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Small Ubiquitin-related Modifier (SUMO)3 and (SUMO)4 Gene Polymorphisms in Parkinson's Disease

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Abstract

Objective: The ubiquitin/proteosome system is one of the main axes of the pathogenesis of Parkinson's disease (PD). Small ubiquitin-related modifier (SUMO) proteins are involved in many biochemical events including regulation of transcriptional activity, modulation of signal transduction pathways, and response to cellular stress indicating a role for SUMO in the ubiquitin/proteosome system. In this study, our aim was to examine the prevalence of SUMO gene variants and their clinical associations in PD.

Methods: Fifty-four consecutively recruited PD patients and 74 age-gender matched healthy controls were included. SUMO1, 2, 3 and 4 genes were screened by a next generation sequencing method using blood samples of participants. Single nucleotide polymorphisms (SNPs) with a significantly altered prevalence were determined by Bonferroni correction.

Results: Two SNPs in the SUMO4 gene rs237025 and rs237024 and two SNPs in the SUMO3 gene rs180313 and rs235293 were found to have altered prevalence in PD. Although there was no association among these SNPs and clinical features of the patients, an increased family history of cancer was found in patients with SUMO3 gene variants.

Conclusion: Several SUMO SNPs were identified for the first time in PD patients suggesting that SUMO is involved in the pathophysiology of the disease. rs237025 has also been associated with diabetes mellitus indicating a pathogenic mechanism for SUMO that is shared with other degenerative disorders.

Keywords: Parkinson's disease; SUMO gene; Sumoylation; Ubiquitin; Genetics

Introduction

Parkinson's disease (PD) is the second most common degenerative disorder of the central nervous system worldwide, with an estimated 7-10 million people affected [1]. Dopaminergic neuron loss and Lewy bodies (LBs) are considered as defining pathological characteristics of PD. The accompaniment of neurofilaments, ubiquitin, and β-amyloid in LBs was demonstrated before the main component α-synuclein was identified in 1997 [2]. Later, immunoreactivity for many other proteins, including parkin, synphilin-1 and the small ubiquitin-related modifier (SUMO) was shown in LBs [3-5].

The mammalian SUMO paralogs SUMO-1 and SUMO-2/SUMO-3, although partially redundant, may fulfill different functions as suggested by various studies on substrate specificity, mono-/polySUMOylation, expression, and oxidative stress [6-9]. It is well known that ubiquitin and SUMO share similarities in respect to tertiary structure and conjugation/deconjugation cycles. SUMO has several different isoforms in mammalians and carries a consensus motif, ubiquitin conjugating enzyme 9 (UBC9) as exclusive SUMO-E2 conjugation enzyme [10]. SUMOylation simultaneously discovered by two groups (Matunis and Mahajan) emerged in recent years as a likely candidate mechanism to regulate a plethora of processes within the cell [11,12]. Surprisingly, research in recent years uncovered the existence of mixing units such as the SUMO-targeted ubiquitin ligases (STUbLs) or E3 ligases with dual functions for SUMO and ubiquitin [13]. Notably, SUMOylation and ubiquitination both regulate α-synuclein degradation and aggregation, a hallmark of PD pathology [14].

Despite its well-established significance in PD pathogenesis, genetic variants of SUMO genes have never been investigated in PD. In this study, our aim was to reveal the presence and prevalence of SUMO gene variants and their associations with clinical features of PD.

Materials and Methods

Patients

In this study, 54 PD (34 male, 20 female) patients and 74 age/gender-matched healthy controls (37 male, 37 female) were included. The diagnosis of the patients was based on clinical PD criteria formulated by the Brain Bank of the United Kingdom PD Association [15]. The study was approved by the Institutional Review Board and signed consent was obtained from all participants. Patients with pyramidal or cerebellar system findings, dyspraxia, autonomic dysfunction, and history of head trauma, encephalitis and exposure to toxic substances were excluded. Also, patients with Parkinson plus syndromes, vascular parkinsonism were not included. Age and gender-matched healthy controls without any known systemic, neurological or psychiatric illness were recruited. In the PD group, severity and clinical course of the disease were assessed by Hoehn-Yahr (H&Y) scale and Unified Parkinson's Disease Rating Scale (UPDRS) [16-18]. All cases were taken on a standard structured interview and neurological examination was performed.

