KDIGO Controversies Conference on Gitelman Syndrome

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Kidney Disease: Improving Global Outcomes (KDIGO) is an international organization whose mission is to improve the care and outcomes of kidney disease patients worldwide by promoting coordination, collaboration, and integration of initiatives to develop and implement clinical practice guidelines. Periodically, KDIGO hosts conferences on topics of importance to patients with kidney disease. These conferences are designed to review the state of the art on a focused subject and to ask conference participants what needs to be done in this area to improve patient care and outcomes. Sometimes the recommendations from these conferences lead to KDIGO guideline efforts and other times they highlight areas for which additional research is needed to produce evidence that might lead to guidelines in the future.

BACKGROUND

Gitelman's syndrome (GS) is a salt-losing tubulopathy characterized by hypokalemic alkalosis with hypomagnesemia and hypocalciuria. With a prevalence of ~1 per 40,000 in Western Countries and 4-9 per 40,000 in Asia, GS is arguably the most frequent inherited tubulopathy detected in adults. The disease is recessively inherited, caused by inactivating mutations in the *SLC12A3* gene that codes for the thiazide-sensitive sodium-chloride cotransporter that is expressed in the apical membrane of the cells lining the distal convoluted tubule. Classically, GS has been considered as a benign variant of salt-losing nephropathies, usually detected during adolescence or adulthood, and is often asymptomatic or presenting with mild symptoms such as weakness, fatigue, salt craving, thirst, nocturia, or cramps. This view has since been challenged by recent reports emphasizing the phenotype variability and the potential severity of the disease. Due the molecular characterization and increasing awareness for GS, a growing number of

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patients are identified worldwide. This increase is paralleled by the need to clarify a vast number of issues related to the disease. These issues include the diagnostic criteria and methods; the clinical work-up and follow-up; the phenotype heterogeneity in terms of age at presentation; nature/severity of the biochemical abnormalities and clinical manifestations; the treatment and long-term consequences of the disease. Since GS is associated with impaired quality of life, practical issues related to its management and patient support are particularly relevant.

In 1966, Gitelman and co-workers described a new familial disorder in which patients presented with hypokalemic alkalosis and a peculiar susceptibility to carpopedal spasm and tetany due to hypomagnesemia.[1] During several decades, the disease has been included in a group of closely related disorders, referred to as hypokalemic salt-losing tubulopathies or Bartter's-like syndromes (BS). All these disorders are associated with secondary aldosteronism which is responsible for hypokalemia and metabolic alkalosis, but they markedly differ in terms of age of onset, severity of clinical manifestations, presence of urinary concentrating defect, other electrolyte abnormalities, and magnitude of urinary calcium excretion. Based on clinical manifestations, it appeared that GS was related to a defect in the distal convoluted tubule (DCT) with thiazide-like manifestations, in contrast with the various types of BS which include disorders affecting the thick ascending limb (TAL) of Henle's loop, with furosemide-like manifestations.[2] In particular, a distinctive feature of GS is the dissociation of renal Ca^{2+} and Mg^{2+} handling, leading to hypocalciuria and hypomagnesemia.[3] GS is associated with loss of function mutations of the *SLC12A3* gene (located on chromosome 16q13) coding for the thiazide-sensitive sodium-chloride cotransporter (NCC) that is expressed in the apical membrane of the DCT.[4]

Genetics

GS is transmitted as an autosomal recessive trait, and the majority of patients are compound heterozygous for different mutations within the paternal and maternal *SLC12A3* allele. The prevalence of heterozygous carriers is approximately 1% in various populations.[5] To date, more than 250 mutations[6,7] scattered through *SLC12A3* have been identified in GS patients. Most (~ 75%) are missense mutations substituting

conserved amino acid residues, whereas nonsense, frameshift and splice-site defects, and gene rearrangements are less frequent.

A significant number of GS patients, up to 40% in some series, are found to carry only a single mutation in *SLC12A3*, instead of being compound heterozygous or homozygous. Because GS is recessively inherited, it is likely that there is a failure to identify the second mutation in regulatory fragments, 5' or 3' untranslated regions, or deeper intronic sequences of *SLC12A3*, or that there are large genomic rearrangements.[8] Vargas-Poussou *et al*. showed that there is a predisposition to large rearrangements caused by the presence of repeated sequences within the *SLC12A3* gene. These large rearrangements, which may account for up to 6% of all *SLC12A3* mutations, can be detected by multiplex legation-dependent probe amplification (MLPA), enhancing the sensitivity of genetic testing to >80%.[6] Mutations in the *CLCNKB* gene, which codes for the basolateral chloride channel ClC-Kb, have been detected in a few patients presenting simultaneous features of classic BS and GS.[9,10] The distribution of ClC-Kb in both the TAL and DCT, and potential compensation by other Cl$^-$ transporters, may probably explain these overlapping syndromes.[2] In any case, GS is indeed genetically heterogeneous, raising the possibility of a concurrent heterozygous mutation in a gene other than *SLC12A3*. In addition to *CLCNKB*, other genes participating in the complex handling of cations by the DCT are potential candidates.[8]

