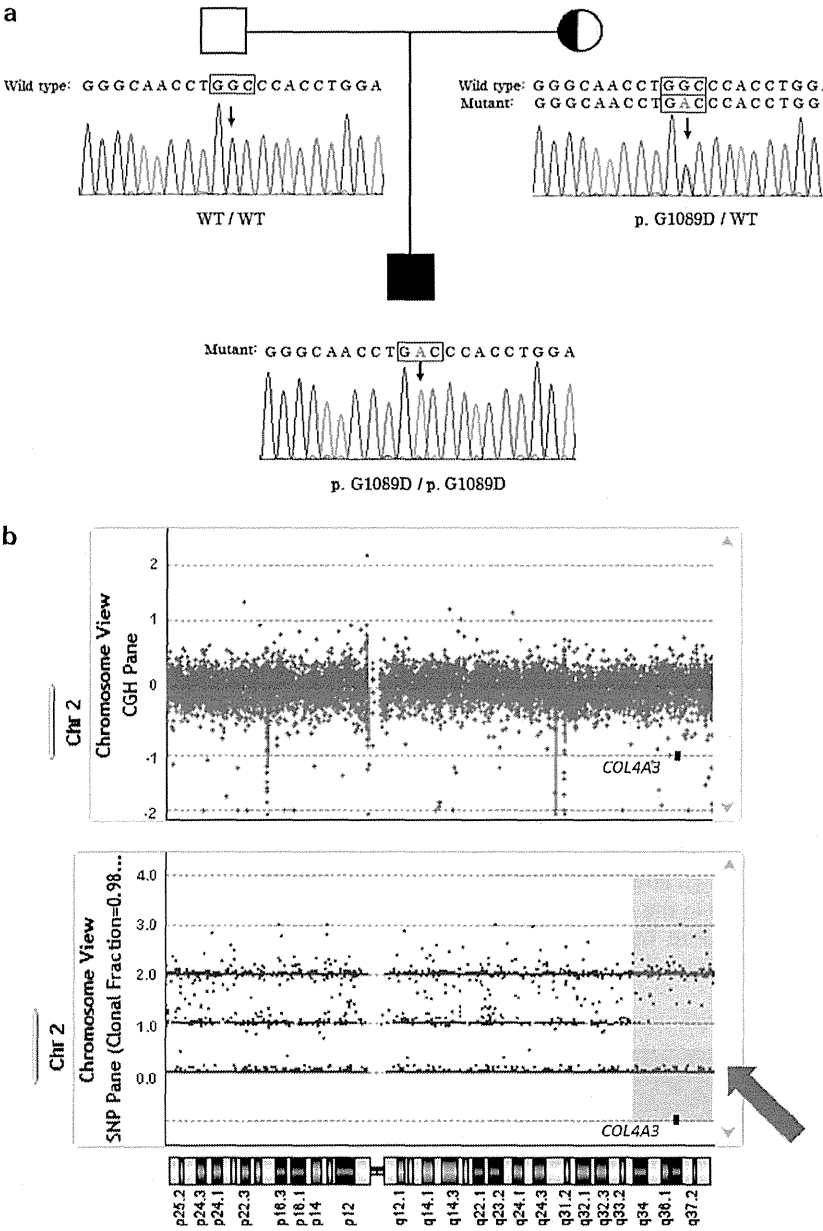

Figure 2. The family pedigree and sequencing of the *COL4A3* gene show a homozygous missense Gly1089Asp mutation in the patient. His mother is a carrier, whereas his father does not carry the mutation (**a**). Agilent CytoGenomics Analysis Software shows that there is no 2q deletion, and LOH is detected in the telomeric end of chromosome 2 (chr2: 207,541,513–243,014,630; **b**). CGH, comparative genome hybridization; SNP, single-nucleotide polymorphism; WT, wild type.

The patient is a 22-year-old Japanese male who was born from nonconsanguineous parents. He presented with persistent proteinuria and microhematuria since the age of 2 years and a history of renal biopsy performed at age 7 years. Renal electron microscopic findings showed thinning of the glomerular basement membrane (Figure 1a). Immunohistochemical staining for type IV collagen α2 (α2 [IV]) expression was observed in the glomerular basement membrane, and staining for α5 [IV] was observed only in Bowman's capsule (Figure 1b). These findings were compatible with typical ARAS findings. At the age of 17 years, he was diagnosed with progressive bilateral sensorineural hearing loss, and at 22 years, he underwent kidney transplantation. He has no other disorders, including physiological developmental delay. The patient's mother had mild proteinuria but normal renal function. She had a normal audiogram and no ocular abnormalities. No other family member suffered from renal disease.

We performed genetic analysis of the patient to confirm the diagnosis. The sequences of the *COL4A3*, *COL4A4* and *COL4A5* genes were examined by direct DNA sequencing and showed a novel homozygous missense NM_000091.4:c.3266G>A, Gly1089Asp mutation in exon 39 of the *COL4A3* gene. Genetic analysis of the mother showed that she is a heterozygous carrier of the mutation, whereas genetic analysis of the father showed only the wild-type sequence (Figure 2a). No mutations were found in the *COL4A4* or *COL4A5* genes. To rule out a hemizygotic mutation of this allele, we performed semiquantitative PCR analysis, which showed that the patient has two copies of the mutant *COL4A3* gene. From these results, we considered the possibility of maternal isodisomy of chromosome 2, including the *COL4A3* gene. To confirm the precise gene copy number and single-nucleotide polymorphism (SNP) haplotype of the region of the *COL4A3* gene, microarray analysis using comparative genome

hybridization (CGH) and SNP microarray (SurePrint G3 Human CGH +SNP Microarray 4×180 K, Agilent Technologies, Santa Clara, CA, USA) analysis were performed for the patient. SNP microarray data analysis for the patient revealed loss of heterozygosity (LOH) located in the chromosome region 2q33.3–2q37.2. The *COL4A3* gene is located in this ~35 Mb LOH region (Figure 2b). The region of LOH was determined to be copy number neutral, with no gain or loss of genetic material. Comparative analysis of SNP genotyping data in the region of LOH confirmed the occurrence of maternal isodisomy. This finding strongly suggests a segmental uniparental isodisomy of maternal origin in this region that includes the *COL4A3* gene. To confirm UPD, we tested polymorphisms using 16 single-nucleotide microsatellite markers spanning the entire length of chromosome 2. Nine markers were uninformative because they could have been inherited from either parent. Four other markers showed a heterozygous pattern compatible with a biparental mode of inheritance. Three markers showed a homozygous maternal inheritance, suggesting that reduction to homoallelism for the mutant *COL4A3* gene allele was due to segmental maternal isodisomy of the telomeric end of chromosome 2. These results confirmed that the patient has ARAS due to maternal isodisomy. Finally, we determined that a homozygous mutation in the *COL4A3* gene was caused by nonMendelian inheritance with segmental maternal isodisomy of the telomeric end of chromosome 2.

The present case is the first reported case of AS due to partial segmental UPD and is the second reported case of segmental maternal isodisomy of the telomeric end of chromosome 2q. The first report described reduction to homoallelism for a primary hyperoxaluria type 1 mutation in the case of a patient with complete liver alanine:glyoxylate aminotransferase deficiency with no symptoms, but liver and kidney dysfunction.[5] Although many cases of UPD—such as Beckwith–Wiedemann syndrome (UPD11) or Prader–Willi syndrome (UPD15)—are related to genomic imprinting and show various phenotypes, including intellectual disability, the present case shows typical ARAS. This outcome may stem from the fact that there are no imprinting regions in chromosome 2.[6] Because the recurrence risk after the birth of a child with segmental UPD seems to be negligible,[7] our result is useful for genetic counseling of the family.

We confirmed that there is a homozygous missense Gly1089Asp mutation of the *COL4A3* gene in the patient. Although this mutation has not been reported in ARAS patients, the PolyPhen-2 score (http://genetics.bwh.harvard.edu/pph2/) is 1.000, which indicates 'probably damaging,' and glycine substitutions in the collagenous domain of the *COL4A3* gene lead to the crucial constitutional changes resulting in the development of renal abnormalities. Although various genetic abnormalities of AS, including missense mutation, nonsense mutation, splicing error, nucleotide deletion and/or insertion, have been reported,[8] to the best of our knowledge, the present case is the first case of a patient with AS due to UPD. We have also reported the cases of 30 Japanese ARAS patients,[9] and among this group, there were no UPD patients other than the present patient (who is Patient number 114 in the literature).

