development of clinical FM.*

Introduction

Fibromyalgia syndrome (FM) is an idiopathic chronic pain syndrome characterised by widespread nonarticular musculoskeletal pain, generalised tender points, in the absence of inflammatory or structural musculoskeletal abnormalities, accompanied by a constellation of symptoms that include fatigue and disturbances of sleep and mood (1, 2). The FM syndrome causes functional disability in all affected age groups and adversely affects quality of life (3, 4). Fibromyalgia is estimated to affect 2% to 4% of the population and it is seen predominantly in women.

Although the etiology of fibromyalgia has yet to be understood (5), the current concept views the syndrome is the result of central nervous system malfunction, resulting in amplification of pain transmission and interpretation (6, 7), while a peripheral pathology responsible for pain generation is not considered essential for the development of this chronic and debilitating disorder.

In a recent review, Buskila has (8) discussed the evidence that there is a common association of FM with other rheumatic and systemic illnesses. Patients with FM were found to have an impaired ability to activate the hypothalamic pituitary portion of the hypothalamic pituitary adrenal axis as well as the sympathoadrenal system. The basal autonomic state of patients with FM is characterised by increased sympathetic and decreased parasympathetic tones (9) and this autonomic dysregulation may have implications regarding the symptomatology, physical and psychological aspects of health status. To summarise, neurohormonal, neurochemical and endocrine factors (10, 11) as well as pain mechanisms (12, 13) have all been implicated in the etiology of this disorder.

Family clustering of FM is commonly observed although few specific genetic factors have been reported (14-17).

Buskila and his colleagues demonstrate that relatives of patients with FM have a higher prevalence of FM and are more tender than the general population, as recently reported and shown in a healthy control group. Family aggregation of FM has led to the notion that genetic factors play a role in this disorder and several candidate gene studies have been reported (14-17).

Strong interest exists for the discovery of genes that cause individual differences in responses to physical and environmental challenges. In the case of pain, both sensitivity and inhibition are traits that vary considerably among individuals, with some of the variability being attributed to genetic factors (18). However, the influence of genes on regulatory processes in the human brain is particularly difficult to resolve. A functional genetic variant may affect not only the protein coded by the gene in question but may also have downstream effects contributing to the overall system response. Furthermore, differences in human resiliency and stress responses determine individual vulnerabilities to many psychiatric and other complex diseases.

Cathechol-O-methyl transferase (COMT) is one of the enzymes that metabolise catecholamines, thereby acting as a key modulator of dopaminergic and adrenergic/noradrenergic neurotransmission. There is a common functional polymorphism of the catechol-O-methyltransferase (COMT) gene that codes the substitution of valine (*val*) by methionine (*met*) at codon 158 ($val^{158}met$) (19). This substitution is associated with a difference in thermostability leading to a three- to four-fold reduction in the activity of the COMT enzyme. The alleles are codominant so that individuals with the *val*/*val* genotype have the highest activity of COMT, those with the met/met genotype have the lowest activity of COMT, and heterozygous individuals are intermediate. The $val^{158}met$ genotypes have been linked to a number of behavioural diseases of complex etiology.

Zubieta *et al*. (20) examined the influence of a common functional genetic polymorphism affecting the metabolism of catecholamines on the modulation of responses to sustained pain in humans. They hypothesized that different levels of COMT activity conferred by $val^{158}met$ genotypes may then have important influences on functions regulated by these neurotransmitters, including μ-opioid system responses.

In the study of Diatchenko and colleagues (21) of healthy female volunteers, SNP rs4818 accounted for 7% of pain sensitivity, SNP rs6269 for 6%, and SNP rs4680 ($Val\text{-}^{158}\text{-}Met$) showed only a marginal relationship with pain sensitivity. In a sample of Spanish patients, Vargas-Alarcon *et al*. (22) reported an association between FM and the COMT haplotype previously associated with high pain sensitivity.

