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The half-life of different aryl hydrocarbon receptor-interacting protein (AIP) variants and their significance on clinical phenotype

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INTRODUCTION & OBJECTIVES

Mutations in the aryl hydrocarbon receptor-interacting protein (*AIP*) gene predisposes to young-onset growth hormone (GH) or prolactin secreting adenomas, usually, manifesting before the age of 30. There are 614 variants of *AIP* reported in the gnomAD database (gnomAD v2.1) and over 100 variants have been described in patients with pituitary adenomas. While the pathogenic role of variants resulting in truncated protein is beyond doubt, determination of the clinical relevance of missense variants could be challenging. In this study, we aimed to functionally assess some *AIP* variants identified in pituitary adenoma patients in order to determine their pathogenic role (**Table 1**).

Table 1. Age of disease onset and adenoma type of patients with the studied *AIP* variants

Variant	Phenotype	Variant	Phenotype
p.R9Q	14y, ACTH, macroadenoma 39y, prolactinoma 21y, GH, macroadenoma 53y, 54y and 58y family, GH macroadenoma	p.R188Q	24y, microprolactinoma
p.D30E	23y, GH	p.K241E	40y, macroprolactinoma 53y, non-functioning pituitary adenoma
p.K103R	6y, corticotrophinoma	p.E245K	24y, prolactinoma
p.R119W	32y, GH	p.A277P	12y, GH, macroadenoma
p.R128H	27y, GH, macroadenoma	p.E283Q	71y, lung carcinoma, somatic mutation
p.W168*	14y, GH, macroadenoma	p.E319K	11y, GH, macroadenoma

EXPERIMENTAL DESIGN

The stability of 12 missense *AIP* variants and one nonsense mutation was investigated through cycloheximide (CHX) chase assays (**Figure 1**). Human embryonic kidney (HEK 293T) cells were transfected with wild type (WT) and the 13 AIP variants. The cells were subsequently treated with CHX and protein lysates were quantified after Western blotting. The results were analysed using a one-phase decay equation and the degradation constants (K) of the AIP variants were compared to the WT AIP (reference for method: Hernández-Ramírez et al., JCEM, 2016).