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Next generation sequencing

128 human genomic DNA samples were sequenced by next generation sequencing method. Exomes regions of SUMO1, SUMO2, SUMO3, SUMO4 genes were sequenced.

DNA was isolated from blood samples by Invitrogen DNA isolation kit (Carlsbad, CA, USA) and stored at -80°C. In PCR operations, amplification was conducted using a total volume of 50 µl comprising: 100 ng genomic DNA; a reaction buffer with magnesium chloride and potassium chloride; 1 U Taq polymerase; 2.5 mM deoxynucleotide; and 10 pmol of forward and reverse primers. Using the primers set forth in Table 1, the following cycles were applied: 95°C for 5 min, 35 cycles at 95°C for 30 sec and at 60°C for 40 sec.

Thermal cycler protocol was applied for 8 min at 68°C and 10 min at 72°C. The obtained PCR products were confirmed with gel electrophoresis. All amplicons obtained after PCR were diluted to be at the same concentration and purified using Agencourt Ampure beads kit (Agencourt Bioscience Corporation, MA, USA). The samples were then sequenced using the Illumina MiSeq platform and chemicals, following the manufacturer's protocols. With an average of 360000 readings per sample, a total of 4019498 2x 150 bp readings and 5.13 Gb data were obtained.

The readings in the target position (Table 2) from the array data obtained after the scrambling were filtered out. The obtained raw sequence data were truncated considering the quality scores (Trimmomatic v0.27). The corrected crude sequence data was aligned to the human genomic reference sequences (GRCh37, GRCh38) (using the Burrows-Wheeler Aligner). Subsequently, a local realignment was performed Genome Analyzer Tool Kit (GATK IndelRealigner v3.3.0) to align the the indel. After merging and alignment, reading count optimization, base quality recalibration was performed by filtering the repetitive readings using the GATK (v3.3.0) application. In the last step, single point variants and indices were determined using GATK (Unified Genotyper).

All candidate variants (small insertions, deletions, single nucleotide changes) obtained as a result of the exon sequencing analysis were first compared to the minor allele frequency (MAF) (MAF ≤ 0.01 variants) using general control databases (ExAC, 1000 Genome) in Table 3. The obtained variants were assessed using Integrative Genomic Viewer (IGV 2.3, Broad Institute) program.

Statistical analysis

Prevalences of SUMO gene variants were compared among patient and healthy control groups by Chi-square test and Bonferroni test. Chi-square, T-test and Mann-Whitney U statistical tests were used for the comparison of clinical and demographic parameters among study groups. $p<0.05$ was considered statistically significant.

Results

SUMO gene variants

As a result of the in-depth sequencing, 48 SNPs were detected in the SUMO1, SUMO2, SUMO3 and SUMO4 genes. Significant ($p<0.05$) SNPs determined by comparing patients and controls were rs237025 and rs237024 in the SUMO4 gene and rs180313 and rs235293 in the SUMO3 gene, respectively. None of the p values remained significant after Bonferroni correction in Table 3. According to these results, 42 of the 54 PD patients were found to display rs237025 and rs237024 variants of the SUMO4 gene. On the other hand, in 8 patients, rs180313 and rs235293 variants were determined in the SUMO3 gene. The SNP and p values in the control group are shown in Table 3.

Comparison of PD patients with and without SUMO variants

PD patients with and without SNPs SUMO genes were compared in terms of clinical and demographic characteristics. The parameters considered in the comparison are summarized in Tables 4A and 4B. There were no statistically significant differences between the groups in terms of age, sex, age at onset, duration of disease, Hoehn-Yahr scores, dementia, depression, and family history of PD. PD patients with rs180313 and rs235293 variants but not rs237025 and rs237024 variants had a significantly higher prevalence of family history of cancer (Table 4B).