In a study analysing 61 fibromyalgia patients and 61 healthy controls, the presence of polymorphisms in the COMT genes was determined (23). Three polymorphisms of the COMT gene were studied, termed LL, LH and HH. In this study the combination of the LH and LL genotypes were more highly represented in patients compared to controls, while the HH genotype was less frequent among patients than among controls. No association was demonstrated between the COMT polymorphisms and psychiatric status. The authors concluded that the polymorphisms of the COMT gene may be of importance in fibromyalgia both regarding the pathogenesis and the pharmacogenetic response to catecholamine-like medications. Tander *et al*. (24) also reported that the allele frequencies of *COMT* gene were not different between patients and controls (24).

In animal models, the chronic activation of dopaminergic neurotransmission and D2 receptors, a situation parallel to that encountered in *met*/*met* homozygotes, reduces the neuronal content of enkephalin peptides and induces compensatory increases in regional μ-opioid receptor concentrations in various brain regions (25). Reductions in D2 receptor-mediated neurotransmission, similar to that achieved by the higher levels of COMT activity in *val*/*val* homozygotes, results in opposite effects on the μ-opioid system (26). Therefore, it can be hypothesized that chronic over activity of the dopaminergic system induced by the low-function *met*/*met* COMT enzyme would be associated with a lesser capacity to activate μ-opioid neurotransmission under provocation conditions by virtue of a lower neuronal content of enkephalin. Compensatory increases in μ-opioid receptor binding should also be observed under these circumstances. The authors hypothesized intermediate effects in heterozygous individuals, while the presence of the higher metabolic capacity of the *val*/*val* COMT genotype would be associated with higher enkephalin content, a superior capacity to activate μ-opioid neurotransmission and possibly compensatory reductions in receptor binding levels.

To summarise, Zubieta *et al*. (20) found that individuals homozygous for the met allele of the catechol-O-methyltransferase (COMT) polymorphism ($val^{158}met$) showed diminished regional μ-opioid system responses to pain compared with heterozygotes in imaging studies. These effects were accompanied by higher sensory and affective ratings of pain and a more negative internal affective state. Opposite effects were observed in val homozygotes. The COMT $val^{158}met$ polymorphism thus influences the human experience of pain and may underlie inter-individual differences in the adaptation and responses to pain and other stressful stimuli.

Prompted by the Zubieta *et al*. study (20) that COMT is involved in the mediation of pain perception in humans, and the hypothesized role of pain perception in FM, the aim of the current study was to examine the role of COMT in FM disorder.

Methods

We recruited altogether 209 FM patients and 152 of their non-affected relatives for a study designed to examine the role of COMT in this disorder. Subjects were diagnosed as having FM if they fulfilled the currently accepted criteria of the American College of Rheumatology (2). We enrolled female patients with FM (and their non-affected relatives) attending the Rheumatology Outpatient Clinic at the Soroka University Hospital, Beersheba, and other locations in Israel.

Table I. Demographic background and health behaviour measures of patients with FM and relatives without.

	FM patients n=209	Relatives without FM n=152	Statistic
Age (years) Range	48.5 ± 11.7 20 – 76	48.4 ± 14.4 20 – 76	NS
Education (years) Range	12.1 ± 14.1 0 – 25	12.9 ± 3.3 0 – 25	NS
Professional status: Working (%)	68.8%	77.6%	NS
Marital status Married (%)	89.6%	86.6%	NS
Disease duration (years) Range	9.1 ± 8.8 (0.5 – 52)		
Physical functioning (!–10 scale, 10=worst)	9.1 ± 8.8		

Table II. Association between FM and catechol-O-methyltransferase *val*^{158}*met* polymorphism using UNPHASED (Cocaphase).

			Cocaphase (FM) – LRS = 8.55217 DF = 1 *p*=0.0034				
Allele	Case	frequency	Control	frequency	Odds ratio	Chi-sq	*p*-vaue
val	210	0.502	186	0.61	1	8.552	0.0034
met	208	0.497	118	0.39	1.561	8.552	0.0034

LRS: likelihood ratio statistic; DF: degrees of freedom.

Table III. Cross tabulation of *val*^{158}*met* allele frequency between FM and control relatives (SPSS).

