Received: 10 April 2023 | Revised: 6 June 2023 | Accepted: 10 June 2023

DOI: 10.1111/cge.14395

SHORT REPORT

CLINICAL GENETICS WILEY

Performance of the ACMG-AMP criteria in a large familial renal glucosuria cohort with identified *SLC5A2* sequence variants

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Funding information
Fundação para a Ciência e a Tecnologia, Grant/Award Number: UID/BIM/00009/2016

Abstract

Familial Renal Glucosuria (FRG) is a co-dominantly inherited trait characterized by orthoglycaemic glucosuria. From 2003 to 2015 we have reported several cohorts validating *SLC5A2* (16p11.2), encoding SGLT2 (Na+/glucose cotransporter family member 2), as the gene responsible for FRG. The aim of this work was to validate the variants identified in our extended FRG cohort of published, as well more recent unreported cases, according to the ACMG-AMP 2015 criteria. Forty-six variants were evaluated, including 16 *novel* alleles first described in this study. All are rare, ultra-rare or absent from population databases and most are missense changes. According to the ACMG-AMP standards, only 74% of the variants were classified as P/LP. The lack of descriptions of unrelated patients with similar variants or failing to test additional affected family members, averted a conclusion for pathogenicity in the alleles that scored VUS, highlighting the importance of both family testing and variant reporting. Finally, the cryo-EM structure of the hSGLT2–MAP17 complex in the empagliflozin-bound state improved the ACMG-AMP pathogenicity score by identifying critical/functional protein domains.

KEYWORDS
glycosuria, proximal tubule, renal, sodium glucose transporter 2

1 | INTRODUCTION

Familial Renal Glucosuria (MIM 233100) is an inherited human trait characterized by persistent isolated glucosuria. In 2003, we submitted two *novel SLC5A2* gene mutations in a FRG affected individual.[1] Previously to submission, only one variant had been reported[2] but soon afterward the molecular findings of the first large FRG cohort were published,[3] therefore validating *SLC5A2* as the gene responsible for FRG. SGLT2 accounts for most of glucose reabsorption in the renal proximal tubule.[4] Several other FRG cohorts have been studied, listed and updated in 2019[5] with a few additional reports meanwhile published,[6,7] that further established FRG as a co-dominantly inherited phenotype instead of a recessive one, as initially assumed. Individuals harboring two *in trans SLC5A2* mutations typically display urinary glucose excretion (UGE) in excess of 10 g/1.73 m^2/day. Although diagnostic criteria for FRG are straightforward, when considering co-dominant inheritance measuring UGE is needed for proper assessment of variants' pathogenicity. For most family members, however, the FRG status is determined by a qualitative or semi-quantitative assessment of glucosuria in the first morning void or random sample urine collection, therefore hindering the establishment of robust geno-phenotype correlations. Genetic heterogeneity is not relevant in FRG, with only one individual reported so far with mutations in *PDZK1IP* (1p13) a gene that codes for the SGLT2 accessory unit MAP17.[8]

In the last decade, the number of variants reported in populational and disease databases has increased exponentially, rendering genetic test interpretation more probabilistic than determinative. With the goal of defining standards for interpreting variants in clinical testing, the American College of Medical Genetics and Genomics and

the Association for Molecular Pathology (ACMG-AMP) endorsed the implementation of a five tiers system of classification of variants relevant for Mendelian diseases.[9] It recommends that the vocabularies of "mutation" and "polymorphism" be abandoned and replaced by the term "variant" with the modifiers of pathogenic (P), likely pathogenic (LP), uncertain significance (VUS), likely benign (LB), or benign (B). This classification is based on differently weighted evidence for criteria either of pathogenicity (supporting, moderate, strong or very strong) or benignity (stand alone, strong or supporting).

It is our purpose to re-assess the *SLC5A2* mutations that our group has identified in a research environment using the ACMG-AMP guidelines and criteria as supported by populational, disease and literature databases, computational predictive algorithms and segregation evidence.

