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ORIGINAL ARTICLE

Poly(ADP-ribose) polymerase-1 (PARP-1) longer alleles spanning the promoter region may confer protection to bilateral Meniere's disease

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Abstract
Conclusion: The longer alleles $(CA)_{17–20}$ of the promoter region of PARP-1 gene may confer some protection against bilateral Meniere's disease (BMD). *Objective:* To analyze microsatellite $(CA)_n$ polymorphisms in the promoter region of PARP-1 gene and seek out risk and protective variants for BMD. *Subjects and methods:* Eighty patients from two ethnically defined groups with definite BMD, according to the diagnostic scale of the American Academy of Otolaryngology Head and Neck Surgery, were compared with a group of 371 normal controls from the same origin in a prospective multicenter study. We developed a specific amplification protocol to determine the PARP1-promotor CA microsatellite polymorphisms. *Results:* We found that the longer alleles $(CA)_{17–20}$ had a very low frequency in BMD (2/160, 1.3%, OR = 7.33 (1.77–30.37, 95% CI), corrected $p = 0.012$), suggesting that it may confer some protection against BMD.

Keywords: *Sensorineural hearing loss, vertigo, inner ear, transcription factor, neuronal death, endolymphatic hydrops*

Introduction

Bilateral Meniere's disease (BMD) is a severe disease affecting the inner ear, which usually results in bilateral severe or profound sensorineural hearing loss and chronic dysequilibrium with loss of vestibular function [1]. Although the etiology is unknown, endolymphatic hydrops is a consistent finding in Meniere's disease and it is probably the result of a disturbance in the ionic homeostasis in endolymph [2].

Poly(ADP-ribose)-polymerase 1 (PARP-1) is a nuclear enzyme that contributes to both neuronal death and survival under stress conditions, a situation occurring in spiral ganglion neurons (SGNs) after the development of endolymphatic hydrops [3]. PARP-1 is the most abundant of several PARP family members, accounting for >85% of nuclear PARP activity, and it is present in spiral ganglion neurons [4]. When activated by DNA damage, PARP-1 consumes nicotinamide adenine dinucleotide (NAD+) to form branched polymers of ADP-ribose on nuclear acceptor proteins, which include DNA ligases, histones, and PARP-1 itself [5]. This process can have at least three important consequences in SGNs, depending on the extent of DNA damage: 1) poly(ADP-ribose) formation on histones and on the enzyme itself is involved in DNA repair, can prevent sister chromatid exchange, and facilitate base-excision repair; 2) poly(ADP-ribose) formation can influence the action of transcription

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factors, such as nuclear factor kappaB (NFkB), and thereby promote inflammation; and 3) extensive PARP-1 activation can promote neuronal death through mechanisms involving NAD+ depletion and release of apoptosis inducing factor (AIF) from the mitochondria. PARP-1 activation is thereby a key mediator of neuronal death during NMDA-mediated excitotoxicity and oxidative stress [6], a proposed model for death in SGNs [3].

The human PARP-1 gene (MIM 173870) is located in chromosome 1q42 and consists of 23 exons spanning 43 kb. The promoter contains a polymorphic CA nucleotide repeat in the 5′ flanking sequence of the N-terminal DNA-binding domain [7] (Figure 1). Interestingly, this multiallelic polymorphism is located close to the binding site of the transcription factor Yin Yang 1, and therefore, it might affect PARP-1 transcription [8]. Additionally, four sequence variations have been identified in this region: C410T, poly(A)n, C1362T, and G1672A [9]. These polymorphisms are part of two unique haplotypes of the PARP-1 promoter, which includes four consecutive PARP-1 polymorphisms: haplotype A (410T-[A10]-short CA repeats [83–87 bp]-1362C) and haplotype B (410TC-[A11]-long CA repeats [89–101 bp]-1362T), the CA microsatellite being the haplotype-defining variant of the whole PARP-1 promoter polymorphism [10]. Our hypothesis was that structural changes in the $(CA)_n$ microsatellite could miss a regulatory binding site at the promoter and this may lead to a change in the transcription rate of PARP-1.

The aim of this study was to analyze the microsatellite $(CA)_n$ in the promoter region of the PARP-1 gene in patients with definite BMD.