		COMT		Total
		VAL	MET	
Controls	Count	186	118	304
	%	61.18	38.82	100
FM	Count	210	208	418
	%	50.24	49.76	100
Total	Count	396	326	722

Chi-Square Tests	Value	df	*p*-value (2-sided)	Exact sig. (2-sided)	Exact sig. (1-sided)
		1.000	0.004		
Pearson chi-square	8.513	1.000	0.004		
Continuity correction(a)	8.077	1.000	0.003		
Likelihood ratio	8.552			0.004	0.002
Fisher's exact test	1.000	0.004			
Linear-by-linear association	8.502				
N. of valid cases	722				

Exclusion criteria consisted of current or recent substance abuse disorders, psychotic symptoms, and significant cognitive impairment likely to interfere with study procedures or with informed consent. All participants gave written informed consent after receiving a detailed explanation of the purpose and design of the study.

The study was approved by the Ministry of Health Genetic Section and the Soroka Hospital IRB committee.

DNA extraction and genotyping

DNA was obtained from all family members and extracted by Master Pure kit (Epicentre, Madison WI). SNPs were assayed using an ABI SNaPshot kit and the products analyzed in an ABI 310 DNA analyzer. The primers for the first and second PCR reactions are shown below. A ReddyMix master mix was used (Abgene, Surrey, UK) at a magnesium concentration of 1.5-2.5 mM MgCl2. The first PCR reaction was carried out as follows. The sample was initially heated at 95ºC for 5' followed by 35 cycles of 95ºC (30 seconds), 55ºC (30 seconds), 72ºC (90 seconds) and a final extension step of 72ºC for 10 minutes. The second reaction was carried out according to the instructions in the ABI SNaPshot kit.

For COMT, the following primers were used in the first reaction: F - 5' ACTGTGGCTACTCAGCTGTG 3' and R- 5' CCTTTTTCCAGGTCTGACAA 3'. In the second reaction (probe) the following primer was used: 3' TGCACACCTTGTCCTTCA 3'.

Statistical methods

We also used the logistic based variant of the transmission disequilibrium test (TDT) so-called ETDT (27) to assess association (and linkage) without confounding effect of population stratification. The TDT, in its simplest version, compares, for one allele, the number of times this allele is transmitted to the number of times where this allele is not transmitted to an affected offspring. Note that only heterozygous parents are informative. This approach can be extended to haplotypes.

The various tests we used are implemented in the program *UNPHASED* (http://www.rfcgr.mrc.ac.uk/~fdudbrid/software/unphased/). UNPHASED (28) is a suite of programs for association analysis of multilocus haplotypes from unphased genotype data. UNPHASED currently includes the following programs: UNPHASED: graphical front end to the analysis programs; TDTPHASE: TDT and HHRR analysis for nuclear families; COCAPHASE: case/control data; QTPHASE: quantitative trait in unrelateds; PDTPHASE:

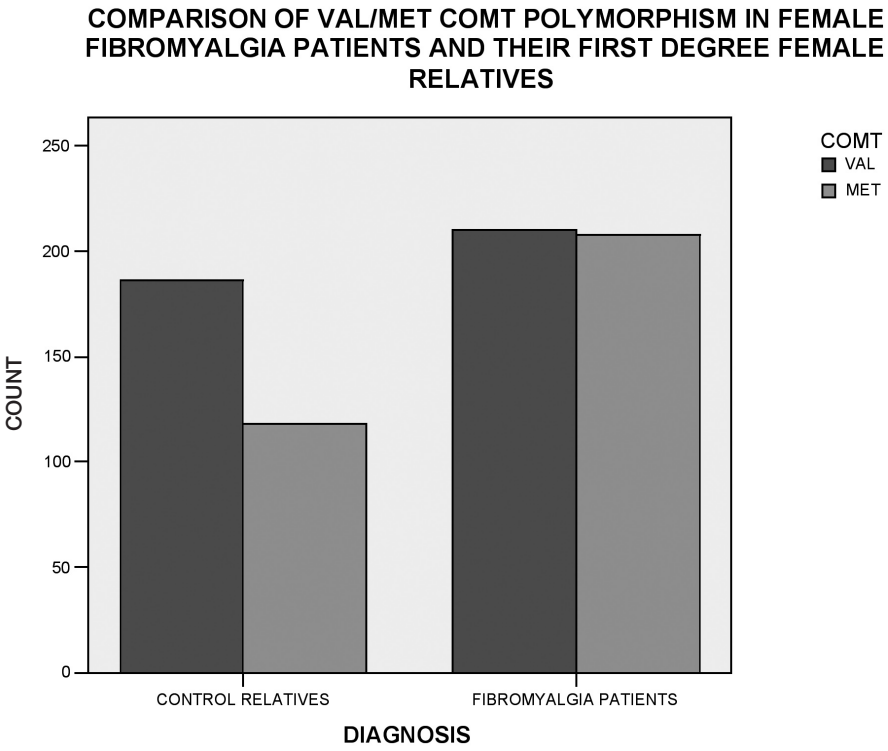

Fig. 1. Comparison of val158met polymorphism in FM patients and female first degree relatives.

Table IV. Test of association between COMT *val*^{158}*met* genotype and number of tender points (SPSS) in FM patients.

Tests of Between-Subjects Effects

Dependent variable: Tender points

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	228.8703	2	114.435	3.493	0.032
Intercept	11925.69	1	11925.686	363.972	0.000
COMT	228.8703	2	114.435	3.493	0.032
Error	6323.716	193	32.765		
Total	19241	196			
Corrected total	6552.587	195			

AR Squared = 0.035 (Adjusted R Squared = 0.025).

pedigree disequilibrium test; QPDT-PHASE: quantitative trait PDT.

Results

Demographic data of the subjects are summarised in Table I. The two groups, FM patients and relative without FM, did not differ significantly on any of the demographic measures.

We first examined association between FM and COMT using the Cocaphase (population based analysis) routine in the UNPHASED program. As shown in Table II there is significant association between COMT and FM. Figure 1 shows the count of the *val*^{158}*met* SNP in the case (FM) and control (female relatives). Although there is no difference between the percentage of *val* versus *met* SNPs in the patients and their unaffected relatives, there is a significant decrease in the percentage of met allele in the control relative group. Similar results were obtained when the data was analysed using SPSS (Table III).

We also examined the relationship between the number of pressure points, core diagnostic criteria for FM, and COMT genotype (Fig. 2).

Significant association was observed both by SPSS ANOVA analysis (p=0.032) for genotype and by UNPHASED (Qtphase: p=0.003). Carriers of the met/met genotype showed an increased number of pressure points compared to val/val genotypes (Tables IV, V).

Discussion

The results from the current study are consistent with and extend a single previous investigation in a group of Turkish patients (29). Although no significant difference was found between *met*/*met* and *met*/*val* separately, the *met*/*met* and *met*/*val* genotypes together were more highly represented in patients than controls (p=0.024). In addition, *val*/*val* genotypes in patients were significantly lower than in the control groups (p=0.04).

In the current investigation we also observe an association between the COMT *val*^{158}*met* polymorphism and FM. However, in comparison to the Turkish study we used as a control group non-affected relatives of the FM patients. We observed that non-affected relatives of FM patients had a reduced percentage of the COMT *met* allele. We theorise that the reduced frequency of the *met* allele in the non-affected relatives acts as a 'protective' allele in this group and prevents the development of clinical FM. It is a reasonable notion that the first degree relatives of FM patients share many of the risk genes in common with their affected relatives raising the question why don't they develop the full blown syndrome? Our results suggest an explanation-since they have reduced frequencies of the *met* allele they are less sensitive to pain and therefore do not present to rheumatology clinics. Interestingly, we do observe a dose response effect of the met allele on the number of pressure points observed in the subjects studied in the current report. Subjects with the met/met combination show the greatest number of pressure points.

Not all investigations reported positive associations between COMT and pain.

