Author’s response to reviews

Title: Novel ANKRD11 gene mutation in an individual with a mild phenotype of KBG syndrome associated to a GEFS+ phenotypic spectrum: a case report.

Authors:

Rita Alves (ritamaria.alves@gmail.com)
Paolo Uva (paolo.uva@crs4.it)
Marielza Veiga (marielzaveiga@gmail.com)
Manuela Oppo (manuelaoppo@tiscali.it)
Fabiana Zschaber (fabianazschaber@hotmail.com)
Giampiero Porcu (giampieroporcu@tiscali.it)
Henrique Porto (henriquefono@hotmail.com)
Ivana Persico (ivana.persico@irgb.cnr.it)
Stefano Onano (ste.ona5@gmail.com)
Gianmauro Cuccuru (gmauro@crs4.it)
Rossano Atzeni (ratzeni@csr4.it)
Lauro Vieira (vieiralauro@yahoo.com.br)
Marcos Pires (euro.marcospires@hotmail.com)
Francesco Cucca (fcucca@uniss.it)
Maria Toralles (m.toralles@uol.com.br)
Andrea Angius (andrea.angius@irgb.cnr.it)
Laura Crisponi (laura.crisponi@irgb.cnr.it)

Version: 1 Date: 07 Dec 2018

Author’s response to reviews:

Monserrato, December 7th, 2018
Dear Editor,

Please find attached the revised version of the manuscript “MGTC-D-18-00412-Novel ANKRD11 gene mutation in an individual with a mild phenotype of KBG syndrome associated to a GEFS+ phenotypic spectrum: a case report”.

We would like to thank both reviewer for their valuable comments and suggestions. Below, you will find a point-by-point description of how each comment was addressed in the manuscript.

Ilaria Parenti (Reviewer 1)

Comment 1 “The variant in SCN9A p.Lys655Arg appears to be quite frequent in the population, and also ClinVar suggests a conflicting interpretation of pathogenicity for this variant. The authors could expand their discussion and speculate on the reason-why the same variant does not cause the same phenotype in the unaffected father (e.g. Incomplete penetrance? Existence of an additional causative variant in the siblings? Existence of a protective variant in the father?)”

According to the comment reviewer, we expand the discussion as follow:

Line 42-47 Page 6

Patients with GEFS+ express a highly variable phenotype combining febrile seizures, absence seizures, partial seizures, myoclonic seizures, or atonic seizures, with a variable degree of severity (Wallace et al., 1998). In a recent paper (Mulley et al., 2013), none of the GEFS+ families analyzed could be explained by highly penetrant SCN9A mutations. Furthermore a non-penetrant individual is not unexpected as they are commonly seen in autosomal dominant diseases and are well documented in febrile seizures pedigrees (60-80% of penetrance Singh et al., 2009).

Comment 2 “The authors should name the kit and the companies used for DNA extraction and array-CGH. Also, the different stages of the genetic analysis should be discussed in more detail.”

We add in the text the requested information and a new reference for the extraction protocol.

Line 9-12 Page 5

“Blood samples were collected from the affected individuals and their parents and genomic DNA was extracted from peripheral blood lymphocytes using a salting out procedure [20].

An array based Comparative Genomic Hybridization (CGH) analysis was done using commercially available Human Genome CGH Microarray (Agilent Technologies, Waldbronn, Germany) with a estimated average resolution of 13Kb (SurePrint G3 Human CGH Microarray).”

And Line 17-24 Page 5
“WES protocols were performed using the Nextera Rapid Capture Expanded Exome Kit (Illumina) which provides 62 Mb of genomic content, including exons, non-translated regions (UTRs) and miRNA. One hundred nanograms of genomic DNA were sheared by tagmentation, and sequencing libraries were prepared according to manufacturer instructions. Quality of post-amplification libraries were assessed using DNA1000 chips on the BioAnalyzer 2100 (Agilent) and Qubit fluorometric quantitation using Qubit dsDNA BR Assay Kits (Invitrogen). Libraries were loaded into Paired Ends v3 flow cells on an Illumina cBot followed by indexed paired-end sequencing (101+7+101 bp) on a HiSeq 2000 using SBS Kit v3 chemistry (Illumina).”

Comment 3 “The authors should check the references throughout the manuscript, since some references are not included in the references list (e.g. Swols 2017).”

We carefully checked the references and update the article.

Palma Finelli (Reviewer 2)

Comment 1 “line 8 pag 5 please specify the aCGH resolution”

We add in the text the requested information.

Line 10-12 Page 5

An array based Comparative Genomic Hybridization (CGH) analysis was done using commercially available Human Genome CGH Microarray (Agilent Technologies, Waldbronn, Germany) with a estimated average resolution of 13Kb (SurePrint G3 Human CGH Microarray).”

Comment 2 “line 61 pag 5 delete (Goldenberg et al., 2016; Low et al., 2016)”

Comment 3 “line 15 and 18 pag 6 delete (Parenti et al., 2016) and (Zhang et al., 2004)”

Comment 4 “line 4 page 11 Figure 1: A-P instead of A-O”

As suggested, we have revised the manuscript.

We thank you for considering our article and we look forward to hearing your response.

Best regards.

Dr Andrea Angius