Author’s response to reviews

Title: Digenetic inheritance of SLC12A3 and CLCNKB genes in a Chinese girl with Gitelman syndrome

Authors:

yuan mei Kong (kongyuanmei@163.com)
Ke Xu (xuke@zhuanhuayixue.org)
Ke Yuan (21618001@zju.edu.cn)
Jianfang Zhu (6507032@zju.edu.cn)
Weiyue Gu (guweiyue@zhuanhuayixue.org)
Li Liang (zdliangli@zju.edu.cn)
Chunlin Wang (hwangcl@zju.edu.cn)

Version: 1 Date: 15 Feb 2019

Author’s response to reviews:

Response to Efstathios Koulouridis (Reviewer 1):

Minor Essential Revisions:

1. Abstract. Lines 31-34: If her grandmother has the same polymorphisms in SLC12A3 and CLCNKB genes why the grandmother doe's not suffer from Gitelman's syndrome phenotype?

Response: Her grandmother is carrier of SLC12A3 p.N359K only, so doe's not suffer from Gitelman's syndrome phenotype.

2. According to your data patient's mother carried two SNP polymorphisms in CLCNKA c.1054-22(IVS11)delG, C and CLCNKB p.L94I. As you know inactivation of Barttin protein and digenic mutations affecting concomitantly CLC-KA and CLC-KB, in Barter syndrome type IV, which affect concomitantly the function of CLC-Ka and CLC- Kb, is coupled with the more severe clinical presentation of Barter syndrome. I would like to have your comment why these
two polymorphisms did not act as "double hit" phenomenon and the mother did not exhibit any phenotypic evidence of salt losing tubulopathy, except mild hypokalemia?

Response: Compound heterozygous pathogenic variants in CLCNKA and CLCNKB genes can cause GS, which was considered a canonical pattern of digenic inheritance, but, interestingly, we did not find any sign of renal tubular dysfunction, nor identify potential pathogenic variant in CLCNKA gene, using bioinformatics method, of the mother who carrying the variants (Fig. 2), which helps to exclude the pathogenicity of CLCNKA c.1054-22 (IVS11) delG.

Compulsory Revisions:

1. Seyberth HW, in 2008, (Nat Clin Pract Nephrol 2008;4:560-567. ) has proposed a new classification of salt loosing tubulopathies in three types: DC-type is referred to distal convoluted tubule dysfunction and comprises loss of function of NCC and CLC-KB. L-type is referred to the Loop of Henle dysfunction and comprises loss of function of NKCC2 and ROMK, and the L-DC type which comprises a mix category with loss of function of CLC-KA and/or CLC-KB and the b-subunit Barttin. Taken in account that your patient exhibits a phenotype relevant to Gitelman syndrome but her genetic abnormality comprises a polymorphism in SCLC12A3 (characteristic of Gitelman syndrome) and a polymorphism in CLC-KB (characteristic of Bartter syndrome) it is more proper to reassume your terminology and classify your case according to Seyberth's terminology and not the classical terminology.

Response: We identified a pathogenic variant in SLC12A3, a potential pathogenic variant CLCNKB p.L94I, and a likely benign variant c.1054-22 (IVS11) delG in CLCNKA gene. Since GS was previously reported related to several genes, we still speculated that a “double hit” in pattern of the digenic inheritance may play a role in our case.

Response to Detlef Bockenhauer (Reviewer 2):

we have referred to professional group to edit the manuscript and this process would take some time, about a week.

1) If the authors really want to propose this paradigm change in the genetics of GS, they better have good dataThe authors should check databases to assesses the frequency of SLC12A3 and CLCNKB variants in the general population. Current papers suggest a frequency of
SLC12A3 mutations around 2-3%. Presumably, there are less variants in CLCNKB, but if one were to include VUS (as the authors do in this paper), the frequency is probably around 0.5%. Which would mean that the frequency of digenic GS would be roughly 1:10,000. Thus, in a country like China with a population of 1 billion, there would be about 100,000 such GS patients! How amazing that nobody has discovered this before!

2) The other way to look at this, is that the likelihood to find such digenic variants is quite high and coincidental to the phenotype.

Response: The CLCNKB p.L94I was annotated a “moderate evidence of pathogenicity”, according to the PM1 rating of ACMG, which means “located in a mutational hot spot and/or critical and well-established functional domain without benign variation”. We did a further research in UniProt database, and found that the CLCNKB (id P51801) p.94 is the first residue located at the second helix in the trans-membrane domain (TMD), yet proving the pathogenicity of CLCNKB p.L94I still need further study.

3) There are a number of reports of GS patients with only heterozygous identified mutations and a few papers from Taiwan suggest that intronic variants on the other allele may be causative. Have the authors looked at this?

Response: Between 18 and 40% of patients with clinical GS are usually found to carry only one mutant allele after SLC12A3 screening. These issues have already been extensively discussed. Mutational screening of the whole coding region and hot spot of intronic variants of the SLC12A3 gene were detected in our research, did not reveal any abnormality except p.N359K.

4) They should also perform functional studies of the VUS in CLCNKB, before asserting pathogenicity.

Response: The putative pathogenicity of variant p.L94I in CLCNKB gene still needs further confirmation.

5) The idea to go into protein interaction databases for SLC12A3 and CLCNKB is really quite bizarre, since the physiology and location of these transport molecules is well known and described!

Response: Our case indicates that, based on the clinical phenotypes, genetic evidence of the pedigree, and previous reported studies, heterozygous variants in SLC12A3 and CLCNKB genes, respectively, result in renal tubular dysfunction because of the digenetic inheritance due to a “double hit” mechanism. The novel findings reported in this study are expected to provide a
possible mechanism of SLC1A3/CLCNKB mediated Gitelman syndrome and to provide a theoretical basis for genetic counseling.

Minor:

1) what is the definition of "slight hypokalaemia"?

Response: 3.0–3.5 mmol/L

Response to Helena Gil (Reviewer 3):

Minor comments:

- Although in-silico predictors can not reproduce real molecular interactions, the authors have used excellent functional predictions that provide a well-evaluated approach to variant tolerance.

However, there are not functional assays to be certain about the pathogenicity of the CLCNKB gene variant, which is an important limitation on the interpretation of the results.

Response: It’s the limitation of our research. The putative pathogenicity of variant p.L94I in CLCNKB gene still needs further confirmation.

- An interesting fact is the growth retardation observed in the diagnosis (-1.62 SD in length), which means a severe form of the disease, although laboratory tests did not reveal extreme values for hypokalemia or metabolic alkalosis. Do the authors believe that the effect of growth may be due to a negative effect of the p.L94I variant of CLCNKB on the p.N359K mutation of SLC12A3?

Response: Her grandmother, father and younger brother were the carriers of SLC12A3 p.N359K, and have been diagnosed with hypokalemia many years ago, but plasma potassium level reached normal after taking in potassium-rich foods. So we believe that the effect of growth may be due to a negative effect of the p.L94I variant of CLCNKB on the p.N359K mutation of SLC12A3.

- The authors mention the HNF1B gene referred to in the Blanchard publication (Kidney Int, 2017). However, they do not explain why this gene should be taken into account for genetic
testing of Gitelman syndrome. They should include the study of Verhave JC (J Am Soc Nephrol, 2016).