Discussion

PD is a neurodegenerative disease that primarily affects the substantia nigra, and accounts for 80% of all parkinsonism cases. Epidemiological studies have shown that about 1% of the population over 65 years old suffer from this disease. At least 60-80% of dopaminergic cells of substantia nigra need to be lost in order for the findings of PD to appear [19]. Precise mechanisms leading to nigral degeneration, responsible for the clinical manifestations of the disease are as yet not entirely clear and genes associated with increased PD susceptibility still continue to be characterized. Hereditary predisposition, environmental

Primer Set	Forward Primer Series	Reverse Primer Series	Amplicon Length	Amplicon Location
SUMO4	TATGTCTGTGTGTTTTGATCCAGG	CCCTACTTAAAGACAGATTGCCCT	1505 bp	chr6:149720817-149722321
SUMO3_2	CAATTTTGGTTGTGCCAATCCTTG	CCTTGCTGAGAACTTTGTAAAGGG	3768 bp	chr21:46226119-46229886
SUMO3_1	GTGAAAATAAGATCTGCCCTCAGC	TAGGGTTCCTCTGAGTCACAAATG	1656 bp	chr21:46232913-46234568
SUMO2_3	ATCAGCAACAAAGGCAAAATCAGA	ATGAAACTTGGAACTTAGTGGAACA	1131 bp	chr17:73163481-73164611
SUMO2_2	TAAAGGATCAGAGAGCATCACGTT	GTAGCTGTGTCTGAAAAGCAGTAA	2056 bp	chr17:73170020-73172075
SUMO2_1	TCCTAAGGGTTTTCACGAGCTATC	TGGAAGCCATTTTGATTATGCTCC	2355 bp	chr17:73176975-73179329
SUMO1_5	AGAGGGTAATATGAAGGGGACTGA	GACAGAATCAGAAGGAAAGACACC	468 bp	chr2:203071761-203072228
SUMO1_4	CCAGCCATTAATGTTACCATCACC	GCATAGGCTTAGAAACAGGTTTGG	735 bp	chr2:203075136-203075870
SUMO1_3	ACTGGTAAGCCATCAAGACAGAAA	TGGTGGAAAAATACAGGTTACAAGG	478 bp	chr2:203078809-203079286
SUMO1_2	GGCCTCTTCTACCTCTAACAGATG	AATGCTGTTTGTATTCTCAGGTGC	343 bp	chr2:203084607-203084949
SUMO1_1	GATTAGTCCTCTGGAAGGAGACG	GGAGAGAGCAATCTAGGTTGTGAG	784 bp	chr2:203102788-203103571

Table 1: Primers used in PCR studies.

Location	Gene	P-value	Bonferroni	FDR P-value Correction	Experiment Group	Experiment Group (%)	Control Group	Control Group (%)
chr21:46228165 T>C	SUMO3	1.87E-03	0.07	0.04	7	12.96	0	0
chr21:46233230 C>T	SUMO3	1.87E-03	0.07	0.04	7	12.96	0	0
chr17:73171791 G>T	SUMO2	4.15E-03	0.1	0.05	11	20.37	3	4.05
chr21:46228930 C>T	SUMO3	4.32E-03	0.17	0.06	8	14.81	1	1.35
chr21:46233071 A>G	SUMO3	6.67E-03	0.26	0.07	9	16.67	2	2.7
chr21:46227798 C>T	SUMO3	9.89E-03	0.39	0.08	7	12.96	1	1.35
chr6:149721965 T>C	SUMO4	0.02	0.11	0.08	40	74.07	40	54.05
chr6:149721690 G>A	SUMO4	0.02	0.16	0.08	42	77.78	44	59.46
chr6:149722189 A>C	SUMO4	0.05	0.33	0.11	19	35.19	15	20.27
chr21:46227364 G>A	SUMO3	0.05	1	0.31	5	9.26	1	1.35

Table 2: Comparison of Significant Variants in The SUMO Gene Between the Patient and Control Groups.

factors, mitochondrial dysfunction and ubiquitin cycling are crucially involved in this process [20].

In this context, significance of SUMO and sumoylation in neurodegenerative disorders has been recently scrutinized. Although PD cases are mostly sporadic, several genes have also been associated with familial types of disease. α-synuclein, DJ-1 and parkin are three of these genes and are target proteins for SUMO, indicating the role of this molecule in the molecular mechanisms of PD pathogenesis [21,22]. SUMO proteins bind to large number of cellular targets. It modulates protein-protein and protein-DNA interactions, modifies intracellular localizations of proteins and protects cells from ubiquitin-induced degradation [11,12]. Sumoylation is functionally a more varied modifier than ubiquitination and unlike ubiquitination, proteins do not directly target the proteasome. Instead, sumoylation blocks proteosomal degradation by competing with ubiquitination for a common lysine residue and substrate samples are used for this process [5,23]. SUMO and ubiquitin also have a variety of functional and structural properties that play a role in the regulation and coordination of different stages of DNA damage recognition and repair, regulation of replication and replication stress, protection of genomic stability, and various other cellular events [14,23].