The mechanisms of monosomy or trisomy rescue result in complete UPD, whereas segmental isodisomy with a normal status on the rest of chromosome as observed in this study indicates a fusion of maternal and paternal chromosomes, and was therefore most likely due to a postzygotic event.[3] Somatic recombination may be the possible mechanism for the segmental isodisomy. In this patient, homologous recombination between paternal and maternal chromatids may have occurred in the very early postzygotic period, as the vast majority of the patient's cells were found to carry the homozygous mutation based on the sequence data. Homologous recombination is one of the mechanisms for the repair of double-strand breaks. In this patient, the region of segmental isodisomy extends to the end of the long arm of chromosome 2, suggesting that break-induced replication, one of the double-strand break repair pathways similar to homologous recombination, was likely to lead to the generation of the large segmental isodisomy.[10]

In conclusion, this is the first reported case of a patient with AS due to UPD. Our observations may lead to an improved understanding of the genetic polymorphism of AS.

HGV DATABASE

The relevant data from this Data Report are hosted at the *Human Genome Variation* Database at http://hgv.figshare.com/genome_variation/13.

ACKNOWLEDGEMENTS

All phases of this study were supported by a grant from the Ministry of Health, Labour and Welfare (Japan) for Research on Rare Intractable Diseases in Kidney and Urinary Tract (H24-nanchi-ippan-041 to KI) in the 'Research on Measures for Intractable Diseases' Project, and a Grant-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Culture, Sports, Science and Technology (Subject ID: 25893131 to KN).

COMPETING INTERESTS

The authors declare no conflict of interest.

REFERENCES

1 Martin P, Heiskari N, Zhou J, Leinonen A, Tumelius T, Hertz JM *et al.* High mutation detection rate in the COL4A5 collagen gene in suspected Alport syndrome using PCR and direct DNA sequencing. *J Am Soc Nephrol* 1998; **9**: 2291–2301.
2 Mochizuki T, Lemmink HH, Mariyama M, Antignac C, Gubler MC, Pirson Y *et al.* Identification of mutations in the alpha 3(IV) and alpha 4(IV) collagen genes in autosomal recessive Alport syndrome. *Nat Genet* 1994; **8**: 77–81.
3 Engel E. A new genetic concept: uniparental disomy and its potential effect, isodisomy. *Am J Med Genet* 1980; **6**: 137–143.
4 Herzfeld T, Wolf N, Winter P, Hackstein H, Vater D, Müller U. Maternal uniparental heterodisomy with partial isodisomy of a chromosome 2 carrying a splice acceptor site mutation (IVS9-2A>T) in ALS2 causes infantile-onset ascending spastic paralysis (IAHSP). *Neurogenetics* 2009; **10**: 59–64.
5 Chevalier-Porst F, Rolland MO, Conchat P, Bozon D. Maternal isodisomy of the telomeric end of chromosome 2 is responsible for a case of primary hyperoxaluria type 1. *Am J Med Genet A* 2005; **132A**: 80–83.
6 Baskin B, Geraghty M, Ray PN. Paternal isodisomy of chromosome 2 as a cause of long chain 3-hydroxyacyl-CoA dehydrogenase [LCHAD] deficiency. *Am J Med Genet A* 2010; **152A**: 1808–1811.
7 Kotzot D. Complex and segmental uniparental disomy updated. *J Med Genet* 2008; **45**: 545–556.
8 Storey H, Savige J, Sivakumar V, Abbs S, Flinter FA. COL4A3/COL4A4 mutations and features in individuals with autosomal recessive Alport syndrome. *J Am Soc Nephrol* 2013; **24**: 1945–1954.
9 Oka M, Nozu K, Kaito H, Fu XJ, Nakanishi K, Hashimura Y *et al.* Natural history of genetically proven autosomal recessive Alport syndrome. *Pediatr Nephrol* (e-pub ahead of print 15 March 2014; doi:10.1007/s00467-014-2797-4).
10 Chen JM, Cooper DN, Férec C, Kehrer-Sawatzki H, Patrinos GP. Genomic rearrangement in inherited disease and cancer. *Semin Cancer Biol* 2010; **20**: 222–233.

Clin Exp Nephrol (2014) 18:4–9
DOI 10.1007/s10157-013-0911-8

SPECIAL ARTICLE

Diagnostic criteria for atypical hemolytic uremic syndrome proposed by the joint committee of the Japanese society of nephrology and the Japan pediatric society

Toshihiro Sawai · Masaomi Nangaku · Akira Ashida · Rika Fujimaru · Hiroshi Hataya · Yoshihiko Hidaka · Shinya Kaname · Hirokazu Okada · Waichi Sato · Takashi Yasuda · Yoko Yoshida · Yoshihiro Fujimura · Motoshi Hattori · Shoji Kagami

Published online: 18 December 2013

Abstract Atypical hemolytic uremic syndrome (aHUS) is rare and comprises the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury. Recently, abnormalities in the mechanisms underlying complement regulation have been focused upon as causes of aHUS. The prognosis for patients who present with aHUS is very poor, with the first aHUS attack being associated with a mortality rate of ~25 %, and with ~50 % of cases resulting in end-stage renal disease requiring dialysis. If treatment is delayed, there is a high risk of this syndrome progressing to renal failure. Therefore, we have developed diagnostic criteria for aHUS to enable its early diagnosis and to facilitate the timely initiation of appropriate treatment. We hope these diagnostic criteria will be disseminated to as many clinicians as possible and that they will be used widely.

Keywords Atypical hemolytic uremic syndrome · Thrombotic microangiopathy · Complement dysregulation · Alternative complement pathway · ADAMTS13

This article has been jointly published in *Clinical and Experimental Nephrology* and *Pediatrics International* by the Japanese Society of Nephrology and the Japan Pediatric Society.

T. Sawai
Department of Pediatrics, Shiga University of Medical Science, Otsu, Japan

M. Nangaku
Division of Nephrology and Endocrinology, The University of Tokyo School of Medicine, Tokyo, Japan

A. Ashida
Department of Pediatrics, Osaka Medical College, Takatsuki, Japan

R. Fujimaru
Department of Pediatrics, Osaka City General Hospital, Osaka, Japan

H. Hataya
Department of Nephrology, Tokyo Metropolitan Children's Medical Center, Fuchu, Japan

Y. Hidaka
Department of Pediatrics, Shinshu University School of Medicine, Matsumoto, Japan

S. Kaname
First Department of Internal Medicine, Kyorin University School of Medicine, Mitaka, Japan

H. Okada
Department of Nephrology, Faculty of Medicine, Saitama Medical University, Saitama, Japan

W. Sato
Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan

T. Yasuda
Division of Nephrology and Hypertension, Department of Medicine, St. Marianna University School of Medicine, Kawasaki, Japan

Y. Yoshida · Y. Fujimura
Department of Blood Transfusion Medicine, Nara Medical University, Kashihara, Japan

M. Hattori
Department of Pediatric Nephrology, Tokyo Women's Medical University, Tokyo, Japan

Introduction

Hemolytic uremic syndrome (HUS) is characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury (AKI) [1]. Approximately 90 % of pediatric patients develop this syndrome after infection with *Shigella dysenteriae*, which produces true Shiga toxins, or *Escherichia coli*, some strains of which produce Shiga-like toxins. Shiga toxin was originally called verotoxin because Vero cells derived from the kidney epithelial cells of the African green monkey are hypersensitive to this toxin [2]. Subsequently, other toxins were called Shiga-like toxin because of their similarities to Shiga toxin in terms of their antigenicity and structure. Shiga-like toxin-1 differs from Shiga toxin by only 1 amino acid, whereas Shiga-like toxin-2 shares 56 % sequence homology with Shiga-like toxin-1. Although Shiga-like toxin-producing *E. coli*-HUS (STEC-HUS) strains most often trigger HUS, certain Shiga toxin-secreting strains of *S. dysenteriae* can also cause HUS. They are currently known as the Shiga toxin family, and the terms are often used interchangeably. HUS occurring from infection with STEC-HUS was formerly called diarrhea + HUS (D + HUS) or typical HUS.

In contrast, HUS that is not related to Shiga toxins and accounts for ~10 % of all HUS cases, is called atypical HUS (aHUS). Although STEC-HUS is relatively common in children, aHUS occurs in individuals of all ages and is often familial. The prognosis is very poor, with the first aHUS attack being associated with a mortality rate of ~25 %, and with ~50 % of cases resulting in end-stage renal disease requiring dialysis [3].

In recent years, abnormalities in the mechanisms underlying complement regulation have been focused on as causes of aHUS. Various genetic abnormalities in complement regulatory factors, including complement factor H, have been noted in 50–60 % of patients. The analysis of the pathology underlying this condition is currently progressing rapidly [4].