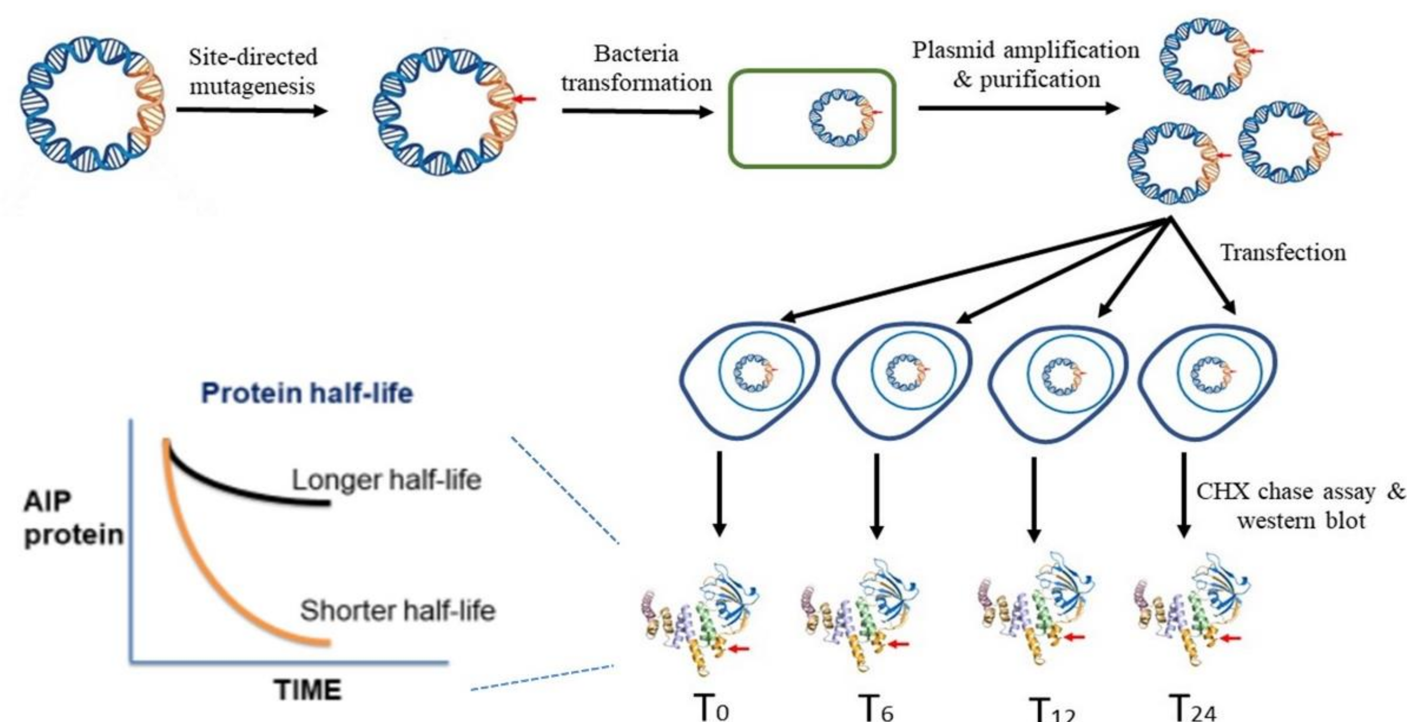

Figure 1. Scheme of the experimental design used for the evaluation of the stability of WT and variant AIP proteins. The desired missense changes were introduced into the pcDNA3.0-myc-AIP vector through site-directed mutagenesis. HEK 293T cells were transfected using polyethyleneimine and subsequently treated with cycloheximide (100µg/mL). Protein lysates were collected at 0, 6, 12, 24 hours after inhibition and blotted using anti-Myc and anti-GAPDH antibodies.

RESULTS & DISCUSSION

Figure 2 shows the results of the CHX chase experiments. The missense *AIP* variants p.A277P and p.K241E were found to be significantly less stable compared to WT AIP, suggesting these are pathogenic variants (**Figure 3A**). Two of the three short half-life variants (p.C238Y and p.K241E) affect amino acids well-known to be crucial for the stability of the C-terminal tetratricopeptide (TPR) motifs (Igreja et al., Hum Mut 2010), resulting in loss of a disulfide bond for p.C238Y and losing a conserved amino acid for p.K241E. Missense changes affecting residues involved in the folding of the TPR domain could result in misfolded proteins, leading to unstable proteins that are degraded by the proteasome pathway.

We also studied a nonsense mutation, p.W168* (**Figure 3B**), which, interestingly, showed only a slightly reduced stability. The N-terminal part of the protein (amino acids 2-166) has been crystallised separately (Linnert et al., 2013), suggesting that it is a stable protein. However, *in vivo* the mRNA of this missense variant is probably degraded by nonsense-mediated decay, so the short N-terminal protein would not be synthetized.