Phenotype variability

Cruz *et al*.[11] showed a high prevalence of patient-reported symptoms, with about half of the patients rating their symptoms as moderate to severe. Furthermore, GS was associated with a significant reduction in the quality of life—comparable in intensity to that associated with congestive heart failure or diabetes. Severe manifestations, such as early onset (before age 6 years), growth retardation, invalidating chondrocalcinosis, tetany, rhabdomyolysis, seizures, and ventricular arrhythmia have been described.[11-13] The phenotype of GS is highly heterogeneous in terms of age at presentation, nature/severity of biochemical abnormalities, and nature/severity of the clinical manifestations.[14] The phenotype variability has been documented not only among all patients carrying a wide variety of *SLC12A3* mutations but also when a common

underlying mutation is present and within families.[15] Considering that most of patients with GS are compound heterozygous harboring a wide variety of mutant *SLC12A3* alleles, the phenotype variability in GS raises the question whether peculiar symptoms could be related to the nature/position of the underlying mutation(s). In particular, nonsense, frameshift, splice-site defects and gene rearrangements will introduce premature translation stop codons that are likely to have a loss-of-function effect on the NCC cotransporter. By analogy with other autosomal recessive disorders, one can predict that the combination of mutations present in each allele may also play a role in the phenotype variability of GS. The possibility that male gender may also play a role in intra-familial variability has been raised.[14]

Clinical diagnosis

The presence of both hypocalciuria and hypomagnesemia is highly predictive for the diagnosis of GS.[16] However, hypocalciuria may be variable and hypomagnesemia may be absent in some patients.[2,17] No specific findings are observed at renal biopsy, apart from occasional hypertrophy of the juxta-glomerular apparatus and markedly reduced expression of NCC by immunohistochemistry. Interestingly, a few cases of GS associated with glomerular abnormalities and/or proteinuria have been described.[18]

The differential diagnosis of GS includes other Bartter-like syndromes, and particularly congenital BS due to mutations in *CLCNKB*, as well as diuretic or laxative abuse, and chronic vomiting. The clinical history and biochemical features, even hypocalciuria and hypomagnesemia, may not be fully reliable to distinguish GS from classic BS. Although implementation of genetic testing should be promoted, such testing in the context of BS and GS bears a significant cost when considering the number of exons to be screened, the lack of hotspots, and the large number of mutations described. A blunted response to a simple thiazide test has been proposed for the diagnosis of GS.[17]

Mechanism of disease: heterologous expression systems and mouse models

A potential mechanism for interfamilial phenotype heterogeneity in GS could be differences in the functional consequences of mutations in *SLC12A3*. Heterologous expression of NCC using *Xenopus laevis* oocytes has permitted functional effects of GS mutants to be tested. Like many integral plasma membrane proteins, the full glycosylation of NCC is essential for its normal function (and thiazide binding) in the cells lining the DCT.[19] Expression of mutant NCC in *Xenopus* oocytes revealed the existence of different types of mutations, which differ in terms of glycosylation, membrane expression and activity in terms of sodium transport.[14] A mouse model with a null mutation in *SLC12A3* on a pure background recapitulates many of the features observed in patients with GS.[20] Another mouse model with a homozygous knock-in mutation provides a good model for both deciphering the molecular pathogenesis of GS and testing potential therapies in vivo.[21]

Treatment

Lifelong oral magnesium and potassium supplementations are the main treatment in patients with GS. Magnesium supplementation should be considered first, since Mg^{2+} repletion will facilitate K^+ repletion and reduce the risk of tetany and other complications related to hypomagnesemia. All types of magnesium salts are effective, but their bioavailability is variable. Spironolactone or amiloride can be useful, both to increase serum K^+ levels in patients resistant to KCl supplements and to treat Mg^{2+} depletion that is worsened by elevated aldosterone levels. Both drugs should be started cautiously to avoid hypotension. Patients should not be refrained from their usual salt craving, particularly if they practice a regular physical activity. More recently, the potassium-sparing diuretic and aldosterone antagonist eplerenone was shown to be useful in the treatment of GS patients. Another option is low-dose indomethacin, a nonselective inhibitor of cyclooxygenase 1 (COX-1) and COX-2 but this treatment comes at the expense of gastrointestinal side effects and lowering of the glomerular filtration rate. Considering the occurrence of prolonged QT interval in up to half GS patients, QT-prolonging medications should be used with caution.[22]