Subjects and methods

Sources of DNA

The present study included 80 patients with BMD and 371 healthy volunteer blood donors. All patients were diagnosed according to the diagnostic scale for MD of the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) [11]. The study group consisted of 42 Mediterranean individuals and 38 patients from Galicia with BMD. Five centers recruited patients for this study: Hospital Clinico Universitario, Santiago de Compostela; Hospital de Pontevedra and Hospital La Fe, Valencia; Hospital Virgen de las Nieves, Granada; and Hospital de Poniente, El Ejido, Almeria. Each subject's informed consent was obtained to participate in the study according to the Declaration of Helsinki and the local institutional committees approved the study. The clinical features of patients with MD affecting both ears were reported previously [12].

PARP-1 polymorphism genotyping

To determine PARP-1 CA microsatellite, standard protocols and polymerase chain reaction (PCR) were used for amplification, with annealing temperatures of 62°C and previously published sense and antisense primers (5′-GATTCCCCATCTCTTTCTTT-3′ and 5′-AAATTGTGGTAATGACTGCA-3′) for PARP-1 CA [7]. Forward primers were 5'-labeled with the fluorescent dye FAM. PCR aliquots (0.5 μl) were added to 14 μl of formamide and 0.5 μl of internal size standard (Genescan-500 LIZ, Applied Biosystems). Samples were analyzed by capillary electrophoresis in a 3130 XL Genetic Analyzer (Applied Biosystems) with Data Collection Software V3.0. Alleles for CA repeats were assigned using Gene Mapper Software V4.0 (Applied Biosystems).

Statistical analysis

Data were analyzed using SPSS Software (SPSS Inc., Chicago, IL, USA). For association studies, a global chi-squared test with Yates's corrections or Fisher's exact test, when it was appropriate, was performed. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated to compare the observed frequencies between patients with BMD and the controls. *p* values were corrected for the number of alleles determined.

Results

The CA microsatellite was selected as a genetic marker to investigate the role of PARP-1 promoter polymorphism in BMD susceptibility. Ten CA microsatellite repeats were found, showing a bimodal distribution, with 12 and 16 repeats being

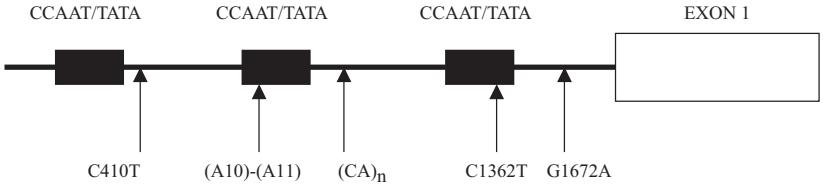

Figure 1. 5′ Flanking region of the PARP1 gene containing the polymorphic CA_n repeats.

Table I. Comparison of the number of repeats of the $(CA)_n$ microsatellite in the promoter of the PARP-1 gene between controls and subjects with BMD.

$(CA)_n$	Control	Bilateral MD	χ^2	OR (95% CI)	Uncorrected p value	Corrected p value
10–12	528 (71.2%)	124 (77.5%)	2.64	0.72 (0.48–1.07)	0.127	NS
13	21 (2.8%)	2 (1.3%)	1.32	2.30 (0.53–9.91)	0.382	NS
14	4 (0.5%)	2 (1.3%)	1.01	0.43 (0.08–2.36)	0.289	NS
15	1 (0.1%)	1 (0.6%)	1.43	0.21 (0.01–3.45)	0.323	NS
16	125 (16.8%)	29 (18.1%)	0.15	0.91 (0.59–1.43)	0.784	NS
17–20	63 (8.5%)	2 (1.3%)	10.32	7.33 (1.77–30.27)	0.002	0.012

the most frequent alleles observed in the control group (Table I).

First, a global test was used to compare the length of the CA microsatellite in patients with BMD from the Galicia and Mediterranean groups, but no difference was found in the distribution of the alleles between the two groups ($p > 0.05$). Then, each allele was compared between all patients with BMD and controls. There were no differences in the frequency of the alleles studied between patients with BMD and controls, except for the $(CA)_{17–20}$ longer alleles, a subtype in the haplotype B. These alleles containing 17–20 repeats have a very low frequency in BMD (2/160, 1.3%, OR = 7.33 (95% CI, 1.77–30.37), corrected $p = 0.012$). However, CA microsatellite repeats grouped in haplotype A or B did not differ between controls and BMD. Comparison of genotypes AA, AB or BB between patients and controls did not show significant differences (Table II).