The differential diagnosis of aHUS from STEC-HUS or thrombotic thrombocytopenic purpura (TTP), another form of thrombotic microangiopathy (TMA) caused by a deficiency of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), is not necessarily easy at the early stages of disease onset. However, if treatment is delayed, there is a high risk of this syndrome progressing to renal failure. Therefore, the Joint Committee of the Japanese Society of Nephrology and the Japan Pediatric Society (JSN/JPS) has developed diagnostic criteria for aHUS to enable its early diagnosis and to facilitate the timely initiation of appropriate treatment [5, 6]. We hope that the diagnostic criteria presented in this report will become familiar to as many clinicians as possible and that they will be used widely.

Definition of aHUS

aHUS is a type of TMA that differs from STEC-HUS and TTP, with the latter being caused by markedly reduced ADAMTS13 activity. aHUS is a syndrome characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and AKI, which is similar to STEC-HUS.

Guidelines for the diagnosis of aHUS

Definitive diagnosis

A definitive diagnosis of aHUS is made when the triad of microangiopathic hemolytic anemia, thrombocytopenia, and AKI is present. The disease should not be associated with Shiga toxins, and TTP should also be excluded.

The Joint Committee of the JSN/JPS defined microangiopathic hemolytic anemia based on a hemoglobin (Hb) level of <10 g/dL. The presence of microangiopathic hemolytic anemia should be confirmed based on increased serum lactate dehydrogenase levels, a marked decrease in serum haptoglobin levels, and the presence of red blood cell fragments in a peripheral blood smear.

Thrombocytopenia is defined as a platelet (PLT) count of <150,000/μL.

The definition of AKI has been updated, with the most recent definition given by the international guidelines group, the Kidney Disease: Improving Global Outcomes that integrates both the Risk, Injury, Failure, Loss, End-stage kidney disease and the Acute Kidney Injury Network classifications to facilitate identification. Thus, we recommend diagnosis based on the most recent guidelines, along with the following definitions. For pediatric cases, the serum creatinine should be increased to a level that is 1.5fold higher than the serum creatinine reference values based on age and gender issued by the Japanese Society for Pediatric Nephrology [7]. For adult cases, the diagnostic criteria for AKI should be used.

Guidelines for the diagnosis of aHUS

Definitive diagnosis

A definitive diagnosis of aHUS is made when the triad of microangiopathic hemolytic anemia, thrombocytopenia,

S. Kagami (✉)
Department of Pediatrics, Graduate School of Medical Sciences, Tokushima University, Tokushima, Japan
e-mail: kagami@tokushima-u.ac.jp

Table 1 Definitions of microangiopathic hemolytic anemia, thrombocytopenia, and AKI that have been established by the joint committee of the JSN/JPS

Microangiopathic hemolytic anemia	Thrombocytopenia	Acute kidney injury
Defined as an Hb level <10 g/dL Presence confirmed based on: Increased serum LDH levels Marked decreases in serum haptoglobin levels The presence of red blood cell fragments in a peripheral blood smear	Defined as a PLT count <150,000/μL	The most recent AKI definition is provided by the international guideline group, the KDIGO, integrating the RIFLE and AKIN classifications to facilitate identification. Thus, diagnosis should be based on the most recent guidelines, and the following definitions should be used. Pediatric cases: Serum creatinine should be increased to a level that is 1.5fold higher than the serum creatinine reference values based on age and gender issued by the Japanese Society for Pediatric Nephrology [7]. Adult cases: Diagnostic criteria for AKI should be used

Hb hemoglobin, *LDH* lactate dehydrogenase, *PLT* platelet, *AKI* acute kidney injury, *KDIGO* kidney disease: improving global outcomes, *RIFLE* risk, injury, failure, loss, end-stage kidney disease, *AKIN* acute kidney injury network

and AKI is present. The disease should have no association with Shiga toxins, and TTP should also be excluded. Table 1 presents the definitions of microangiopathic hemolytic anemia, thrombocytopenia, and AKI that are established by the Joint Committee of the JSN/JPS.

Probable diagnosis

A probable diagnosis of aHUS is made when 2 of the following 3 conditions are found: microangiopathic hemolytic anemia, thrombocytopenia, and AKI. The disease should have no association with Shiga toxins and TTP should be excluded.

Applicability of these diagnostic criteria

When we applied these diagnostic criteria to the Nara Medical University (NMU) TMA cohort, 15 out of 37 individuals who had all the data required for the assessment were diagnosed as having definitive aHUS. Since the data were recorded at one time point only, we speculate that the sensitivity of the diagnostic criteria would increase if we could assess data from multiple time points. The cut-off value for anemia, defined as an Hb level of <10 g/dL, and the cut-off value for thrombocytopenia, defined as a PLT count of <150,000/μL, are equivalent to those employed by the International Registry of Recurrent and Familial HUS/TTP [8]. We had considered using a cut-off value of a PLT count <100,000/μL for thrombocytopenia to reflect that used in the diagnostic criteria for STEC-HUS by the Japanese Society for Pediatric Nephrology (2000), but we only found 1 patient with a PLT count between 100,000 and 150,000/μL in the NMU cohort. Therefore, it is likely that this difference will not have a large impact on the sensitivity or specificity of our diagnostic criteria. Our diagnostic criteria include the category of "Probable" aHUS because we believe that this tentative diagnosis will help in the early diagnosis of aHUS and avoid delays in developing appropriate therapeutic approaches for patients with aHUS.

Evaluation of inappropriate complement activation

Abnormalities in complement regulation are among the main causes of aHUS. The diagnosis of aHUS that is caused by inappropriate complement activation has become more critical because eculizumab, a humanized anti-C5 monoclonal antibody, has been shown to be an effective therapeutic modality [9] that has been approved for the treatment of aHUS patients in Europe and the United States. Recently, Fan and colleagues evaluated genotype–phenotype relationships in 10 Japanese patients with aHUS and identified potentially causative mutations in complement factor H, C3, membrane cofactor protein, and thrombomodulin in 8 of the patients [10]. However, the definitive diagnosis of inappropriate complement activation in aHUS patients is difficult because some patients show normal serum levels of complement components [11] and there are a number of complement regulatory proteins, making it difficult to decide which complement regulatory protein is responsible for a particular patient developing aHUS.

Excluding Shiga toxin-producing *E. coli* infection

STEC-HUS is characterized by diarrhea accompanied by bloody stools. However, diarrhea may also be present in some aHUS cases. Diarrhea in aHUS can be a manifestation of ischemic colitis. In addition, enteritis that is not caused by STEC can trigger aHUS. Therefore, a diagnosis of STEC-HUS cannot be made based on symptoms alone, and the earlier nomenclature that used "D + HUS" to correspond with STEC-HUS and "D-HUS" to correspond

with aHUS is not used at present [11]. The involvement of Shiga toxins should be confirmed by stool culture, the direct detection of Shiga toxins, or the detection of anti-lipopolysaccharide-IgM antibodies.

Excluding TTP

Conventionally, TTP has been diagnosed based on the classic pentad (microangiopathic hemolytic anemia, thrombocytopenia, labile psychoneurotic disorder, fever, and renal failure). However, the discovery of ADAMTS13 led to the finding that 60–90 % of patients with TTP have a marked reduction in the activity of ADAMTS13, to a level of <5 %, regardless of race. Therefore, when diagnosing aHUS, patients who have markedly reduced levels of ADAMTS13 activity (<5 %) should be diagnosed as having TTP, thereby ruling out a diagnosis of aHUS. However, some patients may show the classic TTP pentad and have normal or slightly reduced levels of ADAMTS activity. Therefore, if a patient has levels of ADAMTS13 activity ≥5 %, a differential diagnosis of aHUS or TTP may be necessary to account for other clinical symptoms.

Excluding TMA caused by other distinct factors

Diseases that evidently cause a clinical state of TMA, including disseminated intravascular coagulation, sclerodermatous kidney, and malignant hypertension, should be excluded when diagnosing aHUS.

When a probable case of aHUS is suspected

When a probable case of aHUS is suspected, samples that are necessary to determine the appropriate diagnosis should be collected, and the therapeutic strategy should be established after consultation with an institution that has extensive experience of managing aHUS cases.

Cases where aHUS should be strongly suspected

If there are features that are characteristic of HUS, aHUS should be strongly suspected if the following criteria are fulfilled, regardless of the presence of diarrhea: the patient is younger than 6 months of age; time of onset is unclear (latent onset); the patient has a history of HUS (recurrent case); the patient has a history of anemia of unknown cause; recurrent HUS after kidney transplantation; the patient has a family history of HUS (excluding cases of food poisoning); and, the patient has no diarrhea or bloody stools.

Classification of aHUS causes, excluding TTP caused by the ADAMTS13 defect

Table 2 classifies the causes of aHUS and presents methods to determine the causes.

