

Glutathione S-Transferase Gene Polymorphisms in Children with Down Syndrome and Their Mothers

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ABSTRACT To elucidate the genetic factors causing clinical differences in the children with Down syndrome and evaluate possible maternal risk factors, the researchers have investigated GSTM1, GSTT1, GSTP1 gene polymorphisms. Four groups were defined: group I (n = 52), children with Down syndrome; group II (n = 70), healthy children; group III (n = 52), mothers of the children with Down syndrome; and group IV (n = 69), mothers of the healthy children. Genomic DNA was extracted from the white blood cells and GenID ® GmbH kit used for GST M1, T1 and P1 gene amplification to determine polymorphisms. The researchers did not detect any significant difference in the allele frequencies between groups I and II, nor groups III and IV. The data indicated no relationship between detected GST polymorphisms, neither with Down syndrome nor with the risk of having an infant with Down syndrome.

INTRODUCTION

Down syndrome is the *most common* trisomy among live births was first described by Down et al. in 1866. More than 80 clinical features have been reported about the individuals with Down syndrome. Many studies have been conducted about the factors contributing in the development of the clinical features, and various theories have been proposed to explain diversity of the clinical features (Berg et al. 2001; Antonarakis et al. 2004; Patterson 2007; Roizen et al. 2003; Reeves et al. 2001).

Due to gene-dosage effect, over-expression of genes located on chromosome 21 was suggested to lead to different influences on the growth, maturation, and aging of the tissues (Antonarakis et al. 2004; Gardiner et al. 2000; Reeves 2006). Additionally, polymorphisms in certain genes located on other chromosome were shown to be effective in the development and intensity of various features of Down Syndrome (Amorim et al. 2008; Biselli et al. 2008).

Glutathione (GSH) detoxifies xenobiotics and heavy metals with a reaction catalyzed by glutathione S-transferase (GST). GST is one of the major endogenous enzymes that are important for cellular antioxidant activity (Reed et al. 2008; Hayes et al. 2005).

GSTs have been investigated in two groups: membrane-bound and cytosolic. In vertebrates, seven classes of cytosolic GST have been identified, based on structural differences: alpha, mu, kappa, pi, sigma, theta, and zeta. Different GSTs demonstrate at least 40% amino acid similarity within a class, while at least 30% similarity between the classes (Whalen et al. 1998; Hayes et al. 1995).

Numerous polymorphisms in the genes of GST cause a decrease in the GST enzyme activity. For instance, deletions in both alleles (GSTM1-0 and GSTT1-0) of the GSTM1 and GSTT1 genes may decrease or inhibit GST activity which will detoxify electrophilic carcinogens (Hayes et al. 2005; Sekine et al. 1995; Majumdar et al. 2008).

Polymorphisms of the GST genes, which are effective in the metabolism of GSH, are known to increase predisposition to cancer, inflammatory diseases and cataracts (Whalen et al. 1998; Hayes et al. 1995).

Increased oxidative stress in children with Down syndrome is known to cause particularly "one-carbon metabolism" disorders (Reed et al.

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2008). Even though, the cause has not been identified, the catalytic activity of GST tends to be decreased in children with Down syndrome compared to normal children (Zatorska et al. 2002). Further, Ishibashi et al. reported that oxidative stress may result in congenital anomalies in mice, which may be improved by the addition of GSH (Ishibashi et al., 1997).

Based on these data, the researchers intended to investigate the frequency of glutathione S-transferase gene polymorphisms in children with Down syndrome, and the effects of these polymorphisms on the phenotypic diversity and also on the risk of giving birth to a child with Down syndrome. To meet that purpose, by determining polymorphism rates in GSTM1, GSTT1 and GSTP1 genes of the children with Down syndrome and their mothers, the researchers aimed to determine their correlation with the risk of developing this syndrome and its severity.