Although GS adversely affects the quality of life, we lack information about the long-term outcome of these patients. Renal function and growth appear to be normal, provided there is lifelong supplementation. Progression to renal failure is rare in GS, with only a few cases developing end-stage renal disease being reported. Development of CKD, type 2 diabetes, and secondary hypertension has been reported in some cohorts.[22] During pregnancy, electrolyte disturbances may exacerbate with potential negative impact on obstetric and neonatal outcomes.[23]

CONFERENCE OVERVIEW

The clinical features of the disease (including its potential severity) are increasingly recognized and *SLC12A3* genotyping has been used to ascertain the diagnosis. The important phenotype variability of GS may be explained by types of mutations and their combinations, gender, regulatory or modifier genes, compensatory mechanisms, as well as environmental factors or dietary habits. Despite the remarkable clinical and molecular insights gained since its initial description, there is much mystery surrounding GS. Further efforts are needed to substantiate a number of issues related to the disease. As such the objective of this KDIGO conference is to gather a global panel of multi-disciplinary clinical and scientific expertise to address issues including: the diagnostic criteria and methods; the clinical work-up and follow-up; the phenotype heterogeneity in terms of age at presentation; nature/severity of the biochemical abnormalities and clinical manifestations; and the treatment and long-term consequences of the disease. Since GS is associated with impaired quality of life, practical issues related to its management and patient support are particularly relevant.

The conference also aims to summarize outstanding knowledge gaps and propose a research agenda to resolve standing controversial issues. It is hoped that the deliberations from this conference will inform clinicians of the evidence base for present treatment options and help pave the way for future studies in this area.

Drs. Olivier Devuyst (University of Zurich, Switzerland) and Nine V.A.M. Knoers (University Medical Centre Utrecht, The Netherlands) will co-chair this conference which includes worldwide leading experts with patient participation. The format of the conference will involve topical plenary session presentations followed by focused discussions on questions outlined in the **Appendix: Scope of Coverage**. The conference output will include publication of a position statement to help outline therapeutic management and future research in Gitelman Syndrome.

APPENDIX: SCOPE OF COVERAGE

1. Diagnosis

1. What are the minimum criteria for a clinical diagnosis of GS? Are these criteria different in adults vs. children? What is the predictive value of these criteria? Can we develop exclusion criteria or a flow chart? What is the value of increased/inappropriate urinary excretion of sodium, chloride, potassium? Type of urinary collection? Reference values?

2. What is the differential diagnosis of GS? (Bartter syndrome, Pseudo-Gitelman syndrome and others) Is this age-dependent?

3. What are available diagnostic methods? (including pharmacologic testing)

4. What biochemical analyses should be undertaken to confirm GS diagnosis? (e.g., plasma, urine spot, 24h-urine, renin, aldosterone, etc.) Should these analyses be performed off medications/supplements? For how long? Need to repeat if concurrent disorders?

2. Clinical Characteristics

1. What is the age of onset of symptoms/biochemical abnormalities?

2. What is the spectrum of symptoms? (e.g., tetany, cramps, rhabdomyolysis, seizures, arrhythmias, fatigue, quality of life)

3. What are the symptoms specific to pediatrics? (e.g., growth retardation)

4. What are the symptoms specific to adults? (e.g., chondrocalcinosis)

5. What is the variability in severity of biochemical abnormalities?

6. What is the variability in clinical symptoms?

7. What are the key questions and clinical complaints necessary to ascertain GS? (e.g., history of hydramnios, growth retardation, salt craving, thirst, impaired urinary concentration, joint pain, cramps, muscular manifestations, paresthesia, palpitations, fatigue, impaired urinary concentration, limited sports and physical activity, vertigo, cardiac arrhythmia, stomach and other abdominal pain, personal or familial history of hypokalemia, hypomagnesemia, diabetes or nephropathy)

8. What is the frequency of secondary hypertension[24]? Can we predict this? How best to treat?

3. Genetic Testing

1. Utility
   - What is the validity and clinical utility of genetic testing in the proband?
   - Any importance for diagnostics, research, natural history, practice?
   - When should genetic counselling be provided?
   - In which cases would it be important to perform genetic test as diagnosis of exclusion? (i.e., psychiatric conditions with diuretic abuse, Sjögren syndrome, long QT syndrome)

2. Technique
   - First SLC12A3 and then CLCNKB?
   - Sanger sequencing or next generation gene-panels?
   - Perform MLPA screening for deletions?

