ABSTRACT

Almudena Sacristan-Reviriego, James Bellingham, Chris Prodromou and Jacqueline van der Spuy.

Purpose
Mutations in the photoreceptor/pineal-expressed gene AIPL1 are associated with autosomal recessive Leber congenital amaurosis (LCA), the most severe form of inherited retinopathy that occurs in early childhood. AIPL1 functions as a photoreceptor-specific molecular co-chaperone that interacts specifically with the molecular chaperone HSP90 to facilitate the correct assembly of the retinal cGMP phosphodiesterase (PDE6) holoenzyme. The AIPL1 gene is highly polymorphic. While over 400 variations have been identified throughout the gene, 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 is an important step towards an accurate diagnosis and effective triage of patients for AIPL1-targeted gene replacement therapy.

Approach
AIPL1 variants were engineered in full length AIPL1 mammalian and yeast two hybrid (Y2H) expression vectors. The expression and subcellular localisation was examined in mammalian cells by western blotting and immunofluorescent confocal microscopy. To test the ability of AIPL1 variants to interact with HSP90, we performed directed Y2H interactions, co-immunoprecipitation assays with endogenous HSP90 and quantitative enzyme-linked immunosorbent (ELISA) assays using recombinant HSP90.

Results
Nonsense and frameshift stop coding variations resulting in the large C-terminal truncation of AIPL1 (p.R32X, p.I34DfsX10, p.F35LfsX2 and p.R38LfsX6) were not detected by western blotting and are predicted to be cleared by nonsense-mediated mRNA decay (NMD). p.W72X and p.W88X are also likely true loss-of-function pathogenic mutations as they formed intracellular aggregates of misfolded protein. The C-terminal HSP90 pentapeptide MEEVD is critical for mediating the interaction with the tetratricopeptide (TPR) domain of AIPL1. Our directed Y2H and quantitative ELISA assays confirmed that p.L17P, p.C89R, p.Q163X and p.E282_A283dup were unable to interact with HSP90 efficiently confirming their pathogenicity, whereas p.G64R, p.V71F, p.K214N and p.G262S retained the ability to bind HSP90 in a TPR-dependent manner. However, we recently reported that AIPL1 variations located in the coding region, including c.465G>T (p.Q155H), 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 (Bellingham et al., 2015). We therefore characterised the functional deficits of the resultant AIPL1 isoforms, which included in-frame domain deletions as a result of in-phase exon skipping, frameshift stop mutations, and small insertions and deletions. The loss of function was confirmed for the frameshift stop products that were not detectable by western blotting or formed intracellular aggregates of misfolded protein. All of the AIPL1 protein isoforms showed a significant decrease or loss of HSP90 binding affinity, with the exception of one leading to a small insertion in a loop in the TPR domain that interestingly retained a TPR-dependent HSP90 interaction.

Conclusions
We have validated the disease-association and experimentally confirmed the biochemical defects underlying uncharacterised AIPL1 nonsense, missense and intronic variations, and gained insight into the mechanism of binding between AIPL1 and HSP90. Our findings will be critical for ensuring an early and effective diagnosis and treatment of LCA patients.