Discussion

Nineteen years after Gasser et al. [1] reported HUS, an interesting report was published in the *Lancet* [10]. This report indicated that although C3-predominant activity is initiated in the blood vessels in TMA patients, this is not observed in typical cases of HUS, suggesting that complement activation is involved in aHUS onset [12]. Subsequently, numerous researchers have elucidated further information on the pathology of aHUS. At present, the reported causes of aHUS include, complement regulation abnormalities, cobalamin metabolism disorder, infection with *Streptococcus pneumoniae* and other microorganisms, drugs, pregnancy, and autoimmune diseases.

The complement system plays an important role as part of the immune systems of living organisms. It is activated via 3 pathways, the classical, alternative, and lectin pathways. As a result of the activation of the host's alternative and classical pathways, C5b-9, a membrane attack complex, is generated and destroys cells by forming transmembrane pores. The alternative pathway is involved in the onset of aHUS. Unlike the classical and lectin pathways, activation of the alternative pathway does not require initiators; it is continuously activated by the spontaneous hydrolysis of C3.

When complement proteins are inappropriately activated, there is a risk of inducing cell dysfunction within the host itself. Thus, humoral factors in the circulating plasma and several plasma membrane-bound factors are involved in the regulation of complement activation and act at various stages, such as the inactivation of C3b or C4b, and the inhibition of the generation of membrane attack complexes. The regulators involved in the alternative pathway include complement factors H and I, which are humoral factors, and membrane cofactor protein and thrombomodulin, which are membrane-bound factors. If these factors are abnormal, the subsequent failure of regulation will hyperactivate the complement proteins, leading to the onset of aHUS. Some cases of aHUS develop after trigger events, for example, infections of the respiratory tract and the gastrointestinal tract, and it is likely that activation of the complement cascade by these trigger events and the

Table 2 Classification and determination of the causes of aHUS, excluding TTP caused by the ADAMTS13 defect

Cause of aHUS	Method to determine the cause
Complement regulation abnormality	Hemolysis test, quantification of complement proteins and complement regulatory proteins, and gene analysis. Even if the amounts of complement proteins and complement regulatory proteins are within the normal ranges, it does not serve as a basis for excluding complement-related aHUS
(i) Congenital	
Genetic mutations of complement proteins, factor H, factor I, membrane cofactor protein, C3, factor B, and thrombomodulin	
(ii) Acquired	
Production of autoantibodies, including anti-factor H antibody	Detection of anti-factor H antibody by ELISA, western blot, etc.
(2) Cobalamin metabolism disorder	Age at onset should be considered (<6 months old), and hypomethioninemia or hyperhomocysteinemia is detected on plasma amino acid analysis
(3) Infection	Definitive diagnosis by identification of pathogenic microorganisms and serological examination
(i) Pneumococcus	
(ii) Human immunodeficiency virus	
(iii) Pertussis	
(iv) Influenza	
(v) Varicella	
(4) Drug-induced	Identification of the drug
(i) Anticancer drugs	
(ii) Immunomodulatory drugs	
(iii) Antiplatelet drugs	
(5) Pregnancy-related	
(i) Hemolysis, elevated liver enzymes, low platelet counts (HELLP) syndrome	
(ii) Eclampsia	
(6) Autoimmune disease, collagen disease	Definitive diagnosis by autoantibody test, antiphospholipid antibody test, and serological examination
(i) Systemic lupus erythematosus	
(7) Bone-marrow transplant, organ transplant-related	
(8) Others	

aHUS atypical hemolytic uremic syndrome, *ELISA* enzyme-linked immunosorbent assay

subsequent amplification of complement activation by the alternative pathway cannot be regulated in patients with deficiencies in complement regulation. Gain-of-function mutations in C3 and complement factor B, which are complement-activating factors, also cause hyperactivation of complement proteins and, ultimately, aHUS.

It has been reported that ~50 % of aHUS patients have genetic abnormalities in complement regulatory factors, including complement factor H. The frequency of the presence of certain mutations among aHUS cases, responsiveness to plasma therapy, prognosis of kidney function, and the recurrence rate after kidney transplantation, vary depending on the type of genetic abnormalities present [13]. Although plasmapheresis within 24 h of confirmation of the diagnosis has been recommended as the initial treatment for aHUS [14], its effects are not always satisfactory. The mortality or incidence of end-stage renal disease is considered to be between 70 and 80 %, and the recurrence rate after kidney transplantation may be as high as 80–90 %, particularly in patients with abnormal complement factor H, which is the most frequent abnormality [15].

In 2011, eculizumab (Soliris®, Alexion Pharmaceuticals), a terminal complement inhibitor, was approved as a new drug for the treatment of aHUS in Europe and the US. Eculizumab is a humanized recombinant immunoglobulin G2/4 monoclonal antibody directed against the complement component C5, which was developed as a treatment for paroxysmal nocturnal hemoglobinuria. By binding to complement component C5, the drug inhibits the generation of C5a and C5b-9, and thus subsequently inhibits the complement system.

There are a number of reports stating that only HUS that is associated with complement regulation abnormalities is defined as aHUS. On the basis of the current diagnostic criteria, we have defined aHUS to include all types of HUS that are not related to Shiga toxins or other distinct causes. In cases where aHUS is associated with complement

dysregulation, the introduction of eculizumab may markedly change therapeutic strategies. It should be noted, however, that recommendations of specific therapeutic modalities are beyond the scope of the current diagnostic criteria. However, in cases where complement dysregulation is confirmed as the cause, treatment with eculizumab is established. Thus, it may be desirable to assign HUS associated with complement dysregulation a separate disease name rather than it being classified as "aHUS", as in the case of definitive "complement-mediated TMA".

As described in previous reports, aHUS is a disease that may frequently cause renal failure and be fatal if it is not appropriately diagnosed and treated at the early stages of disease onset. In Japan, aHUS may be misdiagnosed as HUS caused by Shiga toxins because clinicians are not sufficiently aware of aHUS, and consequently, treatment may be delayed. Thus, our diagnostic criteria include the category of "Probable" aHUS to ensure that the clinicians consider aHUS during diagnosis. Many issues should be addressed in the future, including the development of diagnostic strategies to diagnose cases of suspected aHUS, the establishment of insurance coverage for ADAMTS13 activity measurement testing that is necessary to differentiate aHUS from TTP, and the development of treatment guidelines. We hope that our diagnostic criteria will be used widely and will contribute to the diagnosis and treatment of aHUS patients.

Acknowledgments These diagnostic criteria for aHUS were proposed by the Joint Committee of the Japanese Society of Nephrology (JSN) (President: Seiichi Matsuo) and the Japan Pediatric Society (JPS) (President: Takashi Igarashi). The members of the committee are Shoji Kagami (Chair), Akira Ashida, Rika Fujimaru, Hiroshi Hataya, Motoshi Hattori, Yoshihiko Hidaka, Shinya Kaname, Masaomi Nangaku, Hirokazu Okada, Waichi Sato, Toshihiro Sawai, Takashi Yasuda, Yoko Yoshida (Adviser) and Yoshihiro Fujimura (Adviser). This study was supported by the JPS and JSN.

Conflict of interest Advisory role: Yoshihiro Fujimura (Baxter Bioscience and Alexion Pharmaceuticals).Honoraria: Masaomi Nangaku (Kyowa Hakko Kirin Co. Ltd and Daiichi Sankyo Co. Ltd).Subsidies: Masaomi Nangaku (Kyowa Hakko Kirin Co. Ltd, Daiichi Sankyo Co. Ltd, Astellas Pharma Inc., Mitsubishi Tanabe Pharma Corporation, Chugai Pharmaceutical Co. Ltd and Takeda Pharmaceutical Co. Ltd). The other authors have no conflicts of interest.