METHODOLOGY

The present research study was conducted with children, clinically diagnosed and cytogenetically confirmed to have Down syndrome and their mothers, who have been receiving care in the Department of Pediatric Genetics, Dokuz Eylul University Hospital. Healthy children and healthy adult mothers who visited any outpatient clinics were enrolled as the control group. The study was carried out between January to July 2010. Four groups were defined; group I (n = 52), children with Down syndrome; group II (n = 70), healthy children; group III (n = 52) mothers of the children with Down syndrome; and group IV (n = 69), mothers of healthy children. After approval by the institutional Ethics Committee, 2 cc EDTA blood samples were collected from patient and control groups, for testing GST polymorphisms.

Demographic characteristics, cytogenetic analysis, physical examination, eye examination, hearing tests, electrocardiography, celiac antibodies, complete blood count, and thyroid function test results of the children with Down syndrome were recorded during routine control visits. Informed consent was obtained from the participants of the patient and control groups.

NucleoSpin®Dx blood kit was used for DNA isolation, and GenID® GmbH kit for GST M1, T1 and P1 gene amplification. Strips of the study

groups were evaluated according to the formation of bands. By using the evaluation criteria of GenID® GmbH kit, heterozygous and homozygous mutations in the GSTM1, GSTT1, GSTP105, and GSTP114 genes were detected.

Statistical analysis was performed with Windows SPSS 15.0 program, student t-test, Fisher's exact test and chi-square test were used in the evaluation of the parameters, a p-value <0.05 was considered statistically significant.

Differences in terms of gene polymorphism were investigated between the mothers of the children with Down syndrome and healthy women, also between the children with Down syndrome and healthy children. Further, any correlation between the polymorphic sites detected in the GSTM1, GSTT1 and GSTP1 genes and the phenotypic features of the patients with Down's syndrome was explored.

RESULTS

In the study, a total of 243 participants were enrolled in four groups. *Group I*; consisted 52 patients, 24 (46.2%) were female and 28 (53.8%) were male, aged 1 month - 17 years (mean, 6.13 ± 4.86 years), *Group II*; consisted 70 children, 37 (52.9%) were female and 33 (47.1%) were male, aged 1 - 17 years (mean, 9.09 ± 4.98 years), *Group III*; mothers of the children with Down Syndrome aged between 23-56 years (mean 37.27 ± 6.77 years), *Group IV*; mothers of the healthy children aged between 22-50 years (mean, 28.25 ± 6.54 years).

Patients with a diagnosis of Down syndrome were evaluated in terms of pathologies such as cardiac defects, hearing loss, ophthalmic problems (refractive errors, strabismus, cataracts, glaucoma) and hypothyroidism. Among the fifty-two patients with Down syndrome there were 26 (50%) who had cardiac pathology, 12 (23.1%) with hearing loss, 20 (38.5%) with eye pathology and 23 (44.2%) with hypothyroidism.

To investigate a possible association between the polymorphisms of GSTT1, GSTM1 and GSTP1 105-114 and Down syndrome or having a child with Down syndrome, the researchers compared the allelic distributions between the four groups. There was no significant difference neither between group 1 and 2 ($p = 0.88$; 0.95; 0.95; 0.68), nor between group 3 and 4 ($p = 0.54$; 0.44; 0.32; 0.80) (Table 1, 2).

Concerning the relationship between systemic pathologies detected in Down syndrome patients and GST polymorphism; of a total of 26 Down Syndrome patients having cardiac defects; twelve (46.2%) carried a homozygous gene deletion in the GSTM1. Fourteen (53.8%) of these children had no deletion at least in one single allele. The detection method used in this study was not able to differentiate GSTM1 heterozygous children from those presenting with no deletion, therefore, frequency ratios cannot be specified separately. Homozygous deletion of the GSTT1 gene was identified in 10 (38.5%) of these children. At least one single allele of the remaining 16 (61.5%) patients was "wild type".

Fourteen (53.8%) of these children presented with a GSTP1 genotype; one allele had 105Ile, while the other had 105Val. This ratio was found to be 34.6% in the Down syndrome patients without any cardiac defect ($p=0.07$). A polymorphism was detected in one allele of the GSTP1 gene in 7 (26.9%) Down syndrome patients having cardiac defects, whereas this ratio was determined to be 11.5% in the Down syndrome patients without any cardiac defect ($p=0.15$) (Table 3).

