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Genetic Analysis of Matrix Metalloproteinases Gene Polymorphisms in Patients with Hereditary Connective Tissue Diseases

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Abstract

Background: Hereditary connective tissue diseases belong to a heterogeneous group of genetic conditions, associated with the pathology of extracellular matrix fibers. Signs of weakness in the connective tissue are diagnosed in 10—30% of the population, although they do not meet the strict criteria of genetic syndromes, such as Marfan's Syndrome. The genetic defect of these patients is unknown in most cases. **Methods:** Volunteers with signs of weakness of the connective tissue (n=82) and apparently healthy ones (n=70) were included and examined. The frequency of polymorphisms in the genes MMP1 (1607insG), MMP9 (C-1562T), MMP12 (A-82G), TIMP1 (S536T) were analyzed to identify alleles and genotypes associated with hereditary connective tissue diseases. **Results:** The frequency of the mutant allele GG/GG of the MMP1 and mutant allele T of the MMP9 were significantly longer in patients with signs of weakness of the connective tissue. No statistically significant differences were noted in the genes MMP12 and TIMP1 between treatment groups. **Conclusion:** The data in this study confirmed the need for further research to identify the genetic basis of inherited disorders of the connective tissue, for early screening and prevention. IJBM 2011; 1(3): 150-152.

Key words: *matrix metalloproteinases, connective tissue.*

Introduction

Hereditary connective tissue diseases (HDCT) belong to a heterogeneous group of genetic conditions associated with the pathology of extracellular matrix fibers (collagen, elastin, etc.). Marfan syndrome is the most studied representative of this group. Heterogeneity manifestations of Marfan syndrome, Ehlers-Danlos syndrome and Joint Hypermobility syndrome are widely discussed in the literature and formed the basis of the hypothesis of «phenotypic continuum», at one pole of which are sporadic signs of hereditary connective tissue diseases, and at the other, clinically delineated genetic syndromes [1, 2]. According to Russian authors, the prevalence of the HDCT in the Russian population varies from 10 to 30% [3]. These patients express several complaints, but have had no diagnosis [4]. It was assumed that the reason for Marfanoid phenotype formation could be other genes, responsible for metabolism of the connective tissue [5]. In this study the genes of the matrix metalloproteinases (MMP) attracted attention. These are a family of zinc-containing proteins with a specific proteolytic activity against the components of the extracellular matrix [6]. Tissue inhibitors of metalloproteinases (TIMP) are endogenous regulators of MMP. The frequency of polymorphisms were analyzed in the genes MMP1 (1607insG), MMP9 (C-1562T), MMP12 (A-82G), TIMP1 (S536T) to identify alleles and genotypes associated with HDCT.

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Study design

The study sample included 152 unrelated healthy volunteers (age range from 18 to 40 years). The patients were selected between July 2007 and July 2010. The study group comprised 82 people with signs of HDCT, whereas the control group included 70 people without HDCT. HDCT was diagnosed based on the presence of pectus carinatum, pectus excavatum, span to height>1.05, wrist and thumb signs, scoliosis>20°, spondylolisthesis, elbow extension<170°, pes planus, protrusio acetabulae, hypermobility of the joints, cranial deformations, high palate, lumbosacral dural ectasia, ectopia lentis, myopia, iris hypoplasia, aortic root dilatation, aorta dissection, mitral valve prolapse, atrophic stria, and hernias. There were a minimum of two signs associated with asthenic habitus to diagnose HDCT. Not isolated large and small criterion, as compared with Marfan syndrome. Patients were not related to one another and were matched by sex, age and ethnicity. The study protocol was approved by the Regional Ethics Committee.

Genotype determination and statistical analysis

Genomic DNA was extracted from the blood samples of the cases and controls, employing the «salting out» method. Using the isolated genomic DNA as template, polymerase chain reactions were carried out, followed by restriction fragment analyses to determine single nucleotide polymorphisms by horizontal electrophoresis in 3% agarose gel with ethidium bromide. Deviation from the Hardy-Weinberg equilibrium proportions was tested for each genetic marker using Fisher's exact test. The c^2 test with Yates correction was used to compare the genotype and allele frequencies between groups. The strength of the association of the genotypes and alleles with the disease was assessed by the values of the exponent in the odds ratio (OR), and 95% confidence intervals (CI) were calculated. The critical value of significance level (p) was assumed to be 5%.

