The effect of AIP on AHR transcriptional activity – implications for *AIP* mutation pathogenicity

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BACKGROUND

AIP mutations cause sporadic and familial pituitary adenomas, but establishing the pathogenic role of missense *AIP* variants with unknown significance is difficult.[1] The AIP interaction partner AHR – a xenobiotic-activated transcription factor – regulates transcription of xenobiotic-metabolizing enzymes, mediates xenobiotic toxicity, and has been implicated in tumorigenesis.[2,3]

AIM

To describe the effect of *AIP* dosage and mutations on AHR–dependent transcription and use it to assess *AIP* variant pathogenicity.

MATERIALS AND METHODS

Aip was knocked-down (KD) by *Aip*-siRNA transfection or *Aip*-shRNA lentiviral transduction in the GH3 rat somatotrophinoma cell line. Wild-type (WT) or mutant human-AIP were over-expressed by transfection. AIP protein levels were assessed by immunoblotting. Expression levels of *Aip*, *Ahr*, and *Cyp1a1* (an AHR target gene) were measured by RT-qPCR, and normalized to *Gapdh*. AHR-dependent transcription was stimulated with an endogenous ligand, 6-formylindolo[3,2-b]carbazole (FICZ). Data were analysed using the statistical software JMP 11, and threshold for statistical significance was set at 5% confidence level ($p<0.05$).

RESULTS

siRNA *Aip*-KD was demonstrated by RT-qPCR (Figure 1). Immunoblotting confirmed endogenous *Aip*-shRNA KD and efficient expression of transfected human-*AIP* unaffected by RNA-interference (Figure 2).

siRNA *Aip*-KD did not significantly changed *Ahr* mRNA levels (Figure 3), but caused a significant reduction of *Cyp1a1* mRNA levels, in both FICZ and vehicle-treated cells (Figure 4).

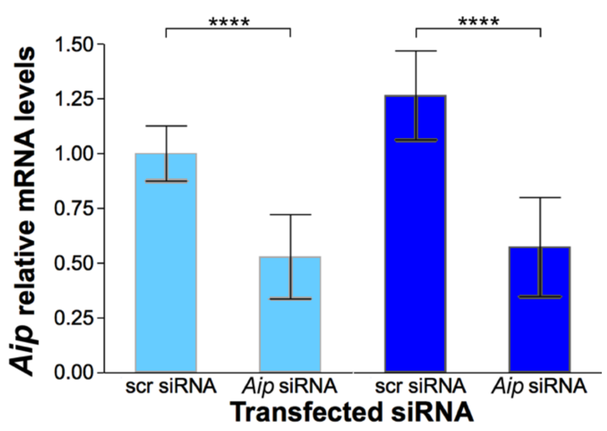

Figure 1: *Aip* mRNA levels in *Aip* siRNA-KD GH3 cells, treated with vehicle (DMSO - light blue) or 10 nM FICZ (dark blue) for 5 hrs. Bars represent means of three experiments; error bars indicate S.E.M. **** $p<0.0001$ (t-test)

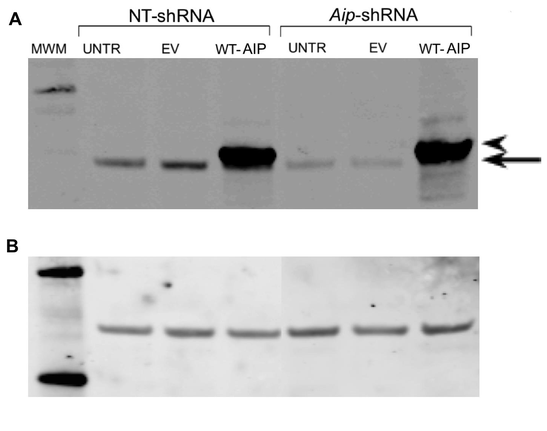

Figure 2: Immunoblotting for stably transduced GH3 cells untransfected (UNTR) or transfected with EV or WT-AIP. Panel A - AIP immunoblotting: arrow indicates the rat AIP band, arrowhead indicates the transfected human WT-AIP band. Panel B - GAPDH (loading control). MWM: molecular weight marker.

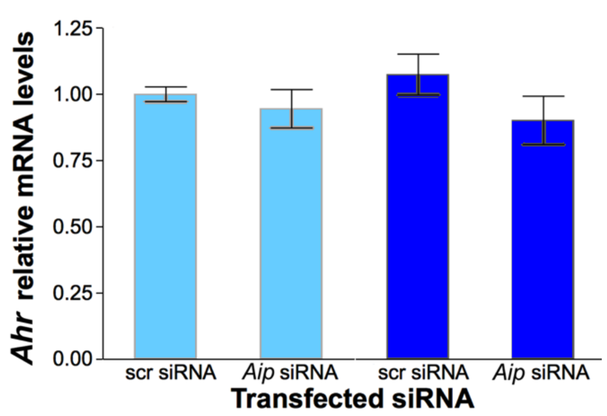

Figure 3: *Ahr* mRNA levels in *Aip* siRNA-KD GH3 cells, treated with DMSO (light blue) or FICZ (dark blue). Bars represent means of three experiments; error bars indicate S.E.M.