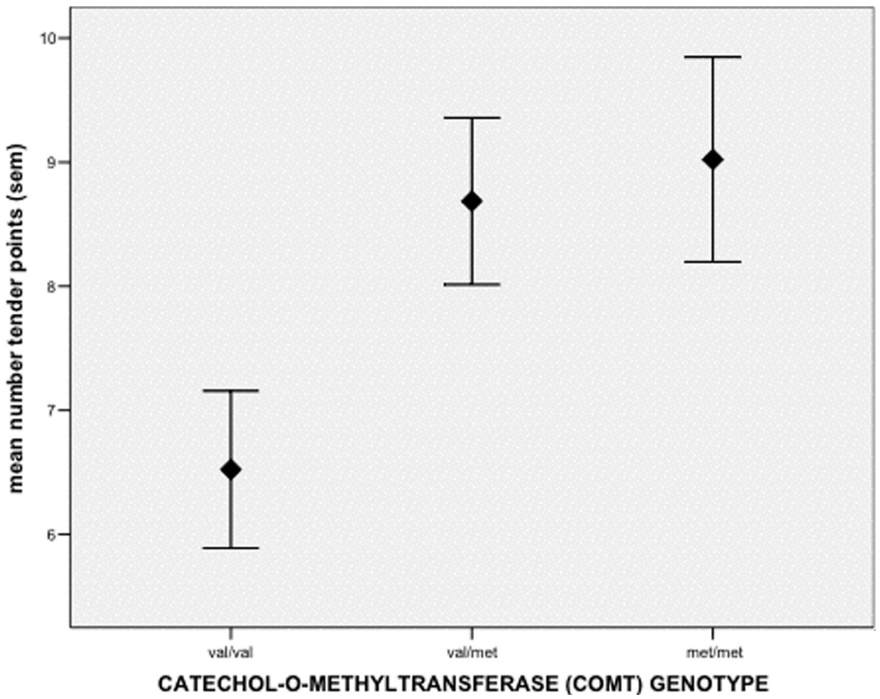

Fig. 2. The relationship between the number of pressure points and COMT genotype.

Table V. Test of association between COMT and number of tender points (UNPHASED; Qtphase) in FM patients.

LRS=8.70024 DF=1 p=0.003

Allele	Count	Frequency	Mean	Variance	Chi-sq	p-value
val	151	0.555	6.954	31.290	8.700	0.003
met	121	0.445	8.992	31.290	8.700	0.003

For example, Kim *et al*. (30) using cold pressor and heat sensitivity found an association between the vanilloid receptor subtype 1 gene (*TRPV1*) in female European Americans with the *TRPV1* $val^{585}val$ allele and males with low harm avoidance showed longer cold withdrawal times based on the classification and regression tree (CART) analysis. CART identified gender, an *OPRD1* polymorphism and temperament dimensions of personality as the primary determinants of heat pain sensitivity at 49°C. However, no association was observed with the COMT val158met polymorphism. This inconsistency between this study and that of Zubieta *et al*. (20) may be attributable to the different characteristics of applied stimuli (cutaneous thermal stimuli vs. hypertonic saline injection). Furthermore, the painful stimuli used in Kim *et al*. (30) study were acutely induced for a short duration compared to the long sustained stimuli in the previous study and clinical pain evidenced in the FM syndrome. This discrepancy is consistent with that pain sensitivities in mice are reported to be genetically dissociable phenomena dependent on the characteristics (duration, inflammation, nerve injury, etc.) of applied stimuli (31).

Rakvig *et al*. (32) analysed the influence from the COMT $val^{158}met$ polymorphism on the efficacy of morphine in a cohort of patients suffering from cancer pain. They genotyped 207 Caucasian cancer patients on morphine treatment with respect to the $val^{158}met$ polymorphism and compared the morphine doses, serum concentrations of morphine and morphine metabolites between the genotype groups. Patients with the *val/val* genotype needed more morphine when compared to the val/met and the *met/met* genotype groups. This difference was not explained by other factors such as duration of morphine treatment, performance status, time since diagnosis, perceived pain intensity, adverse symptoms, or time until death. The observation that cancer patients with the *met/met* genotype needed lower doses of morphine in order to relieve pain compared with patients with the *val/val* genotype is intriguing as individuals with the *met/met* genotype also had low tolerance to pain (20). One possible explanation for the observation of lower morphine requirements in cancer pain patients with the met/met genotype could be that an increase of μ-opioid receptor density causes morphine to be more effective in individuals carrying this genotype. Indeed, Zubieta *et al*. (20) observed increased μ-opioid binding in met/met homozygotes.