In Down patients with or without any hearing loss, no statistically significant difference was detected as shown in Table 4, with regard to GSTM1 ($p=0.158$), GSTT1 ($p=0.157$), GSTP1 105

Table 1: Distribution of GST alleles in group I and group II. * GSTM1 (+) refers to the existence of a deletion at least in one allele, means can not distinguish wild type and heterozygous carriers. **GSTT1 (+) refers to the existence of a deletion at least in one allele, means can not distinguish wild type and heterozygous carriers.

	GSTM1		GSTT1		GSTP1 codon 105			GSTP1 codon 114			Total
	GSTM1 (-)	*GSTM1 (+)	GSTT1 (-)	*GSTT1 (+)	val/val	ile/val	ile/ile	val/val	ala/val	ala/ala	
Group I	23 44.2%	29 55.8%	22 42.3%	30 57.7%	4 7.7%	23 44.2%	25 48.1%	0 0%	10 19.2%	42 80.8%	52 100%
Group II	30 42.9%	40 57.1%	30 42.9%	40 57.1%	5 7.1%	33 47.1%	32 45.7%	1 1.4%	14 20.0%	55 78.6%	70 100%
Total	53 43.4%	69 56.6%	52 42.6%	70 57.4%	9 7.4%	56 45.9%	57 46.7%	1 .8%	24 19.7%	97 79.5%	122 100%

Table 2: Distribution of GST alleles of the mothers in group III and group IV

	GSTM1		GSTT1		GSTP1 codon 105			GSTP1 codon 114			Total
	GSTM1 (-)	*GSTM1 (+)	GSTT1 (-)	*GSTT1 (+)	val/val	ile/val	ile/ile	val/val	ala/val	ala/ala	
Group III	33	19	22	30	6	17		29	6	33	52
	63.5%	36.5%	42.3%	57.7%	11.5%	32.7%		55.8%	11.5%	8.5%	100%
Group IV	58.0%	42.0%	49.3%	50.7%	4.3%	25		41	7	68	69
	58.0%	42.0%	49.3%	50.7%	4.3%	36.2%		59.4%	10.1%	89.9%	100%
Total	73	48	56	65	9	42		70	13	108	121
	60.3%	39.7%	46.3%	53.7%	7.4%	34.7%		57.9%	10.7%	89.3%	100%

Table 3: Allelic distribution of GSTP1 gene in Down Syndrome patients with or without cardiac defects.

		GSTP1codon 105			GSTP1 codon 114		Total
		val/val	ile/val	ile/ile	ala/val	ala/ala	
Cardiac Defect	Not Exist	4 15.4%	9 34.6%	13 50.0%	3 11.5%	23 88.5%	26 100.0%
	Exist	0 0%	14 53.8%	12 46.2%	72 6.9%	19 73.1%	26 100.0%
	Total	4 7.7%	23 44.2%	25 48.1%	10 19.2%	42 80.8%	52 100.0%

and GSTP1 114 polymorphisms (for GSTP1 105, $p=0.45$; for GSTP1 114, $p=0.15$).

When the researchers compared the data of Down syndrome patients who have (38.4%) and have not ophthalmic problems, the researchers couldn't find any significant difference for GSTT1, GSTM1, GSTP1 codon 105, GSTP1 codon 114 polymorphisms (Respectively, $p=0.21$, $p=0.75$, $p=0.18$, $p=0.91$) (Table 4).

Among Down syndrome patients, no statistically significant difference was detected in terms of GSTM1, GSTT1, GSTP1 polymorphisms between 23 (44.2%) children having hypothyroidism and those having normal thyroid functions (respectively, $p = 0.22$, 0.68 , 0.48 , 0.31) (Table 5).