Results

The results of the analysis of the polymorphic loci of the genes of the matrix metalloproteinases and their inhibitor are presented in Table 1.

Comparative analysis of the frequencies of the genotypes and alleles of the genes studied in patients with primary SP revealed several associations.

The frequency of the mutant homozygous genotype MMP1 GG/GG was significantly longer in patients with HDCT. Allele GG was associated with a three-fold increased risk of producing signs of HDCT (OR=3.87, 95% CI: 2.32-6.45). Similar results were obtained with regard to heterozygous and homozygous carriers of the mutant allele T of the gene MMP9, which is associated with a four-fold increased risk of producing symptoms of HDCT (OR=4.41, 95% CI: 1.56-13.0) and occurs in cases without HDCT, only in isolated cases. No statistically significant differences between the treatment groups were noted, with respect to the genes MMP12 and TIMP1.

Table 1.
The frequencies of genotypes and alleles of the polymorphic loci of genes MMP1 (1607insG), MMP9 (C-1562T), MMP12 (A-82G), TIMP1 (C536T).

Genotypes and alleles	HDCT positive (n=82)		HDCT negative (n=70)		χ^2 df=1	p	OR (95% CI)
	n	% (95% CI)	n	% (95% CI)			
MMP1 (-1607insG)							
G/G	10	12.2 (6.0-21.3)	41	58.6 (46.1-70.2)	34.3	0.0005	0.09 (0.04-0.23)
G/GG	43	52.4 (41.6-63.2)	17	24.3 (14.8-36.0)	11.3	0.0015	3.43 (1.62-7.34)
GG/GG	29	35.4 (25.1-46.7)	12	17.1 (9.1-28.0)	5.4	0.019	2.64 (1.15-6.14)
G	63	38.4 (30.9-46.3)	99	70.7 (62.4-78.0)	30.3	0.0005	0.25 (0.15-0.43)
GG	101	61.6 (53.7-69.0)	41	29.3 (21.9-37.6)			3.87 (2.32-6.45)
MMP9 (C-1562T)							
C/C	58	70.7 (59.6-80.2)	64	91.4 (82.3-96.8)	8.9	0.0037	0.22 (0.07-0.63)
C/T + T/T	24	29.3 (19.7-40.3)	6	8.6 (3.2-17.7)			4.41 (1.56-13.0)
C	137	83.5 (76.9-88.9)	134	95.7 (90.9-98.4)	10.3	0.0021	0.22 (0.08-0.60)
T	27	16.5 (11.1-23.0)	6	4.3 (1.5-9.0)			4.40 (1.66-12.3)
MMP12 (A-82G)							
A/A	55	67.1 (55.8-77.0)	54	74.2 (62.4-83.9)	1.42	0.233	0.60 (0.27-1.31)
A/G	20	24.4 (15.6-35.1)	12	17.1 (9.1-28.0)	0.79	0.372	1.55 (0.65-3.74)
G/G	7	8.5 (3.5-16.8)	4	5.7 (1.5-13.9)	0.12	0.720	1.54 (0.38-6.59)
A	130	79.3 (72.2-85.1)	120	85.7 (78.8-91.0)	1.73	0.189	14.6 (8.29-25.9)
G	34	20.7 (14.8-27.7)	20	14.3 (8.9-21.0)			1.56 (0.82-3.00)
TIMP1 (C536T)							
C/C	78	95.1 (87.9-90.4)	66	94.3 (86.0-98.4)	0.000	1.000	1.18 (0.23-5.9)
C/T + T/T	4	4.9 (1.34-12.0)	4	5.7 (1.68-13.9)			0.84 (0.16-4.22)
C	160	97.6 (93.9-99.3)	136	97.1 (92.8-99.2)	0.000	1.000	1.17 (0.24-5.71)
T	4	2.4 (0.67-6.13)	4	2.9 (0.78-0.10)			0.85 (0.17-4.12)

Conclusion

The data from this study confirm the need for further research to identify the genetic basis of the inherited disorders of connective tissue, for early screening and prevention.

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