References

1. Gasser C, Gautier E, Steck A, Siebenmann R, Oechslin R. Hemolytic-uremic syndrome: bilateral necrosis of the renal cortex in acute acquired hemolytic anemia. Schweizerische medizinische Wochenschrift. 1955;85(38–39):905–9.
2. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immun. 1977;18:775–9.
3. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. N Engl J Med. 2009;361(17):1676–87. doi:10.1056/NEJMra0902814.
4. Kavanagh D, Goodship T. Genetics and complement in atypical HUS. Pediatric Nephrol (Berlin, Germany). 2010;25(12):2431–42. doi:10.1007/s00467-010-1555-5.
5. Kagami S, Okada H, Kaname S, Sato W, Nangaku M, Yasuda T, et al. Diagnostic criteria of atypical hemolytic uremic syndrome. Jpn J Nephrol. 2013;55(2):91–3 (in Japanese).
6. Kagami S, Okada H, Kaname S, Sato W, Nangaku M, Yasuda T et al. Diagnostic criteria of atypical hemolytic uremic syndrome. J Jpn Pediatr Soc. 2013; http://www.jpeds.or.jp/uploads/files/saisin_130201.pdf (in Japanese).
7. Uemura O, Honda M, Matsuyama T, Ishikura K, Hataya H, Yata N, et al. Age, gender, and body length effects on reference serum creatinine levels determined by an enzymatic method in Japanese children: a multicenter study. Clin Exp Nephrol. 2011;15(5):694–9. doi:10.1007/s10157-011-0452-y.
8. Noris M, Caprioli J, Bresin E, Mossali C, Pianetti G, Gamba S, et al. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. Clin J Amer Soc Nephrol. 2010;5(10):1844–59. doi:10.2215/CJN.02210310.
9. Legendre C, Licht C, Muus P, Greenbaum L, Babu S, Bedrosian C, et al. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. N Engl J Med. 2013;368(23):2169–81. doi:10.1056/NEJMoa1208981.
10. Fan X, Yoshida Y, Honda S, Matsumoto M, Sawada Y, Hattori M, et al. Analysis of genetic and predisposing factors in Japanese patients with atypical hemolytic uremic syndrome. Mol Immunol. 2013;54(2):238–46. doi:10.1016/j.molimm.2012.12.006.
11. Loirat C, Frémeaux-Bacchi V. Atypical hemolytic uremic syndrome. Orphanet J Rare Dis. 2011;6:60. doi:10.1186/1750-1172-6-60.
12. Stühlinger W, Kourilsky O, Kanfer A, Sraer J. Letter: haemolytic-uraemic syndrome: evidence for intravascular C3 activation. Lancet. 1974;2(7883):788–9.
13. Nester C, Thomas C. Atypical hemolytic uremic syndrome: what is it, how is it diagnosed, and how is it treated? Hematology. 2012;2012:617–25. doi:10.1182/asheducation-2012.1.617.
14. Ariceta G, Besbas N, Johnson S, Karpman D, Landau D, Licht C, et al. Guideline for the investigation and initial therapy of diarrhea-negative hemolytic uremic syndrome. Pediatric Nephrol (Berlin, Germany). 2009;24(4):687–96. doi:10.1007/s00467-008-0964-1.
15. Waters A, Licht C. aHUS caused by complement dysregulation: new therapies on the horizon. Pediatric Nephrol (Berlin, Germany). 2011;26(1):41–57. doi:10.1007/s00467-010-1556-4.

Ann Hematol
DOI 10.1007/s00277-014-2234-6

LETTER TO THE EDITOR

The first two cases of *MYH9* disorders in Thailand: an international collaborative study

Nongnuch Sirachainan · Patcharee Komwilaisak · Katsumasa Kitamura · Suradej Hongeng · Takashi Sekine · Shinji Kunishima

Received: 7 July 2014 / Accepted: 7 October 2014

Dear Editor,

MYH9 disorders are the autosomal dominant platelet disorders characterized by giant platelets, thrombocytopenia, and granulocyte Döhle body-like cytoplasmic inclusion bodies and are due to mutations in *MYH9*, the gene encoding non-muscle myosin heavy chain IIA (NMMHC-IIA). Patients show variable expression of non-hematological complications, such as glomerulonephritis, hearing inability, and cataracts [1, 2]. Although granulocyte inclusion bodies are the laboratory hallmark, they are often overlooked due to their inconspicuous appearance on conventionally stained blood smears. Abnormal NMMHC-IIA protein accumulates in the granulocyte cytoplasm, and an immunofluorescence analysis of NMMHC-IIA localization is now used as a reliable diagnostic test [3–5]. Because the complications are progressive and there are strict genotype/phenotype correlations, an early genetic diagnosis is crucial to confirm and determine the prognosis, and if possible, to select appropriate treatment [6].

We conducted an international collaborative *MYH9* disorders study between Thailand and Japan. Patients were registered in the pediatric macrothrombocytopenia registry in Thailand. The criteria for registration were platelet counts <150,000/μL and large platelets. All patients were investigated for the common causes of macrothrombocytopenia, including the von Willebrand factor indices, flow cytometry for CD42b and CD41/CD61, and platelet aggregation studies. In the present study, peripheral blood smears from 10 patients were sent to Japan, where the immunofluorescence analysis for NMMHC-IIA was performed [3]. Two out of the 10 patients (patients 6 and 8) (20 %) were positive in the analysis, and *MYH9* gene sequencing was further performed [3]. Local institutional ethics committees approved the study, and informed consent was obtained from the patients.

Patient 6 was a 12-year-old female. The hematological examination showed a platelet count of 67,000/μL, MPV of 17.6 fL, giant platelets, and conspicuous granulocyte inclusion bodies (Fig. 1a). Blood chemistry and urinalysis were unremarkable. The hearing test showed a high-tone drop in the left ear. Her father was also hematologically affected (Fig. 1b). He had mild hearing loss in both ears. Cataracts were absent in both individuals. Patient 8 was a 12-year-old female presenting with persistent hypertension, nephritis, and thrombocytopenia. She had a history of intermittent epistaxis, gum bleeding, and menorrhagia. She had previously been treated with prednisolone and nifedipine without any response. The laboratory investigation showed a platelet count of 67,000/μL and MPV of 11.3 fL. A peripheral blood smear showed giant platelets and only faint inclusion bodies in neutrophils (Fig. 1c). The BUN was 64.5 mg/dL (normal range, 5–18 mg/dL), and creatinine was 1.63 mg/dL (normal range, 0.5–1.1 mg/dL). Urinalysis showed protein 2+ and blood 3+. Hearing test was not performed. Mild cataracts were observed. Her father and brother died from bleeding.

The immunofluorescence analysis for NMMHC-IIA revealed type II localization, consisting of several cytoplasmic spots with a circular or oval shape in both patients (Fig. 1d, e, f). Genomic DNA was extracted from the remaining smears.

N. Sirachainan · S. Hongeng
Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

P. Komwilaisak
Srinagarind Hospital, KhonKaen University, KhonKaen, Thailand

K. Kitamura · S. Kunishima (✉)
Department of Advanced Diagnosis, Clinical Research Center, National Hospital Organization Nagoya Medical Center, 4-1-1 Sannomaru, Naka-ku, Nagoya 4600001, Japan
e-mail: kunishis@nnh.hosp.go.jp

T. Sekine
Department of Pediatrics, Toho University School of Medicine, Ohashi Hospital, Tokyo, Japan

Published online: 15 November 2014

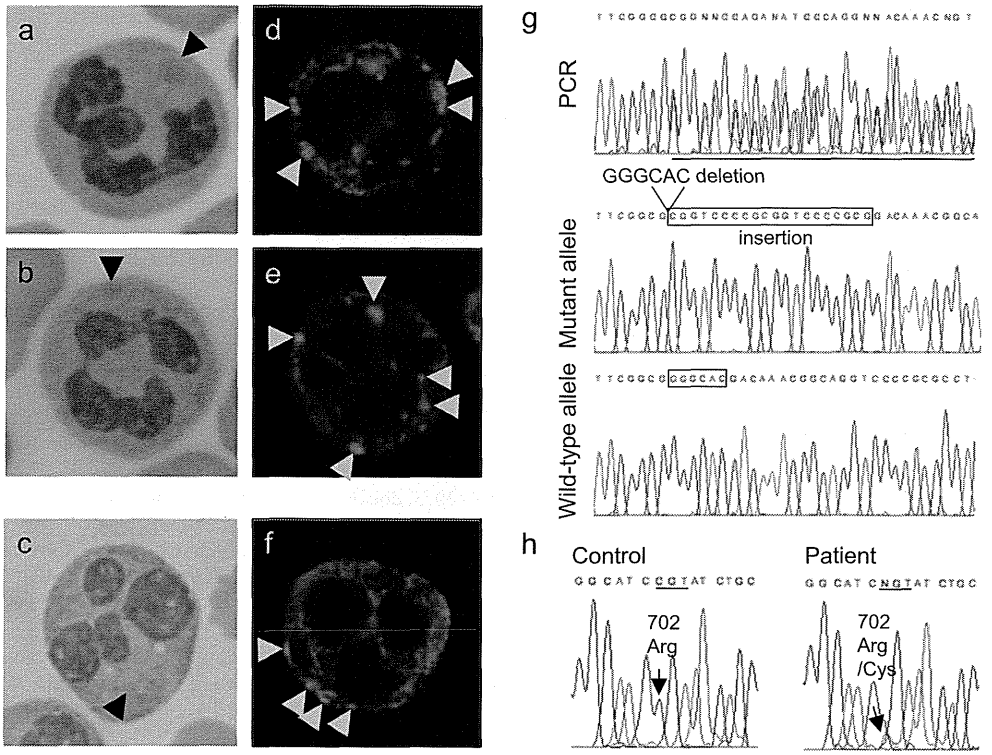

Fig. 1 The results of the NMMHC-IIA and *MYH9* analyses. Light micrographs of May-Grünwald-Giemsa-stained neutrophils from patient 6 (**a**), the father of patient 6 (**b**), and patient 8 (**c**), respectively. Inclusion bodies are indicated by *arrowheads*. Immunofluorescence micrographs of neutrophils immunostained with the anti-NMMHC-IIA antibody in patient 6 (**d**), the father of patient 6 (**e**), and patient 8 (**f**), respectively. The accumulations of NMMHC-IIA are indicated by *arrowheads*. Sequencing of the *MYH9* gene showed 5788_5793delinsCGCGGGGA CCGCGGGGACCG resulting in p.V1930_P1931fsX and 2104 C>T, resulting in p.R702C, in patients 6 (**g**) and 8 (**h**), respectively. Complementary sequences are shown

