GENETICS

Polymorphism of Detoxification Genes and Cell Resistance to Mutagens in Patients with Ehlers-Danlos Syndrome

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Study of polymorphism in 3 genes of the glutathione S-transferase family (GSTM1, GSTT1, and GSTP1) in children with Ehlers—Danlos syndrome whose cells were defective in repair of γ-induced DNA damages revealed accumulation of GSTM1(+) genotypes compared to children of the control group. Generation of reactive oxygen species by neutrophils from patients with this syndrome was higher than in healthy donors. Our results indicate that glutathione S-transferase genes are involved in the resistance to mutagenic agents and demonstrate medical and genetic peculiarities of patients with Ehlers—Danlos syndrome.

Key Words: *GST polymorphism; mutagens; Ehlers—Danlos syndrome*

The resistance of human cells to mutagens is provided by the antioxidant, detoxification, and DNA repair systems [2]. It is important to study polymorphism of genes encoding proteins that determine these functions.

Much attention was paid to polymorphism of DNA repair genes XRCC1-XRCC8, XPD, *etc*. [4] and genes encoding antioxidant enzymes (SOD and CAT) [10] and enzymes of phases I (CYP1A1, CYP2E1, *etc*.) and II of xenobiotic detoxification (glutathione S-transferase (GST), GSTM1, GSTT1, and GSTP1) [5]. The association between accumulation of some alleles and high or low risk of tumor development was evaluated [5]. The effect of polymorphism on resistance to toxicants was described [6].

GST genes play a key role in the protection of cells from oxidative stress [7]. For example, *in vitro* frequency of irradiation-induced chromosomal aberrations in blood lymphocytes from homozygotes for GSTM1 gene deletion was higher than in subjects with positive genotype.

Here we studied the role of polymorphism in GSTM1, GSTT1, and GSTP1 genes in cell resistance to $CdCl_2$ and γ-irradiation. Our study was performed on lymphocytes from patients with Ehlers—Danlos syndrome characterized by inhibition of the repair system for γ-induced DNA damages [3].

MATERIALS AND METHODS

Ehlers—Danlos syndrome is an inherited disorder characterized by metabolic disturbances in con-

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nective tissue elements. Polymorphism of GSTM1, GSTT1, and GSTP1 genes was studied in 37 patients with Ehlers—Danlos syndrome (25 boys and 12 girls). The selection and clinical characterization of children were performed at the Moscow Institute of Pediatrics and Child Surgery. Informed consent for the use of biological materials in this study was obtained from parents. The mean age of patients was 12.8±0.6 years. The control group included 39 children of comparable age, sex, and ethnic characteristics (relative to the group of patients with Ehlers—Danlos syndrome).

DNA was isolated from peripheral blood lymphocytes using Diatom DNA Prep kits (Isogen Laboratory) with lysing reagent, guanidine thiocyanate, and Nucleos sorbent (Isogen Laboratory). Amplification of fragments of GSTM1 and GSTT1 genes in multiplex polymerase chain reaction, sequence of primers, and electrophoretograms were described elsewhere [3].

Amplification with specific outer and inner primers was performed to identify the point mutation (A313G) in the GSTP1 gene. Genepack PCR CORE kits (Isogen Laboratory) consisted of lyophilized dry mixtures for DNA amplification. The amplification mixture (20 μl) included 0.2 μM each primer: F (outer forward primer), 5'-CCCAGTGACTGTGT GTTGATCAG-3'; R (outer reverse primer), 5'-CC GTTACTTGGCTGGTTGATGTC-3'; Fw (inner forward primer), 5'-GACCTCCGCTGCAAATACA-3'; and Rm (inner reverse primer), 5'-TTGGTGTAGA TGAGGGAGAC-3'.

Amplification was performed on a PT-49 programmed thermostat (TDL). The amplification program consisted of 3 cycles: 60 sec at 95°C, 40 sec at 62°C, 120 sec at 74°C (1 cycle); 40 sec at 95°C, 20 sec at 60°C, 90 sec at 74°C (1 cycle); and 20 sec at 95°C, 40 sec at 58°C, 60 sec at 74°C (30 cycles).

Electrophoresis of amplified DNA fragments was performed in 2% agarose gel with ethidium bromide. The gels were analyzed in transmited UV light using a Gel Imager video system.