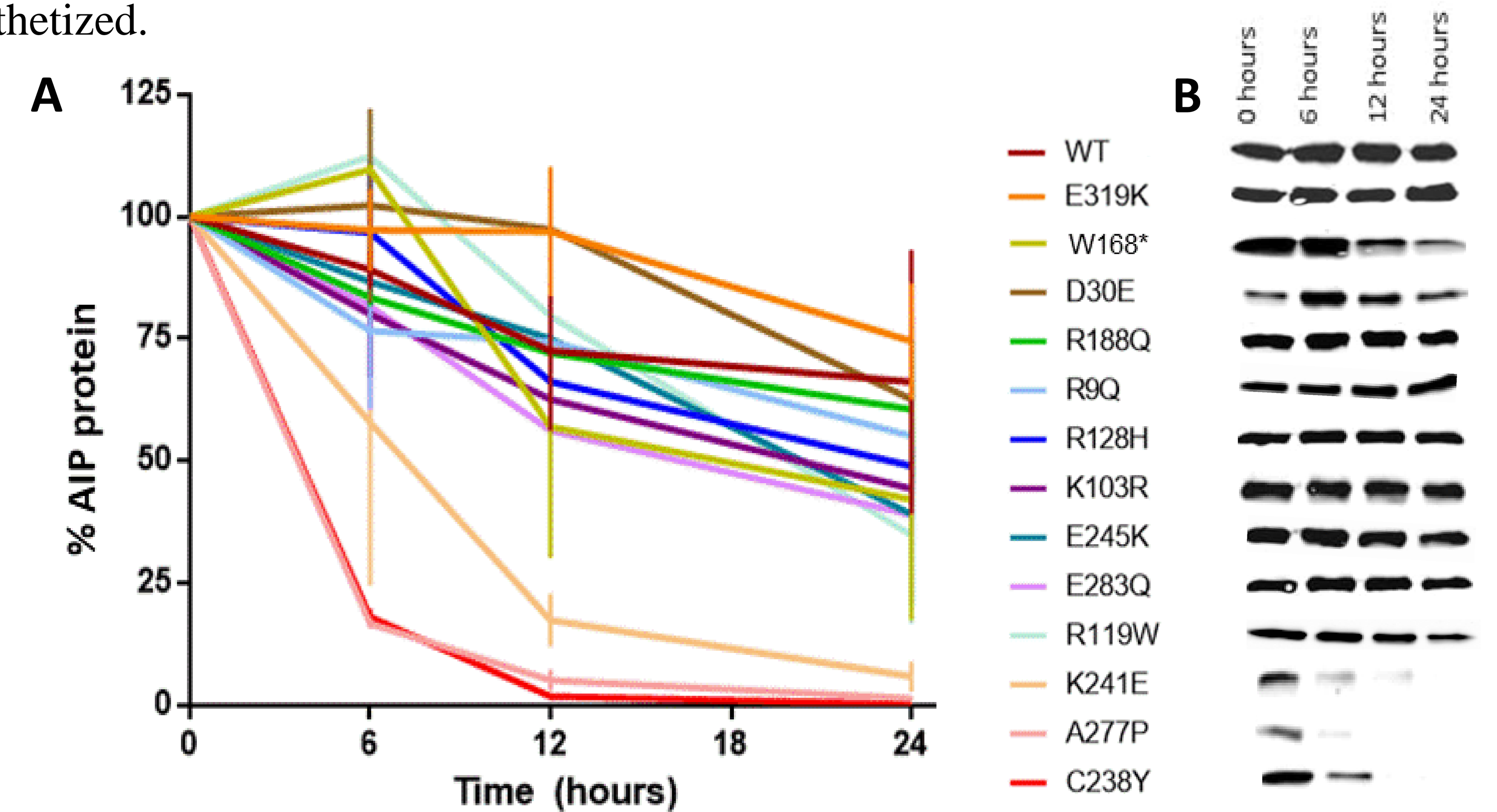

Figure 2. A) AIP protein levels expressed as % of normalised protein levels at time 0h. Each colour corresponds to a variant. **B)** Western blotting of overexpressed WT and AIP variants in HEK 293T cells.