Finally, we note a Spanish study which examined the COMT $val^{158}met$ polymorphism in neuropathic pain and found no evidence for association (33).

To summarise, we observe an association between FM and the COMT $val^{158}met$ polymorphism which is primarily due to a reduction in the percentage of met alleles in the non-affected relatives of the FM patients. We also observed a dose response effect of the COMT genotype and the number of pressure points reported by the subjects in this study (including patients and non-ill relatives). Overall, our results are consistent with carriers of the COMT *met/met* genotype showing increased sensitivity to pain as one mechanism for the role of this gene in conferring risk for FM. Intriguingly, in this group of FM patients and their first degree relatives the role of the COMT met allele (due to its relative reduction in relatives) is to 'protect' non-affected relatives from developing full blown symptomatology of FM.

References

1. BELT NK, KRONHOLM E KAUPPI MJ: Sleep problems in fibromyalgia and rheumatoid arthritis compared with the general population. *Clin Exp Rheumatol* 2009; 27: 35-41.
2. WOLFE F, ROSS K, ANDERSON J, RUSSELL I, HEBERT L: The prevalence and characteristics of fibromyalgia in the general population. *Arthritis Rheum* 1995; 38: 19-28.
3. DA COSTA D, DOBKIN PL, FITZCHARLES MA *et al*.: Determinants of health status in fibro-