Generation of reactive oxygen species (ROS) by blood leukocytes from 5 patients with Ehlers—Danlos syndrome and 1 healthy donor was studied by the method of luminol-dependent chemiluminescence of the whole blood [1]. The medium for measurement included 110 mM NaCl, 10 mM Tris, 5 mM glucose, and 0.65 mM luminol (pH 7.4). The total volume of the cuvette was 1 ml. The medium with luminol and 50 μl heparinized blood from the cubital vein were placed in a cuvette. The data were recorded on a Bioorbit 1251 chemiluminometer at 37°C and constant agitation. Phorbol myristate acetate (PMA, Sigma) in a final concentration of 10^{-6} mg/ml was used to stimulate chemiluminescence.

Genomic damage in lymphocytes (percent of single-stranded DNA) and cell resistance to cadmium salts were studied *in vitro* in 8 patients with Ehlers—Danlos syndrome. Cell culturing and mutagen treatment were performed as described elsewhere [3].

The results were analyzed by Fischer's exact test.

RESULTS

Polymorphism in genes of the GST family was revealed in both groups of children (Fig. 1).

The frequency of homozygotes for GSTM1 gene deletion (0/0 genotype) in the control group of children was 48.71%. Our results are consistent with published data that the frequency of the GSTM1 null genotype in European people is 42-50%. The frequency of homozygotes for GSTM1 gene deletion (0/0 genotype) in patients with Ehlers—Danlos syndrome was much lower than in the control (p=0.033, Fig. 1). These data illustrate the selective importance of GSTM1 gene polymorphism in intrauterine development and death of the organism with defective repair of genomic damage ("selection" of the GSTM1(+) genotype in patients with Ehlers—Danlos syndrome).

Our results are consistent with published data. No relationship was found between CYP2E1 (Rsa 1) and risk of laryngeal cancer [5]. However, the C2 allele was more often identified in the control group (2.8%) compared to tumor patients (1.6%). Hence, the mutant allele has a protective role. These data are consistent with the results of clinical observations on patients with lung cancer. Carriage of the C2 allele was associated with low risk for this disease [9].

The frequency of homozygotes for GSTT1 gene deletion (0/0 genotype) in the group of patients with Ehlers—Danlos syndrome did not differ from the control (Fig. 1).

The frequency of homozygotes for the point mutation in the GSTP1 gene (Ile105Val) in the control group of children was 2.56%. The frequency of heterozygotes for this mutation (GSTP1(Ile/Val) genotype) did not differ in children of the control group and patients with Ehlers—Danlos syndrome. However, the frequency of heterozygotes for GSTP1 gene mutation (GSTP1(Val/Val) genotype) tended to differ between patients with Ehlers—Danlos syndrome and healthy children (p=0.084). The frequency of normal GSTP1(Ile/Ile) genotype in children of the control group was slightly higher than in patients with hereditary disease (Fig. 1).