In our study, two significant SNPs were determined in the SUMO4 gene (rs237025 and rs237024) and two additional SNPs were found in the SUMO3 gene (rs180313 and rs235293). To our knowledge, three of the four significant SNPs have not been previously reported and rs237025 has only been linked to diabetes mellitus, another degenerative disease. None of the identified SNPs appear to be associated with severity or clinical features. Nevertheless, increased prevalence of these SNPs in PD patients might be due to their involvement in the pathogenesis of PD. The rs237025 variant of SUMO4 causes a missense mutation leading methionine to convert to valine. SUMO4 is known to contribute to enhanced cell survival through suppression of inflammation. Moreover, the rs237025 variant of SUMO4 has been suggested to promote intracellular inflammation pathways through NF-κB activation and thus negatively influence cell survival [24]. It is well known that different degenerative disorders might share common molecular mechanisms and due to its multifunctionality, SUMO might be one of these common mechanism factors.

The rs237024 SNP is in the 3'UTR region of the SUMO4 gene and rs180313 and rs235293 genes are found in the intronic area of the SUMO3 gene. Nevertheless, it is well known that mutations and SNPs occurring in non-coding regions may have significant molecular and clinical consequences [25]. Exact mechanisms by which these SNPs contribute to PD pathogenesis need to be further studied.

A notable finding in our study was the association between SUMO3 rs180313 and rs235293 variants and family history of cancer. To our knowledge, SNPs of SUMO genes have not been associated with increased cancer risk. However, SNPs of SUMO-conjugating enzyme UBC9 and E3 SUMO-protein ligase protein inhibitor of activated STAT 3 (PIAS3) genes have been shown to contribute to increased risk of breast cancer [26]. Nevertheless, since only a limited number of patients displayed these variants, our results need to be confirmed by future studies.