As type II NMMHC-IIA localization and visible inclusion bodies are mostly associated with exons 26 and 30 mutations [3], these exons were initially analyzed in patient 6. No mutations were found. An extended analysis revealed a novel exon 40 mutation, p.V1930_P1931fsX24 in both patient 6 and her father (Fig. 1g). The type II NMMHC-IIA localization and faint inclusion bodies in patient 8 suggested exons 1 and 16 mutations. Accordingly, a p.R702C was detected (Fig. 1h). This mutation is known as an early onset of glomerulonephritis and hearing disability [6–8]. Recent investigations have suggested that angiotensin receptor blockers and/or angiotensin converting enzyme inhibitors may have a protective effect against the progression of glomerulonephritis [7, 9]. We thus consider a careful follow-up and treatment plan.

We identified the first two Thai patients with *MYH9* disorders, who had not been definitely diagnosed. The present study should improve the diagnosis and treatment of patients with *MYH9* disorders in areas where a differential diagnosis is unavailable. Patients who present with macrothrombocytopenia, even without granulocyte inclusion bodies and non-hematological complications, should be screened by NMMHC-IIA immunofluorescence, and the presence of an *MYH9* mutation should be confirmed by genetic analysis. Close collaboration between hospitals in areas where these tests are unavailable and institutions with more advanced diagnostic capabilities should be established to facilitate the correct and prompt diagnosis and treatment of these cases [10].

Acknowledgments The authors thank Yoshiko Ohira for valuable technical assistance. This work was supported by grants from the Ministry of Health, Labour and Welfare for research on rare intractable diseases in kidney and urinary tract (to TS), the Japan Society for the Promotion of Science KAKENHI (to SK and TS), Mitsubishi Pharma Research Foundation (to SK), and the 24th General Assembly of the Japanese Association of Medical Sciences (to SK).

The pediatric macrothrombocytopenia registry in Thailand is comprised of 15 centers (Dr. Patcharee Komwilaisak, KhonKaen University, Dr. Somjai Kanjanapongkul, Queen Sirikit National Institute of Child Health, Dr. Chanchai Trivaree, Phramongkutklao Hospital, Dr. Darintr Sosothikul, Chulalongkorn University, Dr. Pimlak Charoenkwan, Chiang Mai University, Dr. Bunchoo Pongtanakul, Mahidol University, Dr. Thirachit Chotsamphancharoen, Prince of Songkla University, Dr. Kittima Kanchanakhumhang, Sawanpracharak Hospital, Dr. Siranee Wongruangsri, Lampang Hospital, Dr. Sumonmaln Klamchuen, Sappasithiprasong Hosptial, Dr. Nattaporntira Phalakornkul, Bhumibol Adulyadej Hospital, Dr. Saranya Busakornruangrat, Somdejprapinklao Hospital, Dr. Pacharapan Surapolchai, Thammasat University, Dr. Angkana Winaichatsak, Maharat Nakhonratchasima Hospital, and Dr. Nongnuch Sirachainan, Mahidol University).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Balduini CL, Pecci A, Savoia A (2011) Recent advances in the understanding and management of *MYH9*-related inherited thrombocytopenias. Br J Haematol 154(2):161–174. doi:10.1111/j.1365-2141.2011.08716.x

2. Kunishima S, Saito H (2010) Advances in the understanding of *MYH9* disorders. Curr Opin Hematol 17(5):405–410. doi:10.1097/MOH.0b013e32833c069c
3. Kunishima S, Matsushita T, Kojima T, Sako M, Kimura F, Jo EK, Inoue C, Kamiya T, Saito H (2003) Immunofluorescence analysis of neutrophil nonmuscle myosin heavy chain-A in *MYH9* disorders: association of subcellular localization with *MYH9* mutations. Lab Invest 83(1):115–122
4. Savoia A, De Rocco D, Panza E, Bozzi V, Scandellari R, Loffredo G, Mumford A, Heller PG, Noris P, De Groot MR, Giani M, Freddi P, Scognamiglio F, Riondino S, Pujol-Moix N, Fabris F, Seri M, Balduini CL, Pecci A (2010) Heavy chain myosin 9-related disease (*MYH9*-RD): neutrophil inclusions of myosin-9 as a pathognomonic sign of the disorder. Thromb Haemost 103(4):826–832. doi:10.1160/TH09-08-0593
5. Kitamura K, Yoshida K, Shiraishi Y, Chiba K, Tanaka H, Furukawa K, Miyano S, Ogawa S, Kunishima S (2013) Normal neutrophil myosin IIA localization in an immunofluorescence analysis can rule out *MYH9* disorders. J Thromb Haemost 11(11):2071–2073. doi:10.1111/jth.12406
6. Pecci A, Klersy C, Gresele P, Lee KJ, De Rocco D, Bozzi V, Russo G, Heller PG, Loffredo G, Ballmaier M, Fabris F, Beggiato E, Kahr WH, Pujol-Moix N, Platokouki H, Van Geet C, Noris P, Yerram P, Hermans C, Gerber B, Economou M, De Groot M, Zieger B, De Candia E, Fraticelli V, Kersseboom R, Piccoli GB, Zimmermann S, Fierro T, Glembotsky AC, Vianello F, Zaninetti C, Nicchia E, Guthner C, Baronci C, Seri M, Knight PJ, Balduini CL, Savoia A (2014) *MYH9*-related disease: a novel prognostic model to predict the clinical evolution of the disease based on genotype-phenotype correlations. Hum Mutat 35(2):236–247. doi:10.1002/humu.22476
7. Sekine T, Konno M, Sasaki S, Moritani S, Miura T, Wong WS, Nishio H, Nishiguchi T, Ohuchi MY, Tsuchiya S, Matsuyama T, Kanegane H, Ida K, Miura K, Harita Y, Hattori M, Horita S, Igarashi T, Saito H, Kunishima S (2010) Patients with Epstein-Fechtner syndromes owing to *MYH9* R702 mutations develop progressive proteinuric renal disease. Kidney Int 78(2):207–214. doi:10.1038/ki.2010.21
8. Kunishima S, Yoshinari M, Nishio H, Ida K, Miura T, Matsushita T, Hamaguchi M, Saito H (2007) Haematological characteristics of *MYH9* disorders due to *MYH9* R702 mutations. Eur J Haematol 78(3):220–226. doi:10.1111/j.1600-0609.2006.00806.x
9. Pecci A, Granata A, Fiore CE, Balduini CL (2008) Renin-angiotensin system blockade is effective in reducing proteinuria of patients with progressive nephropathy caused by *MYH9* mutations (Fechtner-Epstein syndrome). Nephrol Dial Transplant 23(8):2690–2692. doi:10.1093/ndt/gfn277
10. Glembotsky AC, Marta RF, Pecci A, De Rocco D, Gnan C, Espasandin YR, Goette NP, Negro F, Noris P, Savoia A, Balduini CL, Molinas FC, Heller PG (2012) International collaboration as a tool for diagnosis of patients with inherited thrombocytopenia in the setting of a developing country. J Thromb Haemost 10(8):1653–1661. doi:10.1111/j.1538-7836.2012.04805.x

12. 腎・尿路

腎実質性高血圧，腎血管性高血圧

新潟大学医歯学総合病院小児科 池住洋平（いけずみようへい）

治療を要する小児の高血圧の大部分は，腎実質性もしくは腎血管性の二次性高血圧である．年齢の低い児に原因不明の高血圧がみられた場合は，なんらかの器質的疾患の存在を考えて精査を進める必要がある．