Regarding mothers of children with Down syndrome, 33 (63.5%) of these mothers presented the GSTM1 gene, 22 (42.3%) had homozygous GSTT1 gene deletion, 6 (11.5%) had ile105Val exchange in both alleles of GSTP1, while 17 (32.7%) had ile105Val exchange in one single

allele of GSTP1, whereas 6 (11.5%) of the mothers carried the GSTP1 Ala114Val polymorphism in one allele. None of the mothers carried Ala114Val change in both 2 alleles. 40 (58%) of the healthy mothers in the study group presented GSTM1 null allele, 34 (49.3%) mothers had GSTT1 null allele, 3 (4.3%) mothers had polymorphic GSTP1-105 in both alleles, 7 (10.1%) mothers had a change in the single allele of GSTP1 at codon 114.

DISCUSSION

Down syndrome is one of the most common chromosomal disorders (Berg et al. 2001; Antonarakis et al. 2004). In addition to the common symptoms such as distinct facial appearance, mental retardation, hypotonia, which are seen in all patients, other symptoms such as cardiac defects, duodenal atresia/stenosis, Hirschsprung's disease, deafness, speech disorders, immune deficiency, cataracts, AML, gluten-sen-

Table 4: Allelic distribution of GST genes in Down Syndrome patients with or without hearing loss and ophthalmic pathology.

		GSTT1		GSTM1		GSTP1 codon 105			GSTP1 codon 114			Total
		GSTM1 (-)	*GSTM1 (+)	GSTT1 (-)	*GSTT1 (+)	val/val	ile/val	ile/ile	val/val	ala/val	ala/ala	
Hearing loss	Not	Exit	19	21	19	21	41	18	18	61	348	40
			47.5%	52.5%	47.5%	52.5%	0.0%	45.0%	45.0%	5.0%	5.0%	100.0%
	Exist		19	52.5%	19	21	41	18	18	61	34	40
Ophthalmic pathology	Not	Exist	3	9	4	8	0	5	7	4	8	12
			25.0%	75.0%	33.3%	66.7%	0%	41.7%	58.3%	33.3%	66.7%	100.0%
	Exist		13	19	12	20	41	12	16	61	26	32
Total	Not	Exist	9	11	11	9	0	11	9	4	16	20
			45.0%	55.0%	55.0%	45.0%	0%	55.0%	45.0%	20.0%	80.0%	100.0%
	Exist		22	30	23	29	4	23	25	10	42	52
			42.3%	57.7%	44.2%	55.8%	7.7%	44.2%	48.1%	19.2%	80.8%	100.0%

Table 5: Allelic distribution of GST genes in Down Syndrome patients with or without hypothyroidism

		GSTT1		GSTM1		GSTP1 codon 105			GSTP1 codon 114			Total
		GSTM1 (-)	*GSTM1 (+)	GSTT1 (-)	*GSTT1 (+)	val/val	ile/val	ile/ile	val/val	ala/val	ala/ala	
Hypothyroidism	Not	Exist	13	16	15	14	3	14	12	7	22	29
			44.8%	55.2%	51.7%	48.3%	10.3%	48.3%	41.4%	24.1%	75.9%	100.0%
	Exist		9	14	8	15	1	9	13	3	20	23
Total			39.1%	60.9%	34.8%	65.2%	4.3%	39.1%	56.5%	13.0%	87.0%	100.0%
	Exist		22	30	23	29	4	23	25	10	42	52
			42.3%	57.7%	44.2%	55.8%	7.7%	44.2%	48.1%	19.2%	80.8%	100.0%

sitive enteropathy, and atlanto-axial joint instability may be observed. The variable severities of all these pathologies are reflected in the manifestation of clinical picture (Antonarakis et al. 2004; Patterson 2007).

An increase in oxidative stress in children with Down syndrome is known to cause particularly "one-carbon metabolism" disturbances (Reed et al. 2008). Polymorphisms in the GST genes, which are effective in GST metabolism, are known to increase an individual's predisposition to cancer, inflammatory diseases, cataracts, and immune response disorders (Whalen et al. 1998; Hayes et al. 1995; Vlastos et al. 2003). Many polymorphisms in the GST genes may result in a decrease in the GST enzyme activity, and then the GST activity becomes responsible for detoxifying electrophilic carcinogens may decrease or completely cease (Hayes et al. 2005; Whalen et al. 1998; Hayes et al. 1995).