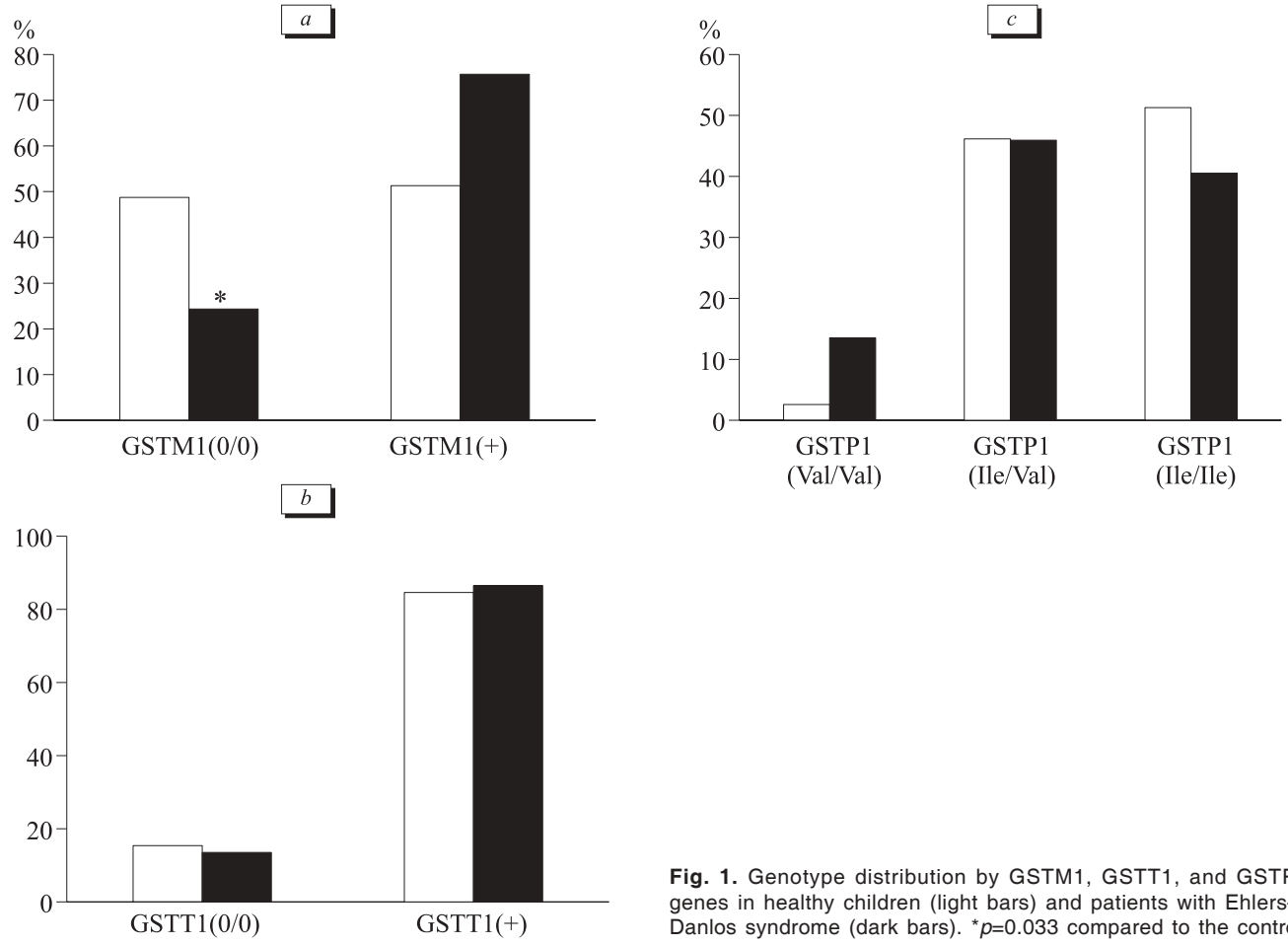

Fig. 1. Genotype distribution by GSTM1, GSTT1, and GSTP1 genes in healthy children (light bars) and patients with Ehlers—Danlos syndrome (dark bars). *p=0.033 compared to the control.

Genotypes with the combination of polymorphic GSTM1 alleles (0/0)/GSTT1(+)/GSTP1(Ile/Ile) in patients with Ehlers—Danlos syndrome were found more rarely than in the control group. The frequency of GSTM1(+)/GSTT1(+)/GSTP1(Val/Val) and GSTM1(+)/GSTT1(+)/GSTP1(Ile/Val) genotypes in

TABLE 1. Frequency Distribution for Combinations of Polymorphic Alleles of GSTM1, GSTT1, and GSTP1 Genes in Healthy Children and Patients with Ehlers—Danlos Syndrome

Genotype	Control	Child patients with Ehlers—Danlos syndrome
GSTM1(+)/GSTT1(+)/GSTP1(Val/Val)	1 (2.56%)	4 (10.81%)
GSTM1(+)/GSTT1(+)/GSTP1(Ile/Val)	7 (17.95%)	11 (29.73%)
GSTM1(+)/GSTT1(+)/GSTP1(Ile/Ile)	7 (17.95%)	8 (21.62%)
GSTM1(+)/GSTT1(0/0)/GSTP1(Val/Val)	—	—
GSTM1(+)/GSTT1(0/0)/GSTP1(Ile/Val)	3 (7.69 %)	1 (2. 70%)
GSTM1(+)/GSTT1 (0/0)/GSTP1(Ile/Ile)	1 (2.56%)	4 (10.81%)
GSTM1(0/0)/GSTT1(+)/GSTP1(Val/Val)	—	1 (2. 70%)
GSTM1(0/0)/GSTT1(+)/GSTP1(Ile/Val)	6 (15.38%)	5 (13. 51%)
GSTM1(0/0)/GSTT1(+)/GSTP1(Ile/Ile)	12 (30.77%)	3 (8%)*
GSTM1(0/0)/GSTT1(0/0)/GSTP1(Val/Val)	—	—
GSTM1(0/0)/GSTT1(0/0)/GSTP1(Ile/Val)	2 (5.12%)	—
GSTM1(0/0)/GSTT1(0/0)/GSTP1(Ile/Ile)	—	—

Note. —, not found. *p=0.018 compared to the control.