2 | MATERIALS AND METHODS

2.1 | Patients and families

We evaluated the variants identified in 49 FRG probands. In addition to previously published pedigrees,[1,10–13] we have included 22 previously unreported families. Written consent was obtained from participating individuals or their legal guardians, according to the ethical committees of participating institutions. In all but five probands, UGE was assessed in a 24 h-urine collection. The *SLC5A2* gene coding region (GeneBank transcript NM_003041.4; Ensembl canonical transcript ENST00000330498.3) was sequenced as previously reported.[1]

2.2 | Population, computational, and predictive data

Minor Allele Frequency for each variant was retrieved from the Genome Aggregation Database (gnomAD v2.1.1; https://gnomad.broadinstitute.org), with variants considered rare (R) if 1e-2 to 1e-4 or ultra-rare (UR), if <1e-4. The Human Genome Mutation Database- HGMD (https://www.hgmd.cf.ac.uk/ac/index.php) as well ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases were mined for the alleles detected in our cohort. Furthermore, we carried out a literature review in order to identified published variants. For missense changes three *in silico* predictive algorithms were used: SIFT (http://sift.jcvi.org), Polyphen2 (http://genetics.bwh.harvard.edu/pph2) and MutationTaster (http://www.mutationtaster.org). Discordance was solved if, at least, 2 out the 3 algorithms scored the variant as deleterious. Most of the *in silico* prediction algorithms were comprehensively evaluated by the *metapredictor* dbNSFP4.2a, a one-stop database of functional predictions and annotations for human non-synonymous and splice site SNVs (http://database.liulab.science/dbNSFP).[15]

2.3 | The ACMG-AMP pathogenicity analysis

FRG is an autosomal co-dominantly inherited trait, with cases exhibiting mild UGE (<10 g/1.73 m^2/day) carrying heterozygous mutations, while compound heterozygous or homozygous individuals display severe UGE (≥10 g/1.73 m^2/day). In order to apply the ACMG-AMP criteria to a co-dominant inherited phenotype, we stratified our approach according to the number of variants, with a recessive model presumed if two were identified. In addition, the recent publication of the cryo-EM structure of the hSGLT2-MAP17 complex in the empagliflozin-bound state[14] enabled the identification of functional domains and critical residues in SGLT2, therefore offsetting the lack of an in vitro or in vivo functional assay (Table S1).

2.4 | Protein structure homology modeling

The 7vsi.1 cryo-EM structure of human SGLT2-MAP17 complex in the empagliflozin-bound state was selected as the template for structure homology-modeling of the *novel* missense SGLT2 mutants identified. The SWISS-MODEL server (https://swissmodel.expasy.org/interactive) was used. The *wildtype* (*wt*) and mutant protein sequences were aligned and analyzed in PyMOL (https://pymol.org/2/).

3 | RESULTS

Two *SLC5A2* variants were identified in 32 probands and in 17 only one was detected (Tables S2 and S3). For those displaying two *SLC5A2* variants and in whom an UGE was available, 89% (24/27) had an UGE ≥10 g/1.73 m^2/day (Figure 1). Meiotic phase was established

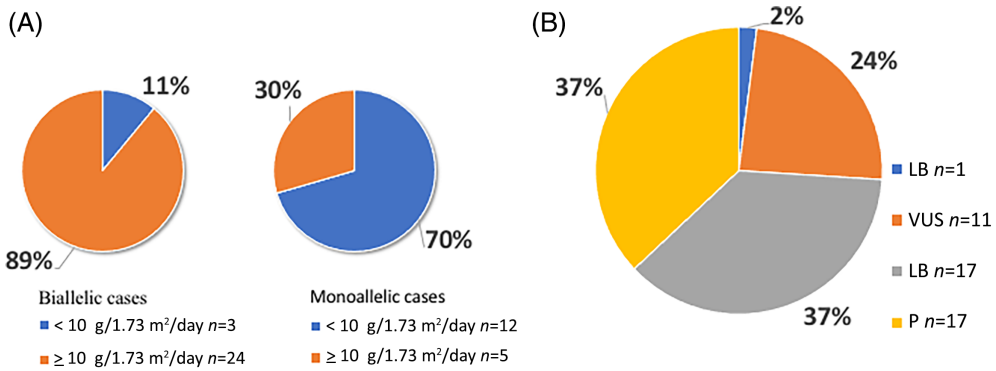

FIGURE 1 FRG cases and *SLC5A2* variants identified. (A) UGE in cases with two or single variants detected. The "*n*" refers to numbers of individuals with UGE below or above 10 g for each group. (B) ACMG-AMP classification of the 46 distinct variants detected. The "*n*" refers to numbers of variants scored in each class. [Colour figure can be viewed at wileyonlinelibrary.com]