In various studies, GSTM1 has been shown to be polymorphic in humans, and despite geographic differences, this ratio was found to be 35-60% (Kuz'mina et al. 2009). Similarly, GSTT1 may express genetic polymorphisms and 10-65% of human populations possess no GSTT1 activity. (Whalen et al. 1998; Chen et al. 1997). Homozygous Ile105Val variant of GSTP1 has 10-20% prevalence in the normal population, while heterozygosity is found in 40-60%. Homozygous Ala114Val change has a prevalence of 0-20% in the normal population, while the heterozygous trait is 10-20% (Ali-Osman et al. 1997). The polymorphism rates that the researchers obtained in the present study groups, were consistent with the literature.

Congenital Heart Disease (CHD) has been detected in 26-61.3% of patients with Down syndrome. Twenty-six (50%) of the 52 patients enrolled in our study had a cardiac pathology, rates consistent with other studies.

According to the data achieved in the study, 31.2% of our patients had an ASD, 15.6% PFO, 12.4% AVSD, 6.2% PDA, 6.2% VSD, 3.1% VSD and PDA, 3.1% VSD and PS, 3.1% AVSD, PS and VSD, 3.1% AVSD, PDA and VSD, 3.1% pericardial effusion, 3.1% valve pathology, 3.1% coarctation of the aorta, and 3.1% complex cardiac diseases and these rates were found to be different in various studies (Abbag 2006; Kallen et al. 1996).

The researchers did not observe any correlation between GST polymorphisms and CHD de-

velopment in the children with Down syndrome. There are no other studies indicating a relationship between the polymorphisms in this gene and the development of CHD in the literature.

Out of the 52 patients enrolled in the study 12 (23%) had hearing loss. Compared with the data of other studies, our rate was found to be lower. Evaluation of hearing loss is considered very important due to its influences on intellectual development and 38-78% of Down syndrome patients may experience this problem, as a conductive, sensorineural or mixed type of hearing loss. In addition to the medical treatment of recurrent otitis media and serous otitis, surgical measures (ventilation tube application, adenotonsillectomy) may be necessary for many patients, especially for the prevention of conductive hearing loss (Van Allen et al. 1999; Smith 2001; Liza-Sharmini 2006). Our patients frequently reported upper respiratory tract infections and otitis media.

When the researchers compared 12 (23%) Down syndrome patients having hearing loss with the Down syndrome patients without any hearing problem, and they found no statistically significant difference with regard to the GSTP1 105 and GSTP1 114 polymorphisms ($p = 0.45$ for GSTP1 105, $p = 0.15$ for GSTP1 114).

Considering the fact that reactive oxygen species are predisposing factor in noise-induced hearing loss, a study was carried out with 58 male workers, who were exposed to noise in the steel factory, and experienced a temporary threshold shift. It was aimed to investigate the effects of genetic mutations of the GSTT1, GSTM1 and GSTP1 on temporary threshold shift. It was concluded that, individuals carrying all genotypes of the GSTT1 null, GSTM1 null and GSTP1 Ile (105) / Ile (105) may be more susceptible to noise-induced hearing loss (Lin et al. 2009).

Nevertheless, assuming reactive oxygen radicals may play an important role in inner ear damage, it was investigated that whether the sudden sensor neuronal hearing loss may be related with the GSTM1 and GSTT1 genotypes; frequency of GSTM1 and GSTT1 null genotype did not differ between patient and control groups (Cadoni et al. 2006). In addition, a significant relationship of the GSTP1, GSTM1 and GSTT1 polymorphisms and age-related hearing loss could not be demonstrated (Ates et al. 2005).

Ophthalmic pathology was detected in 20 (38.5%) of the 52 patients included in the study.