myalgia: a comparative study with systemic lupus erythematosus. *J Rheumatol* 2000; 27: 365-72.
4. MAS AJ, CARMONA L, VALVERDE M, RIBAS B: Prevalence and impact of fibromyalgia on function and quality of life in individuals from the general population: results from a nationwide study in Spain. *Clin Exp Rheumatol* 2008; 26: 519-26.
5. BAIO P, BRUCATO A, BUSKILA D *et al.*: Autoimmune diseases and infections: controversial issues. *Clin Exp Rheumatol* 2008; 26: 74-80.
6. GRACELY R: Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. *Arthritis Rheum* 2002; 46: 1333-433.
7. STAUD R: Abnormal sensitization and temporal summation of second pain (wind-up) in patients with fibromyalgia syndrome. *Pain* 2001; 91: 165-75.
8. BUSKILA D: Fibromyalgia, chronic fatigue syndrome, and myofascial pain syndrome. *Curr Opin Rheumatol* 2000; 12: 113-23.
9. COHEN H, NEUMANN L, SHORE M, AMIR M, CASSUTO Y, BUSKILA D: Autonomic dysfunction in patients with fibromyalgia: application of power spectral analysis of heart rate variability. *Semin Arthritis Rheum* 2000; 29: 217-27.
10. DESSEIN PH, SHIPTON EA, STANWIX AE, JOFFE BI: Neuroendocrine deficiency-mediated development and persistence of pain in fibromyalgia: a promising paradigm? *Pain* 2000; 8: 6213-215.
11. NEECK G, CROFFORD LJ: Neuroendocrine perturbations in fibromyalgia and chronic fatigue syndrome. *Rheum Dis Clin North Am* 2000; 26: 989-1002.
12. EDWARDS RR, BINGHAM CO, 3RD, BATHON J, HAYTHORNTHWAITE JA: Catastrophizing and pain in arthritis, fibromyalgia, and other rheumatic diseases. *Arthritis Rheum* 2006; 55: 325-32.
13. MENSE S: Neurobiological concepts of fibromyalgia--the possible role of descending spinal tracts. *Scand J Rheumatol* 2006; (Suppl.): 11324-9.
14. BUSKILA D, NEUMANN L: Fibromyalgia syndrome (FM) and nonarticular tenderness in relatives of patients with FM. *J Rheumatol* 1997; 24: 641-4.
15. BUSKILA D, NEUMANN L: Genetics of fibromyalgia. *Curr Pain Headache Rep* 2005; 9: 313-5.
16. BUSKILA D, NEUMANN L, HAZANOV I, CARMI R: Familial aggregation in the fibromyalgia syndrome. *Semin Arthritis Rheum* 1996; 26: 605-11.
17. BUSKILA D, NEUMANN L, PRESS J: Genetic factors in neuromuscular pain. *CNS Spectr* 2005; 10: 281-4.
18. MACGREGOR AJ, ANDREW T, SAMBROOK PN, SPECTOR TD: Structural, psychological, and genetic influences on low back and neck pain: a study of adult female twins. *Arthritis Rheum* 2004; 51: 160-7.
19. LACHMAN HM, PAPOLOS DF, SAITO T, YU YM, SZUMLANSKI CL, WEINSHILBOUM RM: Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics* 1996; 6: 243-50.
20. ZUBIETA JK, HEITZEG MM, SMITH YR *et al.*: COMT *val*^{158}*met* genotype affects mu-opioid neurotransmitter responses to a pain stressor. *Science* 2003; 299: 1240-3.
21. DIATCHENKO L, SLADE GD, NACKLEY AG *et al.*: Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Hum Mol Genet* 2005; 14: 135-43.
22. VARGAS-ALARCON G, FRAGOSO JM, CRUZ-ROBLES D *et al.*: Catechol-O-methyltransferase gene haplotypes in Mexican and Spanish patients with fibromyalgia. *Arthritis Res Ther* 2007; 9: R110.
23. GURSOY S EE, HERKEN H, MADENCI E, ALASEHIRLI B, ERDAL N: Significance of catechol-O-methyltransferase gene polymorphism in fibromyalgia syndrome. *Rheumatol Int* 2003; 23: 104-7.
24. TANDER B, GUNES S, BOKE O *et al.*: Polymorphisms of the serotonin-2A receptor and catechol-O-methyltransferase genes: a study on fibromyalgia susceptibility. *Rheumatol Int* 2008; 28: 685-91.
25. UNTERWALD EM; CUNTAPAY M: Dopamine-opioid interactions in the rat striatum: a modulatory role for dopamine D1 receptors in delta opioid receptor-mediated signal transduction. *Neuropharmacology* 2000; 39: 372-81.
26. KING MA, BRADSHAW S, CHANG AH, PINTAR JE, PASTERNAK GW: Potentiation of opioid analgesia in dopamine2 receptor knock-out mice: evidence for a tonically active anti-opioid system. *J Neurosci* 2001; 21: 7788-92.
27. SHAM PC, CURTIS D: An extended transmission/disequilibrium test (TDT) for multi-allele marker loci. *Ann Hum Genet* 1995; 323-36.
28. DUDBRIDGE F: Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol* 2003; 25: 115-21.
29. GURSOY S, ERDAL E, HERKEN H, MADENCI E, ALASEHIRLI B, ERDAL N: Significance of catechol-O-methyltransferase gene polymorphism in fibromyalgia syndrome. *Rheumatol Int* 2003; 23: 104-7.
30. KIM H, NEUBERT JK, SAN MIGUEL A *et al.*: Genetic influence on variability in human acute experimental pain sensitivity associated with gender, ethnicity and psychological temperament. *Pain* 2004; 109: 488-96.
31. LARIVIERE WR, WILSON SG, LAUGHLIN TM *et al.*: Heritability of nociception. III. Genetic relationships among commonly used assays of nociception and hypersensitivity. *Pain* 2002; 97: 75-86.
32. RAKVAG TT, KLEPSTAD P, BAAR C *et al.*: The Val158Met polymorphism of the human catechol-O-methyltransferase (COMT) gene may influence morphine requirements in cancer pain patients. *Pain* 2005; 116: 73-8.
33. ARMERO P, MURIEL C, SANTOS J, SANCHEZ-MONTERO FJ, RODRIGUEZ RE, GONZALEZ-SARMIENTO R: COMT (*Val*^{158}*Met*) polymorphism is not associated to neuropathic pain in a Spanish population. *Eur J Pain* 2005; 9: 229-32.