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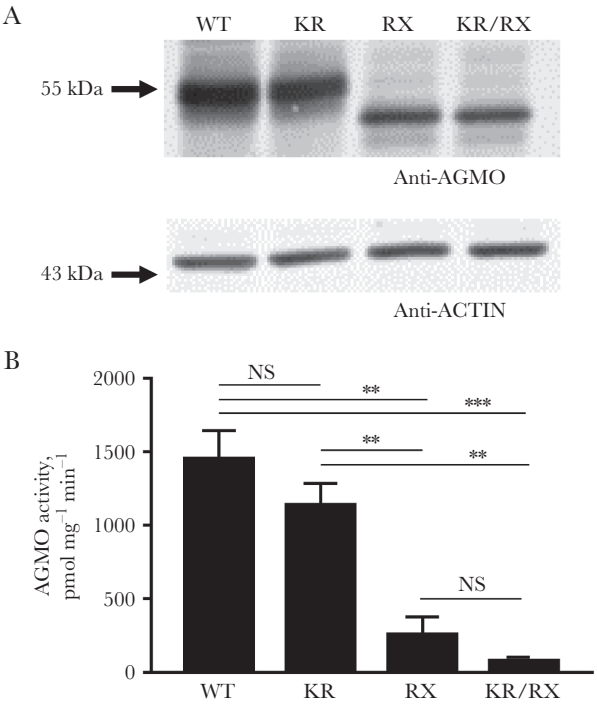

Figure 1. Analysis of cellular alkylglycerol monooxygenase (AGMO) activity and protein expression of wild-type (WT) AGMO versus mutant p.Lys234Arg (KR), mutant p.Arg405* (RX), and double mutant p.[Lys234Arg;Arg405*] (KR/RX) AGMO. *A*, Protein expression of WT, KR, RX, and KR/RX AGMO was analyzed by Western blot in microsomal fractions after transfection in HEK293T cells. The upper panel shows the AGMO signal, and the lower panel shows the signal of actin, the loading control. Marker protein sizes are indicated as arrows. Representative Western blots are shown. *B*, Cellular AGMO activity was measured in parallel samples, using our sensitive fluorescent high-performance liquid chromatography AGMO microassay [7]. Results are means ± standard errors of the mean for 3 independent experiments. NS, not significant. $^{**}P < .01$ and $^{***}P < .001$.

excludes the possibility that this finding was due to chance alone. Further work is required to confirm a possible association between AGMO loss of function alleles and KA relapse. In any event, our data provide experimental evidence for the assumed deleteriousness of variant p.Arg405* co-segregating with the enzymatically silent variant p.Lys234Arg.

Notes

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Reply to Watschinger et al

To the Editor—We thank Watschinger et al for taking an interest in our research and for their biochemical characterization of the alkylglycerol monooxygenase (AGMO) variants [1] that we recently identified [2]. They clearly demonstrate

the functional effect of rs139309795 (p.Arg405*) on both protein production and cellular AGMO activity, providing support for our identification of this mutation as a cause of relapses in visceral leishmaniasis in children from Sudan. The functional effect seems less clear for rs143439626 (p.Lys234Arg) according to their results, although cellular activity seemed to be even lower for the double mutant (p.Lys234Arg; Arg405*). However, we would like to take this opportunity to point out that some of the comments made by Watschinger et al are not relevant, and to provide additional information.

First, as indicated by Watschinger et al, only 1 subject with relapsing kala-azar (KA; individual 5 from family I) carried rs143439626 but not rs135309795. We would like to stress that this is not a genotyping error. Neither exome sequencing nor forward and reverse Sanger sequencing identified rs135309795 in this individual, who does not, therefore, require reassessment. It is true that both variants have been reported to be in complete linkage disequilibrium in the Kenyan LWK population of the 1000 Genomes dataset ($r^2 = 1$, $D' = 1$; www.ensembl.org), but this result is not convincing because it is based on a single sample (NA19030). Besides, the phase 3 data for African individuals in the 1000 Genomes dataset indicate that these 2 variants are not in linkage disequilibrium (ensembl, HaploReg V4.1). Two Africans individuals (HG01912 and HG02861) have been found to carry only 1 variant, as observed for individual 5 in our study. In addition, the frequency of these variants has been estimated at 0.005 for the heterozygous genotype and 0.002 for the rare allele in the African population from 1000 Genomes, rather than the value of 0.18 indicated by Watschinger et al. Moreover, it was not possible to determine from our data whether the rare alleles were positioned in *cis*, contrary to the assertion made in the commentary. It will be necessary to establish haplotypes by genotyping microsatellites located in the surrounding region and within the AGMO gene to resolve this issue.

Second, the biochemical characterization of AGMO variants suggests that p.Lys234Arg is functionally silent and potentially insufficient in itself to explain the KA relapse observed in individual 5 from family I. Our study suggests that AGMO variants cause KA relapses, but this does not rule out a role for other mutations in this disorder. Indeed, it should be borne in mind that this disorder is not monogenic. Individual 5 from family I may therefore carry additional functional mutations of genes involved in the same or a different biological pathway, not necessarily common to all the cases. We are currently testing this hypothesis.

Third, we are agree with Watschinger et al that individuals without KA are not relevant controls. These subjects were included in our study to assess the cosegregation of these variants and the relapse phenotype in each family. Our genotype results are consistent with an autosomal dominant mode of transmission and confirm the presence of these variants only in subjects with KA relapses. For exome sequencing in families, it is sufficient, in our view, to demonstrate the cosegegration of the phenotype and the functional effect of the mutation located in a gene with a relevant function in the disease, without having to provide statistical analysis [3–5].

We believe that the functional analysis of these variants supports our overall findings. AGMO is a crucial enzyme for macrophage-mediated immunity during leishmaniasis and may also play an important role in KA relapses. We are, of course, aware that other mutations may also be involved in this complex phenotype.

Notes

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