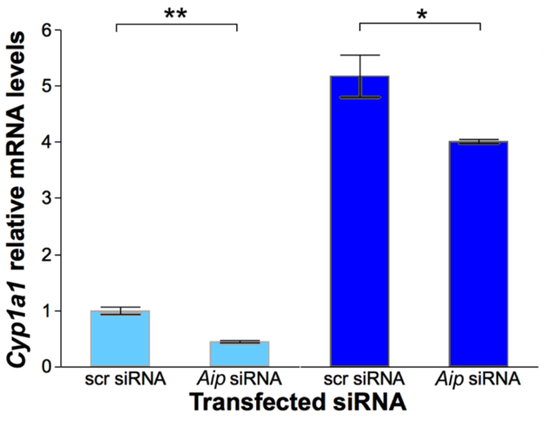

Figure 4: *Cyp1a1* mRNA levels in *Aip* siRNA-KD GH3 cells, treated with DMSO (light blue) or FICZ (dark blue). Bars represent means of triplicates; error bars indicate S.E.M. * $p<0.05$, ** $p<0.01$ (t-test)

In stable *Aip*-KD GH3 cells, reduced *Cyp1a1* expression was rescued by WT-AIP transfection, but not by the pathogenic p.R304* truncation mutant. The p.C238Y and p.R271W pathogenic missense *AIP* variants did not rescue *Cyp1a1* expression, while the likely pathogenic (based on clinical data) p.R304Q and non-pathogenic p.R16H displayed an intermediate rescue (Figure 5).

Transfection of the WT-AIP or the p.R304* truncation mutant in *Aip* shRNA-KD GH3 cells did not significantly changed Ahr mRNA levels (Figure 6).

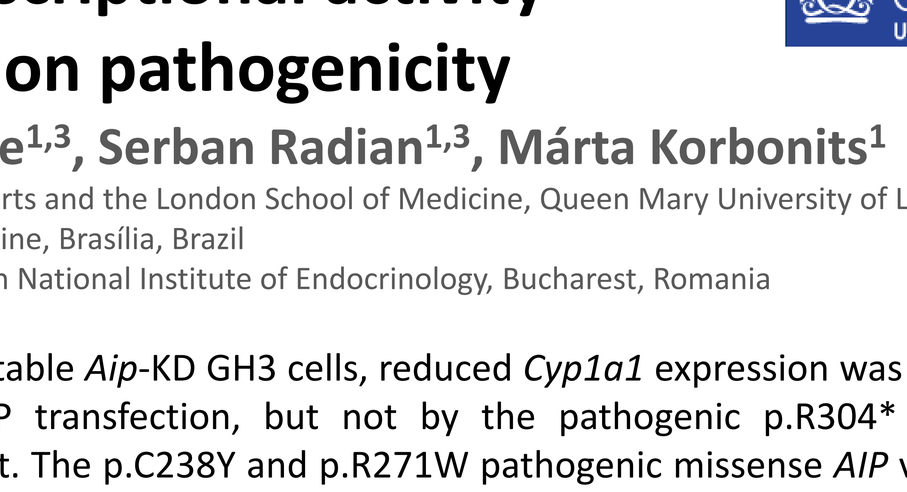

Figure 5: *Cyp1a1* relative mRNA levels in *Aip* shRNA-KD GH3 cells overexpressing AIP, and treated with FICZ 10 nM for 5 hours. Bars represent means of 1 to 3 experiments with 3 to 9 replicates in total; error bars indicate S.E.M. Levels not marked by the same letter are significantly different using ANOVA followed by Tukey-Kramer HSD (honest significant difference) post-hoc test ($p<0.05$)

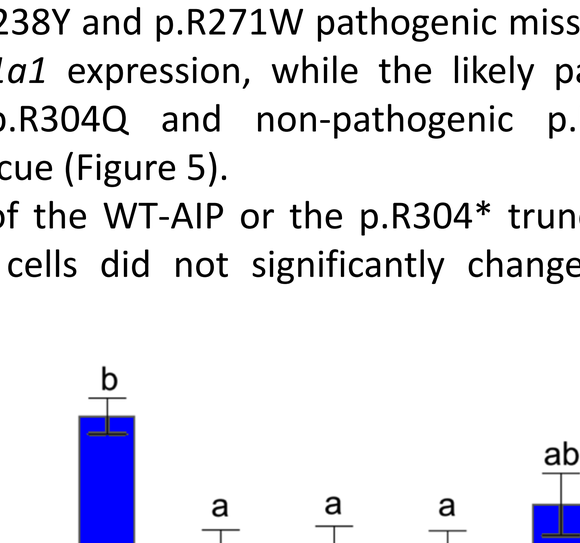

Figure 6: *Ahr* relative mRNA levels in *Aip* shRNA-KD GH3 cells overexpressing AIP, and treated with FICZ 10 nM for 5 hours. Bars represent means of 2 to 4 experiments with 3 to 12 replicates in total; error bars indicate S.E.M.

DISCUSSION

There is considerable controversy regarding the role of AIP in AHR-mediated signaling. Some *in vitro* studies showed increase in Ahr transcriptional activity upon overexpression of *Aip*.[4,5] On the other hand, lack of *Aip* was also associated with elevated *Ahr* transcriptional activity in some reports.[6,7] These differences could be explained by cell type specificity and by different experimental conditions. In our hands, in GH3 cells, *Aip*-KD is associated with reduced transcriptional activity of Ahr.

CONCLUSIONS

***AIP* deficiency or mutated *AIP* is associated with reduced Ahr transcriptional activity, independently of variation in *Ahr* gene expression, in GH3 cells. The effect of *AIP* variants on AHR–dependent transcription is a potential measure of mutation pathogenicity.**

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