TABLE 2. Individual Characteristics of Genomic Damage in Lymphocytes, Cell Resistance to $CdCl_2$ (10^{-6} M, 4 h) *in Vitro*, and GST Gene Status in Patients with Ehlers—Danlos Syndrome

Patient No.	Genotype	DNA breaks in intact lymphocytes (single-stranded DNA, %)	$CdCl_2$-induced DNA breaks (single-stranded DNA, %)
1	GSTM1(0/0)/GSTT1(+)/GSTP1(Ile/Ile)	20.5	22.5
2	GSTM1(0/0)/GSTT1(+)/GSTP1(Val/Val)	15	26
3	GSTM1(0/0)/GSTT1(+)/GSTP1(Ile/Val)	21	21
4	GSTM1(+)/GSTT1(+)/GSTP1(Ile/Val)	23	19
5		17	25
6	GSTM1(+)/GSTT1(+)/GSTP1(Ile/Ile)	19	20
7		13	28
8	GSTM1(+)/GSTT1(0/0)/GSTP1(Ile/Val)	8	21

child patients with the hereditary disease tended to increase compared to healthy children (Table 1).

Study of polymorphism in 3 genes of the GST family in children with Ehlers—Danlos syndrome whose cells were defective in repair of γ-induced DNA damages revealed accumulation of GSTM1(+) and GSTP1(Val/Val) genotypes as compared to children of the control group. These data not only illustrate specific features of cell defense systems in Ehlers—Danlos syndrome, but also characterize the cells defective in repair of γ-induced DNA damages. Genes of the GST family probably play a role in radioresistance of cells and organism. Moreover, function of one defense system may compensate for the deficiency of another system. The combination of certain polymorphic alleles serves as a criterion for the resistance or susceptibility of cells to certain mutagens. Mutant GSTP1(Val/Val) and GSTP1(Ile/Val) genotypes in combination with normal GSTM1(+) genotype determine increased activity of these enzymes and neutralization of free radicals, which reduces the severity of induced DNA damages and decreases the load on repair systems. Our hypothesis is confirmed by published data on the role of GSTM1 in radioinduced chromosomal aberrations. The frequency of chromosomal aberrations in lymphocytes from homozygotes for GSTM1 gene deletion was higher compared to subjects with the GSTM1(+) genotype [7].

Previous studies showed that the number of spontaneous and γ-induced DNA breaks in lymphocytes from patients with Ehlers—Danlos syndrome *in vitro* surpasses the control level [3]. Damage caused by radiation and various chemical toxicants is mediated by the free radical mechanism. Hence, in the present study $CdCl_2$ was used as a mutagen. Independently on haplotypes (combinations of polymorphic alleles), 8 patients with Ehlers—Danlos syndrome were *in vitro* characterized by high variability in individual parameters of genomic damage in lymphocytes (percent of single-stranded DNA) and cell resistance to cadmium salts in (Table 2).

Detoxification genes are involved in ROS generation. ROS production increases in several hereditary diseases that are characterized by impaired of γ-induced DNA damages (Bloom syndrome) [8]. Study of ROS generation by lymphocytes from 5 patients with Ehlers—Danlos syndrome revealed individual differences in induced chemiluminescence. However, the intensity of ROS generation by cells from patients was higher compared to healthy donors (Fig. 2).

The results of our study illustrate molecular and genetic features of polymorphism during Ehlers—Danlos syndrome. Haplotypes for individual characteristics of patients and resistance to mutagenic factors were identified. The interaction between repair and detoxification systems manifested

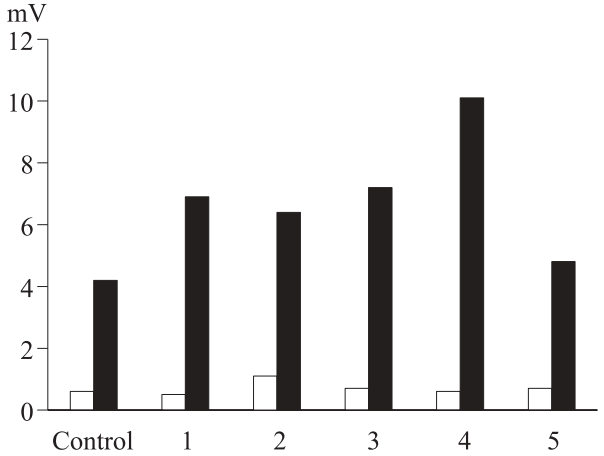

Fig. 2. Spontaneous (light bars) and PMA-induced chemiluminescence (dark bars) of peripheral blood leukocytes from patients with Ehlers—Danlos syndrome. Numerals: patient's number.

in the fact that one defense system may compensate for a deficiency of another system.

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