We detected refractive errors in 75%, strabismus in 35%, nystagmus in 10%, cataracts in 5%, and glaucoma in 5% of these 20 patients. In the present study, the researchers evaluated ophthalmic problems that may cause functional impairment in patients. Liza-Sharmini et al. investigated 60 Down syndrome patients, aged from one month to 17 years, and reported epicanthus in 96.7%, nystagmus in 33.3%, strabismus in 26.7%, bilateral congenital cataracts in 13.3%, blepharoconjunctivitis in 10%, eyelid anomalies in 6.7%, glaucoma in 6.7%, nasolacrimal obstruction in 3.3%, bilateral retinoblastoma in 1.7%, chronic uveitis in 1.7% of the cases (Liza-Sharmini et al. 2006).

In humans, GSTT1 and GSTM1 null genotypes (T0M1, T1M0 and T0M0) have been associated with pathological processes related to certain eye diseases. In a study conducted with glaucoma patients, all three polymorphisms of GST were identified significantly more in glaucoma patients compared to the control group. Decreased GST function may interfere with the oxidative metabolism, and consequently, exacerbate either directly or indirectly negative influences of oxidative stress on the optic nerve. This may suggest that, GST polymorphisms may become a risk factor for glaucoma (Abu-Amero et al. 2008).

Likewise, GST polymorphisms have been considered as risk factors for the development of senile cataracts. In the meta-analysis conducted to explore the relationship between the GSTM1 and GSTT1 null genotypes and the risk for senile cataract, all the studies addressing the relationship between GSTM1/GSTT1 polymorphisms and the risk for senile cataracts were included. The relationship between GSTM1 null genotype and the risk for senile cataract was not statistically significant, and the relationship between GSTT1 null genotype and the risk of senile cataracts was statistically negligible. Subgroup analysis showed a statistically significant relationship between the GSTM1 null genotype and the risk for senile cataracts in Asians, but not in Caucasians. Similar results were observed in the relationship between GSTT1 null genotype and the risk for senile cataracts. This meta-analysis showed GSTM1 and GSTT1 null genotypes were linked with the risk of senile cataracts in Asian populations (Sun et al. 2010).

In the study, 23 (44.2%) out of 52 patients had hypothyroidism. All of the patients were

taking L-thyroxine therapy. Fort et al. (Fort et al. 1984) detected the frequency of Down syndrome in newborns with congenital primary hypothyroidism 28 times higher compared to the normal population. Cutler et al. (Cutler et al. 1986) reported a 27% increase in TSH in the study conducted with 49 Down syndrome patients at ages ranging from 4 months to 3 years. Zori et al. (Zori et al. 1990) detected thyroid dysfunction in 66% of adult patients with Down syndrome; of these, 57% had increased TSH values (5 mIU / mLW), while 28% had positive thyroid autoantibodies.

The relationship between congenital hypothyroidism and polymorphisms of some genes was explored. For instance, recent studies have indicated the correlation between FOXE1 polyaniline tract (FOXE1-polyAla) length polymorphism and a genetic predisposition to thyroid dysgenesis causing congenital hypothyroidism.

All of the patients in the study had varying degrees of mental retardation. When the patients were evaluated in terms of motor development based both clinically and on their medical history, hypotonia and delayed motor development compared to peers were noted. Five of the patients have been followed with the diagnosis of epilepsy; one patient had cerebral palsy due to birth trauma. None of the patients had Alzheimer's disease, which might be related to the age range of the study group.

CONCLUSION

Polymorphisms in the GST genes, which are participate in glutathione metabolism, are known to cause cancer, inflammatory diseases, increased predisposition to developing cataracts, immune response disorders; further, many polymorphisms in the GST genes are known to cause decreased enzyme activity of GST.

In the present study, the prevalence of GST polymorphism in patients with Down syndrome was consistent with the normal population. When these rates were compared with the data of healthy children, no statistically significant differences were observed.

The effects of GST polymorphisms on phenotypic characteristics seen in Down syndrome could not be demonstrated.

The frequency of GST polymorphisms detected in the mothers of both healthy children and children with Down syndrome were found to be similar. (Compared to the normal popula-

tion and no differences were detected between the two groups.)

These polymorphisms were considered to have no effect on having a child with Down syndrome.

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