in 18 cases and in 17 the variants were found to be in *trans*. Conversely, in those 17 cases carrying a single variant, 70% (12/17) displayed an UGE <10 g/1.73 m^2/day. Furthermore, if diabetic *mellitus* probands 10, 12 and 15 (Table S3) are excluded, up to 86% of cases with one variant had an UGE <10 g/1.73 m^2/day. Therefore, this cohort validates the UGE threshold of 10 g/1.73 m^2/day for discriminating between compound heterozygosity/homozygosity from heterozygosity. Overall, when we applied the ACMG-AMP guidelines to the 46 different variants, 37%[16] were classified as P, 37%[16] as LP, 24%[10] as VUS and 2%[1] as LB, rendering only 74% of them suitable for genetic counseling (Figure 1). This scenario remains unchanged whether these variants concerned monoallelic or biallelic cases. The most recurrent alleles are listed in Table 1. Together, those 6 variants represent 29% of the *SLC5A2* chromosomes. In particular, the c.885 + 5G > A;IVS7 + 5G > A allele was detected in 9 unrelated pedigrees. It potentially disturbs a donor splice site of intron 7 as predicted by MutationTaster (https://www.mutationtaster.org).

TABLE 1 Prevalent *SLC5A2* variants.

SLC5A2 variant	Number of chromosomes	AF-gnomAD
c.885 + 5 g > a;IVS7 + 5G > A	9	3.09e-4
c.1961A > G;p.N654S	5	5.60e-3
c.1566C > A;p.C522X	4	2.41e-5
c.305C > T;p.A102V	4	4.00e-6
c.1033-1060del;p.V346AfsX17	3	4.15e-6
c.1405G > A;p.A469T	3	4.57e-5

Abbreviation: AF, allele frequency.

The 16 *novel* variants detected are depicted in Table 2. Missense changes account for the majority of cases (12/16), while 81% (13/16) were categorized as LP/P. For the 12 *novel* missense variants here reported, structural homology modeling was tested against the *wt* SGLT2 7vsi.1 cryo-EM structure of human SGLT2-MAP17 complex in the empagliflozin-bound state.[14] Of relevance, the V95I substitution failed to dock with empagliflozin (Figure S1). The V95 is a critical residue for sugar binding and when mutated found to impair glucose transport in an in vitro model[14]; still, the V95I substitution was predicted to be tolerated by SIFT.

4 | DISCUSSION

The aim of this work was to evaluate the performance of the ACMG-AMP criteria when applied to the variants identified in our extended cohort of 49 FRG individuals, including 22 new cases accounting for 16 *novel SLC5A2* variants. These *novel* variants not only further expand the spectrum of allelic heterogeneity in FRG but have provided, at least in one instance, clinical validation for in-vitro experiments that ensued the determination of the cryo-EM structure of the hSGLT2-MAP17 transporter complex in the empagliflozin-bound state that has identified the V95 as a critical SGLT2 residue.[14]

These results are in line with previous findings of *SLC5A2* missense variants being the most common mechanism of disease in FRG and that biallelic disease causes a more severe phenotype. In this cohort, UGE was measured in a 24 h urine collection in 90% of the probands, strengthening these geno-phenotype observations. The number of probands with two identified variants exceeded those of individuals heterozygous for *SLC5A2* alleles by a ratio of 2:1. The higher renal threshold for glucose excretion observed in individuals

TABLE 2 Summary of the 16 novel variants identified in the current study.

SLC5A2 variant	rs dbSNP	AF-gnomAD	In silico	PVS	PS	PM	PP	BP	ACMG-AMP
c.304–20 g > a;IVS3-20 g > a	rs534857379	2.42e-5	B	–		2	4	2,4	LB
c.1297A > G; p.I433V	rs150546732	1.7e-03	D	–	4	2	2,3		LP
c.1475G > C;p.G492A	n.p.	n.p.	D	–	4	2	2,3,4		LP
c.26C > G;p.S9W	rs564249983	3.19e-05	B	–		2,5	2,4	4	LP
c.283G > A;p.V95I	n.p.	n.p.	D	–		1,2	2,3,4		LP
c.412A > T;p.I138F	n.p.	n.p.	D	–		2,3	1,2,3,4		LP
c.500A > T;p.Q167L	n.p.	n.p.	B	–		2,3	1,2,4	4	LP
c.571A > C; p.T191P	rs771791831	3.98e-06	D	–		2,3	1,2,3		LP
c.1343A > T;p.Q448L	rs959853124	n.p.	D	–	4	2,3,5	1,2,3,4		P
c.1409 T > C;p.V470A	rs139661242	2.45e-04	D	–	4	2,3	1,2,3,4		P
c.1460G > A;p.W487X	n.p.	n.p.	LOF	1		2	4		P
c.1566C > A;p.C522X	rs199795513	2.41e-5	LOF	1	4	2,3	2,4		P
c.1946G > A;p.W649X	rs893453236	8.05e-6	LOF	1		2	4		P
c.1750C > A; p.P584T	n.p	n.p.	B	–		2	2,4	4	VUS
c.281C > T;p.A94V	n.p.	n.p.	D	–		2	2,3,4		VUS
c.989 T > C; p. M330T	n.p.	n.p.	D	–		2	2,3,4		VUS