CHR	REGION	GENE	TYPE	REFERENCE	ALLELE	P VALUE	BONFERRONI	FDR P VALUE CORRECTION	SAMPLE # (CASE)	TOTAL # (CASE)	SAMPLE FREQUENCY (CASE)	SAMPLE # (CONTROL)	TOTAL # (CONTROL)	SAMPLE FREQUENCY (CONTROL)	dbSNP / rs NUMBER	1000G	MAF
6	**149400554**	**SUMO4**	**SNV**	**G**	**A**	**0,025967806**	**0,233710254**	**0,058427563**	**43**	**54**	**79,62962963**	**46**	**74**	**62,16216216**	**237025**	**0,354**	**0.354 G**
6	**149400829**	**SUMO4**	**SNV**	**T**	**C**	**0,025967806**	**0,233710254**	**0,058427563**	**43**	**54**	**79,62962963**	**46**	**74**	**62,16216216**	**237024**	**0,298**	**0.298 T**
21	**44807883**	**SUMO3**	**SNV**	**C**	**T**	**0,034386497**	**1**	**0,756402927**	**8**	**54**	**14,81481481**	**3**	**74**	**4,054054054**	**180313**	**0,965**	**0.035 T**
21	**44809015**	**SUMO3**	**SNV**	**C**	**T**	**0,034386497**	**1**	**0,756402927**	**8**	**54**	**14,81481481**	**3**	**74**	**4,054054054**	**235293**	**0,973**	**0.027 T**
21	44808250	SUMO3	SNV	T	C	0,106078076	1	0,794894473	11	54	20,37037037	8	74	10,81081081	7283639	0,93	0.070 C
6	149399822	SUMO4	SNV	A	G	0,148891461	1	0,223337191	24	54	44,44444444	25	74	33,78378378	34097428	0,804	0.196 G
6	149401053	SUMO4	SNV	A	C	0,148891461	1	0,223337191	24	54	44,44444444	25	74	33,78378378	9498344	0,804	0.196 G
21	44813315	SUMO3	SNV	C	T	0,213242759	1	0,794894473	11	54	20,37037037	10	74	13,51351351	2838696	0,933	0.067 T
17	75175696	SUMO2	SNV	G	A	0,271711759	1	0,65625	7	54	12,96296296	6	74	8,108108108	149700459	0,925	0.052 T
17	75175696	SUMO2	SNV	G	T	0,275238546	1	0,65625	15	54	27,77777778	16	74	21,62162162	149700459	-	-
21	44807449	SUMO3	SNV	G	A	0,31813023	1	0,794894473	9	54	16,66666667	9	74	12,16216216	73232962	0,944	0.056 A
21	44807688	SUMO3	SNV	T	C	0,321434473	1	0,794894473	22	54	40,74074074	26	74	35,13513514	9984357	0,237	0.237 C
21	44807957	SUMO3	SNV	C	A	0,380953577	1	0,794894473	13	54	24,07407407	15	74	20,27027027	2838693	2838693	0.293 A
17	75182038	SUMO2	SNV	A	G	0,38285644	1	0,656252	2	54	3,703703704	1	74	1,351351351	548029059	0,998	0.002 G
21	44813057	SUMO3	SNV	A	G	0,403359823	1	0,794894473	18	54	33,33333333	22	74	29,72972973	17217834	0,937	0.063 G
17	75181714	SUMO2	SNV	G	T	0,42041558	1	0,65625	5	54	9,259259259	5	74	6,756756757	75642533	0,974	0.026 T
17	75167687	SUMO2	SNV	C	T	0,421875	1	0,65625	1	54	1,851851852	0	74	0	533678937	0,999	<0.01 T
17	75167713	SUMO2	SNV	A	G	0,421875	1	0,65625	1	54	1,851851852	0	74	0	118066102	0,994	0.006 G
17	75174283	SUMO2	SNV	T	C	0,421875	1	0,65625	1	54	1,851851852	0	74	0	187263668	0,996	0.004 C
17	75174620	SUMO2	DEL	T	-	0,421875	1	0,65625	1	54	1,851851852	0	74	0	-	-	-
17	75174993^75174994	SUMO2	INS	-	T	0,421875	1	0,65625	1	54	1,851851852	0	74	0	-	-	-
17	75175161	SUMO2	SNV	T	A	0,421875	1	0,65625	1	54	1,851851852	0	74	0	-	-	-
17	75175249	SUMO2	SNV	C	T	0,421875	1	0,65625	1	54	1,851851852	0	74	0	-	-	-
17	75175651	SUMO2	SNV	A	G	0,421875	1	0,65625	1	54	1,851851852	0	74	0	-	-	-
17	75175860	SUMO2	SNV	T	C	0,421875	1	0,65625	1	54	1,851851852	0	74	0	-	-	-
17	75175956	SUMO2	SNV	T	A	0,421875	1	0,65625	1	54	1,851851852	0	74	0	-	-	-
17	75181348^75181349	SUMO2	MNV	GC	AA	0,421875	1	0,65625	1	54	1,851851852	0	74	0	-	-	-
17	75181878	SUMO2	SNV	G	A	0,421875	1	0,65625	1	54	1,851851852	0	74	0	-	-	-
17	75181977^75181978	SUMO2	INS	-	G	0,421875	1	0,65625	1	54	1,851851852	0	74	0	567293884	0,006	0.006 G
17	75182888	SUMO2	SNV	G	C	0,421875	1	0,65625	1	54	1,851851852	0	74	0	563782248	0,999	<0.001 C
21	44805481	SUMO3	SNV	C	T	0,421875	1	0,794894473	1	54	1,851851852	0	74	0	2838692	0,867	0.133 T
21	44806880	SUMO3	SNV	G	A	0,421875	1	0,794894473	1	54	1,851851852	0	74	0	533039230	0,997	0.003 A
21	44806939	SUMO3	SNV	G	C	0,421875	1	0,794894473	1	54	1,851851852	0	74	0	0	0	0
21	44807181	SUMO3	SNV	C	G	0,421875	1	0,794894473	1	54	1,851851852	0	74	0	146097096	0,996	0.004 G
21	44807507	SUMO3	SNV	G	A	0,421875	1	0,794894473	1	54	1,851851852	0	74	0			
21	44809654	SUMO3	SNV	C	T	0,421875	1	0,794894473	1	54	1,851851852	0	74	0	568371060	0,995	0.005 T
21	44813234	SUMO3	SNV	C	T	0,421875	1	0,794894473	1	54	1,851851852	0	74	0	-	-	-
21	44813674	SUMO3	SNV	A	T	0,421875	1	0,794894473	1	54	1,851851852	0	74	0	-	-	-
21	44813702	SUMO3	SNV	A	G	0,421875	1	0,794894473	1	54	1,851851852	0	74	0	-	-	-
21	44813967	SUMO3	SNV	G	A	0,421875	1	0,794894473	1	54	1,851851852	0	74	0	375002240	0,999	0.003 A
21	44814454	SUMO3	SNV	T	C	0,421875	1	0,794894473	1	54	1,851851852	0	74	0	-	-	-
2	202238678	SUMO1	SNV	G	A	0,443984885	1	0,794894473	38	54	70,37037037	50	74	67,56756757	3754931	0,627	0.063 T
21	44808833	SUMO3	SNV	C	T	0,444624014	1	0,794894473	19	54	35,18518519	24	74	32,43243243	873301	0,937	0.063 T

