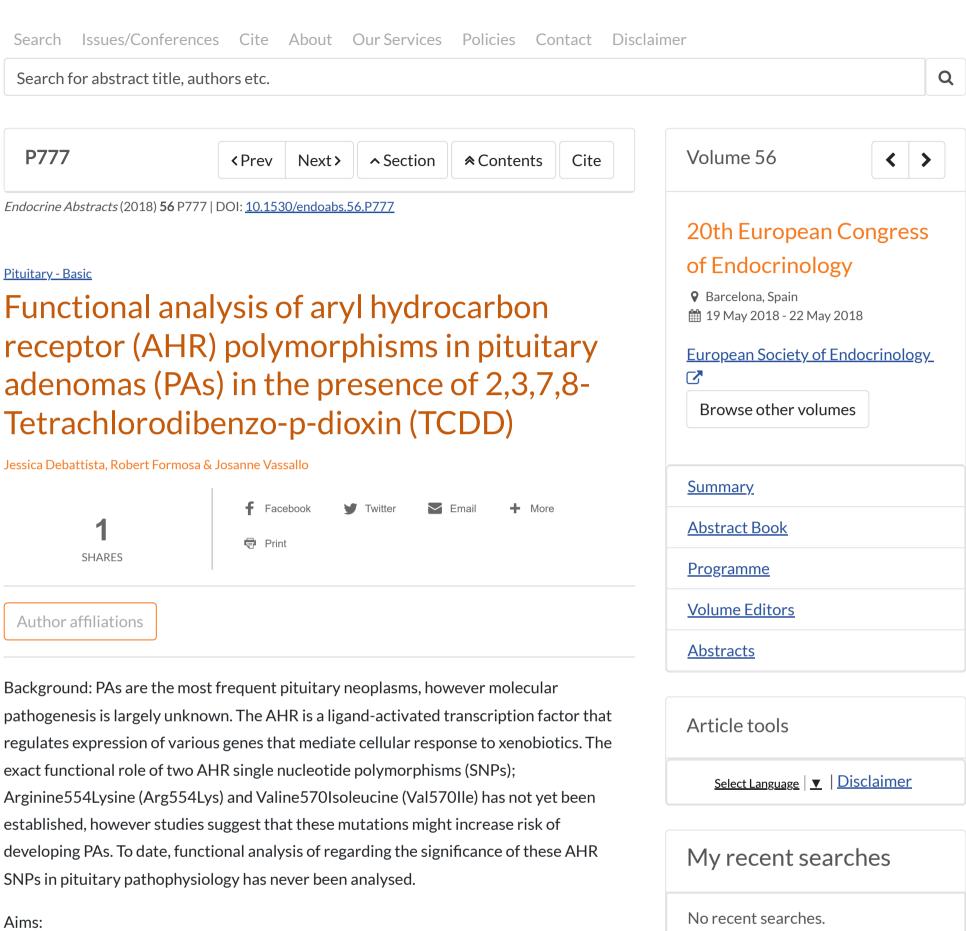
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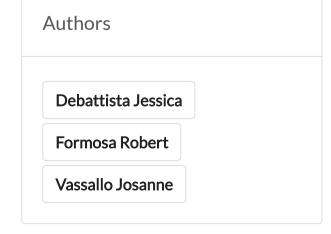
- Elucidate the effect of wildtype and polymorphic AHR on GH3 cell proliferation and on AHR-transcriptional response in the presence and absence of TCDD.
- Determine the allele frequency of the most common AHR SNP; the Arg554Lys in PA patients and in a small cohort of the Maltese population.

Method: The two missense mutations were introduced within the AHR-expressing vector and transfected in GH3 cells by magnetofaction, followed by the exposure to TCDD. Cell viability of GH3 transfected cells was measured using the MTT assay. Functional analysis of GH3 transfected cells treated with TCDD was carried out using luciferase assay and real-time PCR to detect and quantify the AHR-transcriptional activity. Genotyping of the Arg554Lys was performed on PA patients and neonatal controls using allele specific PCR. The Mann-Whitney test was used to compare two groups and Kruskall-Wallis test was used to compare three groups or more.

Results: In the absence and presence of low TCDD concentrations (1 and 10 nM), overexpression of wildtype AHR (wtAHR) did not affect GH3 cell proliferation. GH3 cells transfected with the AHR mutants did not exhibit any significant differences in their



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proliferative ability when compared with the wtAHR, both in the presence and absence of TCDD. Luciferase reporter analysis showed that there was a significant difference between the treated and untreated wtAHR (P=0.016), however this difference was not observed between the treated and untreated AHR mutants. Statistically significant difference in Cyp1a1 gene expression analysis was detected between the treated and untreated wtAHR (P=0.021), Arg554Lys (P=0.005) and Val570IIe (P=0.054). Genotyping of the Arg554Lys in patients with PA gave a minor allele frequency (MAF) of 3% vs 0% in neonatal controls.

Conclusion: Gene expression and quantification analyses of AHR-target genes suggests that these AHR mutants might interfere with AHR target gene expression. Genotyping results suggested that this mutation is quite rare and may be similar to the frequencies of other European populations.

Endocrine Abstracts

ISSN 1470-3947 (print) | ISSN 1479-6848 (online)

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