Abbreviations: AF, Allele Frequency; B, benign; D, deleterious; LOF, loss of function; n.p. not present.

with monoallelic *SLC5A2* mutations (of ~88 mg/dL) may indeed be superior to fasting plasma glucose concentrations and hence glucosuria may be absent if first morning urine void is used for screening purposes.[13]

Nearly one quarter of the variants identified in FRG would be ineligible for genetic counseling as they were classified VUS/LB. With the exception of the c.1750C > A;p.P584T variant, all VUS missense changes were predicted *in silico* to be deleterious, scored PM2, PP2, PP3, and PP4. However, either because there were no reports of unrelated patients displaying the same variant or the lack of additional tested family members, they could not be upgraded to LP. These findings emphasize the importance of variant reporting as well performing co-segregation studies for proper scoring, but also highlight that while applying the standards[9] there is often insufficient data for a conclusion of pathogenicity. FRG is such a specific phenotype, revealing an excellent geno-phenotype correlation, that the observation of predicted pathogenic variants (LP/P) being lower than those actually sequenced, emphasizes the need for adapting the ACMG-AMP guidelines according to the phenotype being evaluated, as shown recently for the Alport Syndrome.[16]

We selected SIFT, Polyphen2 and Mutation Taster based on customary practice as the ACMG-AMP guidelines while requiring multiple lines of computational evidence, does not discriminate. However, different tools may reach distinct thresholds for either pathogenicity or benignity.[17] The ClinGen Working Group recently recommended stratifying tools according to the levels of strength and use those with at least strong evidence for pathogenicity and moderate for benignity, which is not the case for SIFT or Polyphen2. Additional suggestions include the preferential use of a single genome-wide predictor, minimize double counts (frequency data) and endorse gene specific evaluations.[17]

Inferring the impact of *SLC5A2* variants by measuring UGE has limitations. First, the compensatory increase in SGLT1-mediated glucose reabsorption occurring distally to SGLT2 is not considered. *Sglt1* can reabsorb up to 70% of the filtered glucose in *Sglt2* KO mice,[18,19] an observation that could explain why probands displaying two LOF alleles often have a lesser than maximal expected UGE. Second, and as reported for SGLT1 and the Glucose-Galactose Malabsorption syndrome,[20] only heterologous expressions experiments can assess each variants' effect on SGLT2 transport biology and further differentiate protein trafficking from transport activity defects. Nevertheless, in the clinical setting, the availability of the cryo-EM structure of the human SGLT2-MAP17 complex in the empagliflozin-bound has partially circumvented the absence of a functional assay in FRG, since it characterized several critical functional domains found to be mutated in our cohort.

AUTHOR CONTRIBUTIONS

Rui Barata, Marc Fila, Fabienne Dalla-Vale, Roberto Bogarin, Paula Nunes and Joaquim Calado collected the data; José Ramalho and Joaquim Calado performed the molecular analysis. José Rueff and Joaquim Calado wrote the manuscript. The authors are grateful to participating individuals and their families.

ACKNOWLEDGMENTS

This work was supported by grant UID/BIM/00009/2016, Portuguese Science and Technology Foundation.

CONFLICT OF INTEREST STATEMENT

None of the authors declare any conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/cge.14395.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Barata R, Fila M, Dalla-Vale F, et al. Performance of the ACMG-AMP criteria in a large familial renal glucosuria cohort with identified *SLC5A2* sequence variants. *Clinical Genetics*. 2023;1-5. doi:10.1111/cge.14395