Table 3: The genetic characteristics and statistical results of the identified variants.

	PD patients with rs237025 and rs237024 variants (42)	PD patients without rs237025 and rs237024 variants (12)	p-value
Age	62.8 ± 11.8	62.8 ± 17.2	0.497*
Gender (M/F)	27/15	7/5	0.970**
Age of disease onset	52.6 ± 12.9	54.6 ± 15.3	0.347*
Duration of disease	10.1 ± 6.2	8.2 ± 5.5	0.150*
Hoehn-Yahr score	1.8 ± 0.6	2.1 ± 0.7	0.094†
UPDRS toral-score	64.5 ± 66.2	66.2 ± 32.1	0.443†
History of PD in the family	6	3	0.660**
History of cancer in the family	7	5	0.148**
History of DM type II in the family	9	5	0.299**
Postural instability††	1	1	0.923**
Dementia	3	1	0.889**
Depression	5	2	0.664**
DM: diabetes mellitus; M: male; F: female.			
* Student's t-test; ** chi-square test; † Mann-Whitney U test; †† first symptom of PD.			

Table 4A: Distribution of demographic and clinical features of Parkinson's disease (PD) patients according to the presence of rs237025 and rs237024 variants in the SUMO4 gene.

	PD patients with rs180313 and rs235293 variants (8)	PD patients without rs180313 and rs235293 variants (46)	p-value
Age	62.0 ± 10.3	62.9 ± 13.5	0.415*
Gender (M/F)	5/3	29/17	0.976**
Age of disease onset	55.0 ± 9.9	52.7 ± 13.9	0.294*
Duration of disease	7.0 ± 4.9	10.2 ± 6.2	0.165*
Hoehn-Yahr score	1.8 ± 0.5	1.9 ± 0.7	0.273†
UPDRS toral -score	50.4 ± 31.0	67.6 ± 46.2	0.101†
History of PD in the family	2	7	0.864**
History of cancer in the family	6	6	0.001**
History of DM type II in the family	4	10	0.212**
Postural instability††	0	2	0.164**
Dementia	1	3	0.551**
Depression	1	6	0.966**
DM: diabetes mellitus; M: male; F: female.			
* Student's t-test; ** Chi-square test; † Mann-Whitney U test; †† First symptom of PD.			

Table 4B: Distribution of demographic and clinical features of Parkinson's disease (PD) patients according to the presence of rs180313 and rs235293 variants in the SUMO3 gene.

Conclusion

In conclusion, in this study, we have determined certain SNPs in SUMO genes for the first time in PD patients, have found a link between SUMO4 and a neurological disease for the first time and thus provided further evidence for involvement of sumoylation in the pathophysiology of the disease. The mechanisms by which identified SUMO3 and SUMO4 SNPs contribute to the pathogenesis of PD need to be further studied by functional experiments. Our results also indicate that sumoylation molecules may be potential targets for novel therapeutics of PD. This notion requires a better understanding of biochemical activators of SUMO genes, which have been vastly understudied.

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Compliance with Ethical Standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of Istanbul University, Istanbul Faculty of Medicine, Clinical Research Ethical Committee (Project Number 2011/672-528) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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