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...
factors, such as nuclear factor kappa B (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:

### 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

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**Figure 1.** 5’ Flanking region of the PARP1 gene containing the polymorphic CA\(\_\)n repeats.
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
longer alleles, a subtype in the haplotype B. These
alleles containing 17 repeats have a very low
frequency in BMD (2/160, 1.3%, OR = 7.33 (95%
CI, 1.77–30.27), 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 Polz and b, DNA topoisomerase
II, MSH2, and the replication factor complex 1
(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 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.

<table>
<thead>
<tr>
<th>(CA)n</th>
<th>Control</th>
<th>Bilateral MD</th>
<th>χ²</th>
<th>OR (95% CI) Uncorrected p value Corrected p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10−12</td>
<td>528 (71.2%)</td>
<td>124 (77.5%)</td>
<td>2.64</td>
<td>0.72 (0.48–1.07)</td>
</tr>
<tr>
<td>13</td>
<td>21 (2.8%)</td>
<td>2 (1.3%)</td>
<td>1.32</td>
<td>2.30 (0.53–9.91)</td>
</tr>
<tr>
<td>14</td>
<td>4 (0.5%)</td>
<td>2 (1.3%)</td>
<td>1.01</td>
<td>0.43 (0.08–2.36)</td>
</tr>
<tr>
<td>15</td>
<td>1 (0.1%)</td>
<td>1 (0.6%)</td>
<td>1.43</td>
<td>0.21 (0.01–3.45)</td>
</tr>
<tr>
<td>16</td>
<td>125 (16.8%)</td>
<td>29 (18.1%)</td>
<td>0.15</td>
<td>0.91 (0.59–1.43)</td>
</tr>
<tr>
<td>17−20</td>
<td>63 (8.5%)</td>
<td>2 (1.3%)</td>
<td>10.32</td>
<td>7.33 (1.77–30.27)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Bilateral MD</th>
<th>χ²</th>
<th>OR (95% CI) Uncorrected p value Corrected p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haplotype A</td>
<td>528 (71.2%)</td>
<td>124 (77.5%)</td>
<td>2.64</td>
<td>0.72 (0.48–1.07)</td>
</tr>
<tr>
<td>Haplotype B</td>
<td>214 (28.8%)</td>
<td>36 (22.5%)</td>
<td>2.64</td>
<td>0.72 (0.48–1.07)</td>
</tr>
<tr>
<td>Genotype AA</td>
<td>198 (26.7%)</td>
<td>51 (31.9%)</td>
<td>1.77</td>
<td>1.29 (0.89–1.86)</td>
</tr>
<tr>
<td>Genotype AB</td>
<td>132 (17.8%)</td>
<td>22 (13.8%)</td>
<td>1.52</td>
<td>0.73 (0.45–1.20)</td>
</tr>
<tr>
<td>Genotype BB</td>
<td>412 (55.5%)</td>
<td>87 (54.4%)</td>
<td>0.7</td>
<td>1.09 (0.74–1.48)</td>
</tr>
</tbody>
</table>
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 mitochondria, 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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References