



Reply to Watschinger et al

Sandrine Marquet, Alain Dessein

► To cite this version:

Sandrine Marquet, Alain Dessein. Reply to Watschinger et al. Journal of Infectious Diseases, 2018, 217 (11), pp.1847 - 1848. 10.1093/infdis/jiy091 . hal-01891925

HAL Id: hal-01891925

<https://amu.hal.science/hal-01891925>

Submitted on 10 Oct 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Reply to Watschinger et al

Sandrine Marquet, Alain Dessein

► To cite this version:

Sandrine Marquet, Alain Dessein. Reply to Watschinger et al. Journal of Infectious Diseases, Oxford University Press (OUP), 2018, 217 (11), pp.1847 - 1848. <10.1093/infdis/jiy091>. <hal-01891925>

HAL Id: hal-01891925

<https://hal-amu.archives-ouvertes.fr/hal-01891925>

Submitted on 10 Oct 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

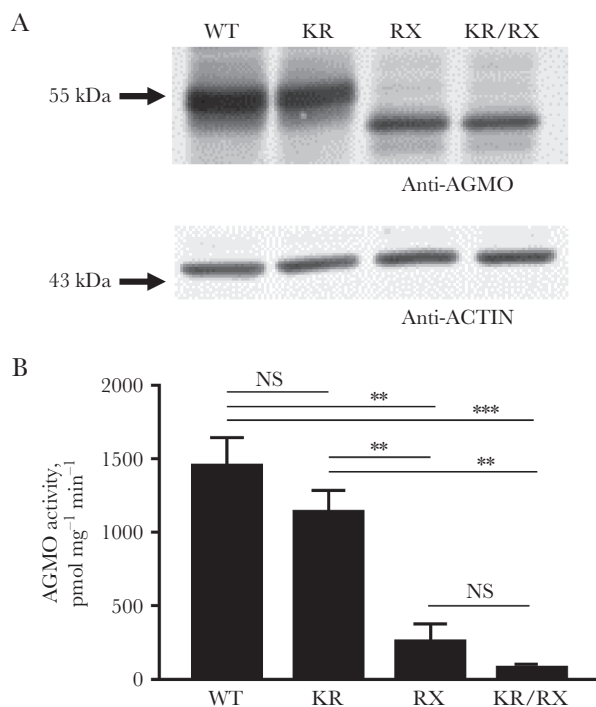


Figure 1. Analysis of cellular alkylglycerol monooxygenase (AGMO) activity and protein expression of wild-type (WT) AGMO versus mutant p.Lys234Arg (KR), mutant p.Arg405* (RX), and double mutant p.[Lys234Arg;Arg405*] (KR/RX) AGMO. *A*, Protein expression of WT, KR, RX, and KR/RX AGMO was analyzed by Western 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 \pm 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

Acknowledgments. We thank Petra Loitzl, Rita Holzknacht, and Nina Madl, for expert technical assistance.

Financial support. This work was supported by the Austrian Science Fund (FWF): projects P28769 [to E. R. W.] and P30800 [to K. W.].

Potential conflicts of interest. All author: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts

of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Katrin Watschinger,¹ Markus A. Keller,² Georg Golderer,¹ Stefan Coassin,³ Johannes Zschocke,² and Ernst R. Werner¹

¹Division of Biological Chemistry, Biocenter, and ²Division of Human Genetics and ³Division of Genetic Epidemiology, Department of Medical Genetics, Molecular and Clinical Pharmacology, Medical University of Innsbruck, Austria

References

1. Marquet S, Bucheton B, Reymond C, et al. Exome sequencing identifies two variants of the alkylglycerol monooxygenase gene as a cause of relapses in visceral leishmaniasis in children, in Sudan. *J Infect Dis* **2017**; 216:22–8.
2. Watschinger K, Keller MA, Golderer G, et al. Identification of the gene encoding alkylglycerol monooxygenase defines a third class of

tetrahydrobiopterin-dependent enzymes. *Proc Natl Acad Sci U S A* **2010**; 107:13672–7.

3. Watschinger K, Keller MA, McNeill E, et al. Tetrahydrobiopterin and alkylglycerol monooxygenase substantially alter the murine macrophage lipidome. *Proc Natl Acad Sci U S A* **2015**; 112:2431–6.
4. Tokuoka SM, Kita Y, Shindou H, Shimizu T. Alkylglycerol monooxygenase as a potential modulator for PAF synthesis in macrophages. *Biochem Biophys Res Commun* **2013**; 436:306–12.
5. Auton A, Brooks LD, Durbin RM, et al. A global reference for human genetic variation. *Nature* **2015**; 526:68–74.
6. Watschinger K, Fuchs JE, Yarov-Yarovoy V, et al. Catalytic residues and a predicted structure of tetrahydrobiopterin-dependent alkylglycerol mono-oxygenase. *Biochem J* **2012**; 443:279–86.
7. Werner ER, Hermetter A, Prast H, Golderer G, Werner-Felmayer G. Widespread occurrence of glyceryl ether monooxygenase activity in rat tissues detected by a novel assay. *J Lipid Res* **2007**; 48:1422–7.

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 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 cosegregation 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

Financial support. This work was supported by the French Research Ministry, the Institut National de la Santé et de la Recherche Médicale (INSERM), the European Union (grant number IC18-CT98 0373), and the French Parasitology Alliance for Health Care (ParaFrap; grant number ANR-11-LABX-0024-01).

Potential conflicts of interest. Both authors: No reported conflicts of interest. Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Sandrine Marquet¹ and Alain J. Dessein^{1,2}

¹INSERM UMR906, GIMP, Labex ParaFrap, Aix-Marseille University; and ²GenePred Biotechnologies, Marseille, France

References

1. Watschinger K, Keller MA, Golderer G, et al. Biochemical characterization of AGMO variants implicated in relapses in visceral leishmaniasis. *J Infect Dis* **2018**; 217:1846–7.
2. Marquet S, Bucheton B, Reymond C, et al. Exome sequencing identifies two variants of the alkylglycerol monooxygenase gene as a cause of relapses in visceral leishmaniasis in children, in Sudan. *J Infect Dis* **2017**; 216:22–8.
3. Heinz L, Kim GJ, Marrakchi S, et al. Mutations in SULT2B1 cause autosomal-recessive congenital ichthyosis in humans. *Am J Hum Genet* **2017**; 100:926–39.
4. Tang S, Wang X, Li W, et al. Biallelic mutations in CFAP43 and CFAP44 cause male infertility with multiple morphological abnormalities of the sperm flagella. *Am J Hum Genet* **2017**; 100:854–64.
5. Srour M, Shimokawa N, Hamdan FF, et al. Dysfunction of the cerebral glucose transporter SLC45A1 in individuals with intellectual disability and epilepsy. *Am J Hum Genet* **2017**; 100:824–30.