3. Family Studies
   - Do we test parents? If so, which one or both?
   - Do we need to test the siblings?
   - Do we need to perform carrier testing in partner of proband?
   - What is the validity of the observation that heterozygous carriers of SLC12A3 mutations are protected from hypertension?

4. Treatment

1. When do we need to treat? Which target?

2. What is the role of salt intake? Should we include low salt intake to attenuate hypokalemia? What is the role of diet, high NaCl supplements, and vitamin D in treatment for GS?

3. In regard to potassium supplements: Which one? Optimal dose? Precautions? What are the side effects?

4. In regard to magnesium supplements: Which form? What about issues concerning bioavailability? What are the side effects?

5. How should one scale up treatment if resistant hypokalemia or hypomagnesemia is presented? What is the role of spironolactone, amiloride, eplerenone, indomethacin, ACE inhibitors, angiotensin II receptor antagonists? How and when to wean?

6. Which drugs to exclude: Furosemide, hydrochlorothiazide? Drugs influencing cardiac conduction? Proton pump inhibitors?

7. How should we treat stomach pain and other abdominal pain? What could be the etiology?

8. How should we treat chondrocalcinosis?

5. Systemic Management and Follow-Up

1. What is the optimal management of GS? Optimal frequency of the follow-up? What are specific measures for aging patients? What is the acute management for severe hypokalemia and hypomagnesemia?

2. What is the risk of non-compliance to K^+ or Mg^{2+} supplements? What is the risk of chronic hypokalemia, alkalosis, hypomagnesemia? What are the risks resulting

from these electrolyte disturbances? (including metabolic syndrome, cardiac arrhythmias, kidney failure or cysts, sudden death, epilepsy)

3. Can we provide recommendations for pregnancy management?

4. Can we provide recommendations for electrolyte corrections before surgery with general anesthesia?

5. Should there be an evaluation for growth hormone disturbances? Is treatment with growth hormone useful? This would be difficult as GH stimulation tests may be normal and only nocturnal peaks are low which implies expensive and uncomfortable work-up (frequent blood sampling during night time)

6. What is the optimal management of chondrocalcinosis?

7. How can quality of life and patient support be better provided for GS patients?

8. Can we provide recommendation for sport practice? For example, in the presence of a documented exercise-induced LV dysfunction, should there be additional precautions; should physical activity be limited/prohibited?[25]

9. How can we best educate patients for self-management?

10. Could this disease compromise school performance or work life? If yes, is it justified to get compensation (e.g., in Germany you can get an upgrade of your school performance depending on the degree of "disability"? Are there social/employment consequences related to the disease state?

11. What are the long-term consequences of hydrochlorothiazide? What are the adverse effects of spironolactone, eplerenone, indomethacin?

12. Can we draw insights for more common conditions: e.g., chronic use of diuretics, dietary modifications (high K+ intake), long-term supplementation in case of renal or digestive loss, reimbursement for such supplements, compliance for oral supplements; GI toxicity of indomethacin?

13. What is the prognosis for GS patients? Is life expectancy altered?

14. What is the appropriate work-up following clinical diagnosis? e.g., renal ultrasound to exclude nephrocalcinosis, cysts, etc; cardiac work-up: EKG to assess conduction problems and, at least for adult patients, exercise LV function evaluation and QTc interval dynamic changes; ultrasound or x-ray for chondrocalcinosis; ophthalmology work-up (magnesium deficiency has been associated with several ophthalmologic diseases)

15. What biochemical analyses should be undertaken to look for complications in GS patient? (e.g., glucose, oral glucose test, lipid, creatinine, proteinuria testing, etc.)

16. Is there a utility in developing a clinical score based on the multi-systemic manifestations?

6. **Identification of Knowledge Gaps and Proposals for a Research Agenda to Resolve Standing Controversial Issues**

Most of the guidance we intend to provide from this conference output will be based on clinical practice or case reports, and is therefore derived from low grade evidence. We will certainly do our best but perhaps we should try to analyze our own cohort of patients in order to include more objective data (e.g., describing biological presentations of patients with positive genetic testing as compared to the negative ones). It may be helpful if we could design a simple data sheet where we can gather information from several hundreds of patients of all age groups.

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