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Functional assessment of AIPL1 variations identifed in Leber Congenital Amaurosis patients

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Purpose: Mutations in the photoreceptor-expressed gene *AIPL1* cause autosomal recessive Leber congenital amaurosis (LCA). AIPL1 facilitates the correct assembly of retinal cGMP phosphodiesterase (PDE6) acting as a co-chaperone for HSP90. While over 400 variations have been identified throughout *AIPL1*, only a handful have been experimentally validated and the disease-causing status is often based on in silico predictions of pathogenic probability. Therefore, the functional assessment and confirmation of likely pathogenic AIPL1 variants in this study is an important step towards an accurate and early diagnosis and treatment of LCA patients.

Methods: Expression and subcellular localisation of AIPL1 variants was examined by western blotting and immunofluorescent confocal microscopy. To test their ability to interact with HSP90, directed yeast two hybrid (Y2H) and quantitative enzyme-linked immunosorbent (ELISA) assays were performed.

Results: The C-terminal HSP90 pentapeptide MEEVD is critical for mediating the interaction with the tetratricopeptide (TPR) domain of AIPL1. The AIPL1 variants p.L17P, p.C89R, p.Q163X and p.E282_ A283dup were unable to interact with HSP90 efficiently, whereas p.G64R, p.V71F, p.K214N and p.G262S retained the ability to bind HSP90 in a TPR-dependent manner. *AIPL1* variations located in the coding region, including c.642G>C(p.K214N) and c.784G>A(p. G262S), or in the non-coding regions of *AIPL1* (c.97_104dup, c.98_99insTGATCTTG, c.276+1G>A, c.276+2T>C, c.277-2A>G, c.785-10_786del12) cause aberrant pre-mRNA splicing leading to alternative transcripts that could encode functionally deficient protein isoforms. Alternative protein isoforms, which included in-frame domain deletions, frameshift stop mutations, and small insertions and deletions, showed a significant decrease or loss of HSP90 binding affinity, with the exception of one isoform with a small insertion in the TPR domain that retained a TPR-dependent HSP90 interaction.

Conclusions: The present study has validated the disease-association and experimentally confirmed the biochemical defects underlying uncharacterised AIPL1 nonsense, missense and intronic variations.

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