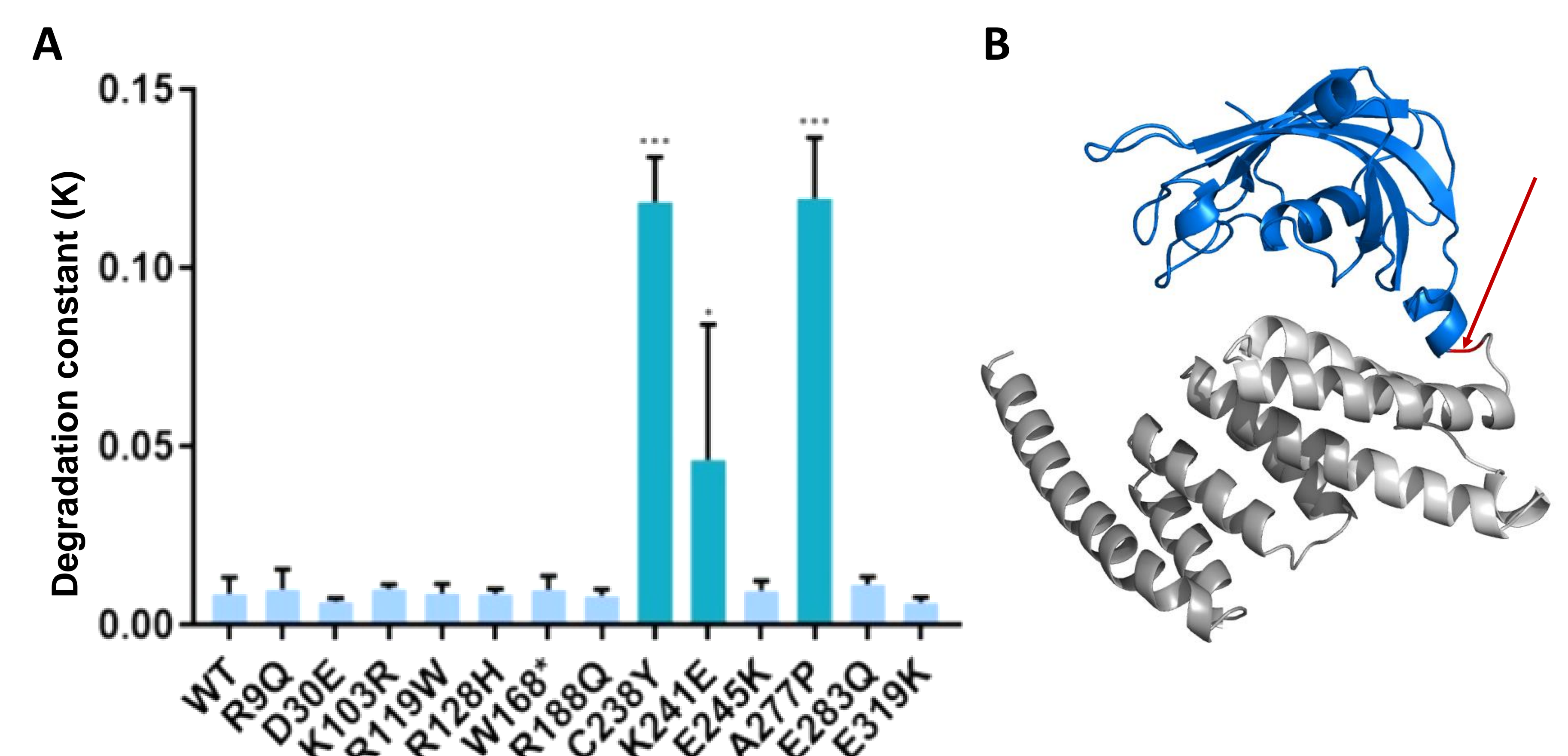

Figure 3. A) Degradation constant values of WT and AIP variants. Values are represented as mean ± SEM (biological replicates=2). ***=p<0.001, *=p<0.05 compared to the WT (one-way ANOVA and Dunnett's multiple comparisons test). **B)** Model of AIP showing in red the W168 residue, where a stop codon would leave the N-terminus FKBP-type peptidyl-prolyl cis/trans isomerase (PPIase) domain intact (blue part of the protein), while the grey C terminal TPR motif part is missing entirely.

CONCLUSIONS

- **Human AIP is a stable protein, while missense variants can have short, normal or close to normal half-life.**
- **Variants p.A277P, p.K241E & p.C238Y present with significantly higher degradation rate (K) compared to the WT, suggesting that these are pathogenic variants. Family members of patients carrying these *AIP* mutations should undergo genetic screening and carriers referred for clinical assessment.**
- **Missense variants with a normal half-life need further assessment to characterize their possible pathogenicity.**

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