診断のポイント

治療を要する小児高血圧症の大部分は二次性高血圧であり，瘢痕化腎や急性糸球体腎炎，腎動脈狭窄などに代表される腎実質性または血管性の腎性高血圧が約80%を占める．

1. 腎実質性高血圧 糸球体腎炎に起因する高血圧は多くの場合，血尿や蛋白尿など，なんらかの尿所見異常を伴う．ただし，わが国の小児の慢性糸球体腎炎の多くは学校検尿で早期に発見され，高血圧症を呈することは比較的まれである．一方，慢性糸球体腎炎をはじめとする腎実質性障害に起因する高血圧は，すでに腎機能の低下を伴っている場合が多い．このような糸球体濾過量（glomerular filtration rate：GFR）の低下に基づく高血圧は，循環血流量の増大（溢水）によりしばしば浮腫を伴い，血液検査で心房性Na利尿ペプチドの上昇がみられる他，胸部X線検査で胸水や心拡大を認める場合がある．

先天性腎尿路奇形（congenital anomalies of kidney and urinary tract：CAKUT）や瘢痕化腎などの腎の形態異常の診断には，超音波検査，CT検査や腎シンチグラフィなどの画像検査が有用である．

2. 腎血管性高血圧 腎血管性高血圧の原因として，小児では線維筋性異形成（fibromuscular dysplasia：FMD）の頻度がもっとも高く，その他大動脈炎症候群，神経線維腫症やもやもや病などが原因となる．

腎血管性高血圧の患児における血圧は収縮期200 mmHg前後のきわめて高値を呈する場合が多く，高血圧脳症や心不全などの重篤な合併症状で発見される場合も多い．また，一般に薬物療法ではコントロールが困難であり，2種類以上の降圧薬が必要となる．安静時の末梢血検査で血漿レニン活性（plasma renin activity：PRA）の上昇を認めるが，高値を認めるのは本症の約50%程度である．また，レニン－アンジオテンシン（renin-angiotensin：RA）系の亢進により低カリウム血症を呈する場合が多く，診断の補助となる．

アンジオテンシン変換酵素（angiotensin converting enzyme：ACE）阻害薬であるカプトプリル（カプトリル®）0.7 mg/kg（最大50 mg）を投与し，投与前と投与1時間後のPRA値を測定する方法（カプトプリル負荷試験）は，より診断に有用であるが，両側腎動脈狭窄では陰性となることも多い．カプトリル負荷レノグラムは，カプトプリル負荷によって狭窄側の腎血流がさらに低下し，健側の血流が増加することから左右差が増強することを利用した方法で検出率が高い．しかし，小児においては再現性が得にくく，血管造影検査をしのぐ診断法とはいえない．また，高血圧緊急症など緊急的な対応を要する状況下で行うことは困難である．

造影CT検査は解像度が優れており，細部にわたり狭窄部位の検索が可能である．また，小児に対しても迅速に行えるという利点がある．しかし，多量の造影剤を使用することから，腎機能障害がある場合には施行しにくい．また，MRIによる血管撮影検査（MRA）についても，ガドリニウムを造影剤として使用する方法は，腎機能障害を伴う患者では腎性全身性線維症を発症する危険性があり，施行が困難である．一方，近年造影剤を使用しないMRI（非造影MRA）の研究・開発が進んでおり，診断能力も飛躍的に向上している．両側の腎機能障害を伴う両側性腎動脈狭窄や，移植腎の血管閉塞などを検出する場合に有用である[1]．

確定診断には，腎動脈血管撮影および腎静脈レニンのサンプリングがもっとも有用となるが，侵襲性の問題があり，上記のスクリーニング検査で診断のつかない症例や，腎動脈狭窄の詳しい評価が必要な場合に行われる．小児に頻度の高いFMDでは，末梢病変を見落とす可能性があり，選択性腎動脈造影の適応となる．

0386-9806/14/¥100/頁/JCOPY

表1　小児高血圧に対する代表な的経口降圧薬

一般名	種　類	使用量	最大量
ニフェジピン	Ca 拮抗薬	0.2～1.0 mg/kg/日 分1～2	60 mg/日
アムロジピン*	Ca 拮抗薬	6歳以上 2.5 mg/日 分1，適宜増減	5 mg/日
エナラプリル*	ACE 阻害薬	生後1か月以上 0.08 mg/kg/日 分1	10 mg/日
リシノプリル*	ACE 阻害薬	6歳以上 0.07 mg/kg/日 分1	20 mg/日
カプトプリル	ACE 阻害薬	0.3～0.5 mg/kg/日 分3	6 mg/kg (150 mg/日)
バルサルタン*	ARB	6歳以上	
		体重 35 kg 未満 20 mg/日 分1	40 mg/日
		体重 35 kg 以上 40 mg/日 分1	160 mg/日
ロサルタン	ARB	0.7～1.4 mg/kg/日 分1	100 mg/日

ACE：アンジオテンシン変換酵素，ARB：アンジオテンシンⅡ受容体拮抗薬
*：わが国で小児高血圧症に対して保険適用を有する薬剤

基本病態

1．腎実質性高血圧　腎実質の障害は腎機能低下（GFR の低下）を伴う場合が多く，GFR の低下に伴う RA 系の活性化により，末梢血管抵抗が増大するとともにアルドステロンの分泌が促進し，Na および水分の再吸収を促進するため，循環血液量が増大し血圧は上昇する．

2．腎血管性高血圧　腎に流入する血管の狭窄による腎組織中の血流の低下は，傍糸球体装置により感知され，腎実質性高血圧と同様に RA 系の活性化をもたらす．

治療の実際

1．腎実質性高血圧　慢性に経過する場合が多く内科的治療が主体となるが，治療薬は循環血液量増加による高血圧，レニン依存性高血圧などの病態に応じて選択する．また，国内外で比較的広く小児に対して使用され，安全性が確認されているものを優先して使用する（表1）．頭痛，めまい，悪心などの症状を伴う急激な血圧の上昇（高血圧緊急症）は降圧薬の静脈内投与を含めた救急治療の対象となる．

2．腎血管性高血圧　RA 系阻害薬を主体とした内科的治療を行うが，コントロールが困難な症例も多い．小児の腎血管性高血圧は FMD に起因する例が多く，粥状動脈硬化の多い成人例と比較して成功率が高いことから，バルーンカテーテルを用いた経皮経管的腎血管形成術（percutaneous transluminal renal angioplasty：PTRA）のよい適応となる[2]．本法は比較的侵襲が少なく繰り返し施行できる利点がある．ステントの挿入は，PTRA 直後や成功後早期に再狭窄を生じる症例については適応があるが，長期にわたる抗凝固薬の内服が必要になることや，長期的な予後が明らかでないことなどから，小児に対して行われる頻度は低い．

PTRA での血行再建が困難な場合や再狭窄をきたす症例では，外科的に狭窄部位を切除する血管再建術や自家腎移植術，あるいは高度機能不全を認める腎については摘出術も検討する必要がある．

最新ガイドライン/エビデンス

小児の高血圧判定基準ならびに治療指針は，日本高血圧学会の「高血圧治療ガイドライン 2009」（JSH2009）および日本循環器科学会や日本小児腎臓病学会などからなる合同研究班の「小児期心疾患における薬物療法ガイドライン」（JCS2012）に示されている[3)4)]．両者の大きな違いは，JSH2009 が日本人小児を対象として自動血圧計を用いて測定した血圧値に基づいた判定基準であるのに対し，JCS2012 は水銀法で測定された血圧値に基づく米国の小児高血圧ガイドラインを採用している点である．米国ガイドラインにおける血圧基準値は，自動血圧計によって測定された JSH2009 の「管理用基準」と比較し，拡張期の基準値が約 10 mmHg ほど高く設定されている．一方，性別，年齢の他，身長の影響も考慮されており，血圧値による明確な治療指針が示されている点で有用である．しかし，小児の血圧測定を正確に行うことは困難な場合も多く，また二次性高血圧の多くは血圧が異常高値を示すことが多いことからも，JSH2009 に示されている比較的高めに設定された小児高血圧の「診断用基準」を用いるのが簡便である（表2）[3)]．

近年のトピック

近年，片側の腎血管狭窄とこれに伴う片側虚血により，著明な高血圧，低カリウム血症，さらに低ナトリウム血症，代謝性アルカローシス，蛋白尿，多飲多尿をきたす病態，hyponatremic hypertensive syndrome（HHS）が知られ，小児例の報告も増えてい