Discussion

This study demonstrates that the CA microsatellite polymorphism containing 17–20 repeats located at the promoter of PARP-1 gene is very uncommon in BMD. The lack of larger alleles of the PARP-1 promoter, which may bind different transcription factors, will decrease the PARP-1 transcription rate and its ability to repair damaged DNA and will increase death in SGNs.

CA microsatellite alleles were previously grouped into two haplotypes: CA repeats between 10 and 12 $(CA)_{10–12}$, considered as haplotype A, and $(CA)_{13–20}$ repeats grouped as haplotype B [10]. Haplotype A was significantly associated with celiac disease (CD), with a dose effect, showing homozygous individuals for haplotype A with a higher risk for CD [13]. It was hypothesized that the different lengths of CA microsatellite could affect the promoter structure, modifying the transcription rate and thus interactions with other molecules, such as different NF-κB complexes. Recent reports provided strong evidence that PARP-1 is required for NF-κB-dependent gene expression in a stimuli-dependent manner and acts as a transcriptional co-activator of NF-κB [14]. A shorter CA microsatellite could miss a regulatory binding site at the promoter and this also led to a change in the transcription of PARP-1. The potential targets for PARP-1 have increased recently, and 12 proteins have been documented to interact with PARP-1 in the complex machinery replication process: proliferating cell nuclear antigen (PCNA), DNA topoisomerase I, DNA ligase I, DNA Polα and β, DNA topoisomerase II, MSH2, and the replication factor complex 1–5 (RFC1, RFC2, RFC3, RFC4, RFC5). PARP-1 may regulate these complexes as a whole rather than regulate one or more individual components [15]. Larger alleles of the PARP-1 promoter may interact with different transcription factors, increasing PARP-1 transcription rate, facilitating DNA repair and the resistance to death in SGNs, so conferring protection against BMD.

NMDA-mediated excitotoxicity has recently been proposed as a key mechanism in the loss of SGNs in ototoxicity and endolymphatic hydrops [3,16,17]. It is likely that hydrops-associated ionic disturbances in the cochlea lead to oxidative stress within all cells of

Table II. Haplotype and genotypes frequencies of the $(CA)_n$ polymorphism in controls and subjects with BMD.

Parameter	Control	Bilateral MD	χ^2	OR (95% CI)	Uncorrected p value	Corrected p value
Haplotype A	528 (71.2%)	124 (77.5%)	2.64	0.72 (0.48–1.07)	0.127	NS
Haplotype B	214 (28.8%)	36 (22.5%)	2.64	0.72 (0.48–1.07)	0.100	NS
Genotype AA	198 (26.7%)	51 (31.9%)	1.77	1.29 (0.89–1.86)	0.217	NS
Genotype AB	132 (17.8%)	22 (13.8%)	1.52	0.73 (0.45–1.20)	0.264	NS
Genotype BB	412 (55.5%)	87 (54.4%)	0.7	1.05 (0.74–1.48)	0.859	NS

the organ of Corti [2,18]. However, cells such as SGNs that receive the added input of glutamate would be subjected to additional stress due primarily to NMDA receptor-induced Ca^{2+} influx and PARP-1 activation. The length of the promoter may be crucial, since the loss of binding sites at the promoter may determine the level of transcription of PARP-1 and its activity. The poly(ADP)-ribosylation of histones causes chromatin loosening and is thought to thereby facilitate DNA repair and transcriptional regulation and coordinate interactions among proteins involved in DNA repair in the CNS [6].

Moreover, the activation of PARP-1 leads to an intrinsic cell death program where PAR polymer appears to be a pro-death signaling molecule that releases AIF from the mitochocondria, translocates to the nucleus, and causes nuclear condensation and cell death in neurons after NMDA excitotoxicity [19]. In fact, NMDA glutamate receptor excitotoxicity appears to require AIF, as neutralizing AIF antibodies can reduce NMDA excitotoxicity [20]. Further studies are required to determine the level of PARP-1 activity in SGNs in animal models of endolymphatic hydrops and patients with MD.

Conclusions

The longer alleles $(CA)_{17–20}$ of the PARP-1 gene have a very low frequency in patients with BMD, suggesting that they may confer some protection against BMD.

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