Investigation of the Prion protein in subjects with chronic tinnitus

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1 Objectives

The prion protein (PrP) plays a major role in central nervous excitability, cellular adhesion, and neurite outgrowth (figure 1). PrP interacts or associates with the 67-kD laminin receptor (table 1), the 37-kD laminin receptor precursor, or the ECM glycoprotein lamin (Rieger et al., 1997; Gruner et al., 2000; Gauczynski et al., 2001). PrP has also been identified in a complex with the neural cell adhesion molecule (NCAM) by chemical cross-linking (Schmitt-Ulms et al., 2001). More recent data confirm that PrP interacts directly with NCAM and associates with NCAM at the neuronal surface. Here, both cis and trans interactions promote recruitment of NCAM to lipid rafts and thereby regulate activation of fyn kinase, an enzyme involved in NCAM-mediated signaling (Santuccione et al., 2005). When these interactions are disrupted, neuronal outgrowth is arrested.

In PrP knockout mice, most phenotypic changes reported have been subtle, perhaps because of backup systems that can at least partly compensate for a lack of PrP.

PrP 0/0 animals feature alterations in circadian rhythm, hippocampal neuronal function, spatial learning, brain copper and cuproenzyme levels, oxidative tissue damage, phagocytosis and inflammatory response, haematopoietic-stem-cell renewal, neural-stem-cell differentiation and stress responses. There have also been claims and counter-claims regarding the modulation of cellular apoptosis by PrP, and the neurotoxicity of PrP peptides. We hypothesized that PrP variants may modify vulnerability to chronic tinnitus, a condition frequently associated with maladaptive neuronal repair in the auditory pathway.

2 Methods

1 39 Caucasian patients who presented with tinnitus lasting more than 6 months were recruited from a tinnitus clinic, underwent detailed neurootological examinations and donated venous blood for the extraction of genomic DNA from lymphocytes. Subjects with a history of vestibular Schwannoma, Meniere’s disease or pathological middle ear conditions were excluded. Tinnitus severity was graded using the Tinnitus Questionnaire (TQ). A polymorphism (rs124241) encoding residue 117 of PrP was genotyped by PCR-based RFLP.

Briefly, the following primers were used to generate a 754bp amplicon: 5'-AGGGTGACATTGGTCCTGAG-3' (forward) and 5'-TGCAACAGGTTGTCTGTTG-3' (reverse). Restriction fragments were obtained with Alu (MBI Fermentas, St. Leon-Rot, Germany). Following electrophoresis on 3% agarose gels prestained with ethidium bromide, results were visualized under UV transillumination. Bands corresponding to fragments of 458, 178 and 118bp indicated carriers of the wildtype allele (A), bands corresponding to fragments of 636 and 118bp indicated carriers of the variant allele (G) (fig. 2).

3 Results

When carriers of the minor PrP allele (AG) were compared to the remaining subjects (AA), we noted only a moderate difference in mean TQ scores (33.4 ± 4.9 vs. 37.4 ± 1.5, p<0.05, figure 3).

4 Conclusion

The present results do not support a significant effect of PrP variant rs124241 on the manifestation of tinnitus as measured by subjective ratings. However, functional PrP variants may act on additional outcome parameters, e.g. on the response to treatment. Future studies will need to address the interplay of these variants, and their effects on specific tinnitus phenotypes.

5 References


Table 1: Cellular distribution and activities of PrP in cell types in which known or putative functions have been described

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Function</th>
<th>Mechanisms, agents and pathways</th>
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<tbody>
<tr>
<td>Neuron</td>
<td>Neuronal functions</td>
<td>Neuronal survival; axonal transport; synaptic plasticity</td>
</tr>
<tr>
<td>Blood cell</td>
<td>Haematopoietic-stem-cell renewal; neural-stem-cell differentiation</td>
<td>PrP interacts with LRP/LR and HSPG by means of separate sites</td>
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<tr>
<td>Lymphocyte/monocyte</td>
<td>Copper-binding</td>
<td>Copper homeostasis</td>
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<tr>
<td>Lymphocyte/monocyte</td>
<td>Cell survival</td>
<td>PrP upregulation upon mitogen-induced activation</td>
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<tr>
<td>Leukocyte</td>
<td>Homing</td>
<td>PrP alters leukocyte recruitment to site of inflammation</td>
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Figure 1: Neurite outgrowth is modulated by PrP interactions with NCAM and STI-1, which can lead to activation of fyn kinase, an enzyme involved in NCAM-mediated signaling (Santuccione et al., 2005). When these interactions are disrupted, neuronal outgrowth is arrested.

Figure 2: 39 Caucasian patients who presented with tinnitus lasting more than 6 months were recruited from a tinnitus clinic, underwent detailed neurootological examinations and donated venous blood for the extraction of genomic DNA from lymphocytes. Subjects with a history of vestibular Schwannoma, Meniere’s disease or pathological middle ear conditions were excluded. Tinnitus severity was graded using the Tinnitus Questionnaire (TQ). A polymorphism (rs124241) encoding residue 117 of PrP was genotyped by PCR-based RFLP.

Figure 3: Subjective ratings of tinnitus stratified by PrP genotype.