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We thank Roucher-Boulez et al. for their interest in our article characterizing the CYP11A1 nonsynonymous variant p.E314K (1). They argue that alternative RNA splicing rather than protein instability is the major pathogenic mechanism resulting from the CYP11A1 p.E314K variant. We appreciate the evidence provided; however, we believe that our conclusion of the mutation causing protein instability as published remains a convincing possibility.

The authors recently showed that a splicing event occurred in the testicular tissue of an affected patient. In their study, Goursaud et al. (2) showed a decrease in RNA abundance in the p.E314K allele, a trace of aberrant splicing with primers specific to exons 3 and 6, and an increase in aberrant splicing in nascent/premature RNA compared with control. Although these data indicate that the p.E314K variant causes aberrant splicing, it also occurred at low levels in the normal control testicular tissue, and several unanswered questions remain. First, given that a random primer was used for reverse transcription, the RT-PCR results reflected total RNA rather than mRNA. More importantly, even if a decrease in mRNA occurred due to the p.E314K variant, it remains unclear whether the level of decrease is sufficient for phenotypic adrenal insufficiency, an autosomal recessive disease. Although a Sanger chromatogram suggested a decrease (probably 50% to 70%) of the mutant allele RNA, a more reliable quantitative method, such as quantitative polymerase chain reaction or Western blot, would have been more definitive in measuring the level of decrease in gene expression. Finally, as acknowledged by the authors, RNA splicing is very tissue specific, and thus quantitative mRNA and gene expression studies in relevant adrenal tissue are needed to determine whether aberrant splicing caused by the p.E314K variant is the major cause of adrenal insufficiency in affected patients.

Our work was based on clinical findings and an in vitro assay to evaluate the overall effects of the CYP11A1 p.E314K variant on P450scc enzyme stability and activity. We demonstrated that the p.E314K variant reduced the enzyme half-life to ~50%, with a 40% decrease in P450scc enzyme activity per transfection. We speculate that a reduction in enzymatic activity occurred because unstable enzyme was present, coupled with increased protein turnover, because we could not rescue the p.E314K protein by using a proteasomal degradation pathway inhibitor.

The authors claim different results regarding enzymatic activity. We observed reduced activity when using epithelial-like human embryonic kidney cells (HEK293T), whereas they found no enzyme activity effect when using monkey kidney fibroblast like cells (COS-1). We agree with the authors that this difference could be attributed to the use of different cell lines. HEK293T cells have been shown to be suitable to investigate the characteristics and expression of steroidogenic enzymes, suggesting that HEK293T cells were a more appropriate model to assess P450scc activity (3).

Protein instability and aberrant RNA splicing caused by the p.E314K variant may both exist. Further studies, including more definitive RNA analyses, are needed. Evaluating missense variants is important in
expanding our understanding of the genetic causes of adrenal insufficiency.

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References