私の治療方針

腎実質性高血圧

1．循環血液量増加による高血圧　以下の①または②を単独，または併用して用いる．

①利尿薬：フロセミド（ラシックス®細粒 40 mg/g）0.5～2 mg/kg/日（最大 80 mg）分 2．

②Ca 拮抗薬：ニフェジピン（セパミット R®細粒 20 mg/g）0.5～2 mg/kg/日（最大 40 mg）分 2．

2．レニン依存性の高血圧　以下の①～③を単独，または併用して用いる．

①ACE 阻害薬：エナラプリル（レニベース®錠）0.08 mg/kg/日（最大 10 mg）分 1．

②アンジオテンシンⅡ受容体拮抗薬（angiotensin Ⅱ receptor brocker：ARB）：ロサルタン（ニューロタン®錠）0.7～1.5 mg/kg/日（最大 100 mg）分 1．

③Ca 拮抗薬：アムロジピン（ノルバスク®錠）2.5 mg/日，適宜増減（最大 5 mg）分 1．

腎血管性高血圧　内科的治療は腎実質性高血圧と同様．小児の腎血管性高血圧は薬物療法でコントロールが困難な場合が多く，可能な限り PTRA による根治を目標とする．

高血圧性緊急症　以下の①を用い，意識障害など内服困難な場合は②を用いる．

①ニフェジピン（セパミット®細粒 10 mg/g）0.3 mg/kg/回，頓用．

②ニカルジピン（ペルジピン®注）0.5 μg/kg/分から開始，維持 1～3 μg/kg/分で持続静注．

表2　小児高血圧判定基準（文献3）より引用）

		収縮期血圧（mmHg 以上）	拡張期血圧（mmHg 以上）
乳　児		100	65
幼　児		120	70
小学校	低学年	130	80
	高学年	135	80
中学校	男　子	140	85
	女　子	135	80

る[5]．この病態では，患側腎虚血による RA 系の亢進に伴い著明な高血圧や低カリウム血症，代謝性アルカローシスをきたす一方，健側腎では圧利尿による尿中への Na 排出の増加と多尿によって低ナトリウム血症を生じる．また，アンジオテンシンⅡ作用が口渇を招き，多飲による循環血漿の希釈が低ナトリウム血症を助長する．さらに，健常糸球体での圧の上昇と，透過性の亢進によって蛋白尿を生じると考えられている[6]．

ピットフォールと対策

ACE 阻害薬や ARB は腎性高血圧の治療薬として有用であるが，腎機能が低下している症例や RA 系が亢進している症例では急速な GFR の低下や高カリウム血症をきたす場合がある．少量から開始し，血清 Cr や K 値のモニタリングが必要である．腎機能の低下例では，肝代謝型である Ca 拮抗薬を用いるほうが安全である．また，ACE 阻害薬や ARB は催奇形性を有することから，妊娠可能な年齢の女性には用いてはならない．

文　献

1) Angeretti M et al.: *Acta Radiol* **54**: 749-756, 2013
2) Tullus K et al.: *Lancet* **371**: 1453-1463, 2008
3) 日本高血圧学会高血圧治療ガイドライン作成委員会・編：小児の高血圧．高血圧治療ガイドライン 2009．日本高血圧学会，83-86，2009
4) 日本循環器学会・他：小児期心疾患における薬物療法ガイドライン．循環器病の診断と治療に関するガイドライン 2012（2010-2011 年度合同研究班報告），日本循環器学会，89-301，2012
5) Kovalski Y et al.: *Pediatr Nephrol* **27**: 1037-1040, 2012
6) Nicholls MG: *Pediatr Nephrol* **21**: 887-890, 2006

著者連絡先

〒951-8520　新潟県新潟市中央区旭町通一番町 754
新潟大学医歯学総合病院小児科
池住洋平

Nucleosides, Nucleotides and Nucleic Acids, 0:1–5, 2013

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2013.857781

Taylor & Francis
Taylor & Francis Group

IDENTIFICATION OF A HYPOURICEMIA PATIENT WITH SLC2A9 R380W, A PATHOGENIC MUTATION FOR RENAL HYPOURICEMIA TYPE 2

Toshinori Chiba,[1] Hirotaka Matsuo,[1] Shushi Nagamori,[2] Akiyoshi Nakayama,[1] Yusuke Kawamura,[1] Seiko Shimizu,[1] Masayuki Sakiyama,[1] Makoto Hosoyamada,[3] Sayo Kawai,[4] Rieko Okada,[4] Nobuyuki Hamajima,[5] Yoshikatsu Kanai,[2] and Nariyoshi Shinomiya[1]

[1]*Department of Integrative Physiology and Bio-Nano Medicine, National Defense Medical College, Tokorozawa, Saitama, Japan*
[2]*Division of Biosystem Pharmacology, Department of Pharmacology, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan*
[3]*Department of Human Physiology and Pathology, Teikyo University School of Pharmaceutical Sciences, Tokyo, Japan*
[4]*Department of Preventive Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[5]*Department of Healthcare Administration, Nagoya University Graduate School of Medicine, Nagoya, Japan*

□ *Hypouricemia is characterized by low serum uric acid (SUA) levels (≤3.0 mg/dL) with complications such as urolithiasis and exercise-induced acute renal failure. We have previously reported that urate transporter 1 (*URAT1/SLC22A12*) and glucose transporter 9 (*GLUT9/SLC2A9*) are causative genes for renal hypouricemia type 1 (RHUC1) and renal hypouricemia type 2 (RHUC2), respectively. In the series of experiments, two families have been revealed to have RHUC2 due to* GLUT9 *missense mutations R198C or R380W, respectively. Thus far, however, no studies have reported other RHUC2 families or patients with these pathogenic mutations. This study is aimed to find other cases of RHUC2.*

We performed mutational analyses of GLUT9 *exon 6 (for R198C) and exon 10 (for R380W) in 50 Japanese hypouricemia patients. Patients were analyzed out of a collection of more than 2000 samples from the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study).*

We identified a novel male patient with heterogeneous RHUC2 mutation R380W. The SUA of this hypouricemia patient was 2.6 mg/dL, which is similar to that of our previous report (SUA: 2.7 mg/dL).

This is the second report indicating RHUC2 patient due to GLUT9 mutation R380W. This mutation occurs in highly conserved amino acid motifs and is reported to be an important membrane topology determinant. R380W is a dysfunctional mutation which completely diminishes the urate

Received 15 September 2013; accepted 16 October 2013.
Address correspondence to Hirotaka Matsuo, Department of Integrative Physiology and Bio-Nano Medicine, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan. E-mail: hmatsuo@ndmc.ac.jp

transport activities of GLUT9. Our study revealed a second hypouricemia patient with GLUT9 *R380W, a pathogenic mutation of RHUC2, which may help to expand our understanding of RHUC pathogenesis.*

Keywords: SLC transporters; GLUT family; GLUT9L; GLUT9S; renal urate reabsorption

INTRODUCTION

Renal hypouricemia is characterized by low serum uric acid (SUA) levels (≤3.0 mg/dL), and confers risk of severe complications such as exercise-induced acute renal failure or nephrolithiasis.[1, 2] Renal hypouricemia is mainly caused by impaired renal urate reabsorption. We previously reported that URAT1/SLC22A12[3] and GLUT9/SLC2A9[4] are key regulators of SUA, and play an essential role in urate reabsorption in the human kidney. The dysfunctional mutations of *URAT1* or *GLUT9* cause renal urate hypouricemia, called renal hypouricemia type 1 (RHUC1) and renal urate hypouricemia type 2 (RHUC2), respectively. [5] Previously, two families have been revealed to have RHUC2 due to *GLUT9* missense mutations R198C or R380W, respectively. Thus far, however, no studies have reported other RHUC2 families or patients with these pathogenic mutations. Here, we report another hypouricemia patient with the pathogenic RHUC2 mutation.

MATERIALS AND METHODS

For the hypouricemia patients, 50 Japanese patients with lower SUA (≤3.0 mg/dl) were identified out of more than 2000 samples from the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study). We performed mutational analysis of *GLUT9* exon 6 (R198C) and exon 10 (R380W) in these 50 hypouricemia patients.

For the *GLUT9* sequence determination, we used following primers described previously:[4] for exon 6, forward 5′-GTCCTCTGAAATGCACCTCC-3′, and reverse 5′-GCACAGAAGATGCCTAAACAAACACA-3′; for exon 10, forward 5′-GGTGACCATATCCATCCAG-3′, and reverse 5′-GAAGGAG-CACCTTAAGGTTG-3′. High molecular weight genomic DNA was extracted from peripheral whole blood cells,[6] and was amplified by PCR. The PCR products were sequenced in both directions using a 3130xl Genetic Analyzer (Applied Biosystems).[7]

RESULTS

The human *GLUT9* gene consists of 14 exons (1 noncoding and 13 coding) and the alternative splicing of the *GLUT9* gene results in two main transcripts: GLUT9 isoform 1 (long isoform, GLUT9L) and isoform 2 (short isoform, GLUT9S). Two heterozygous missense mutations of R380W and

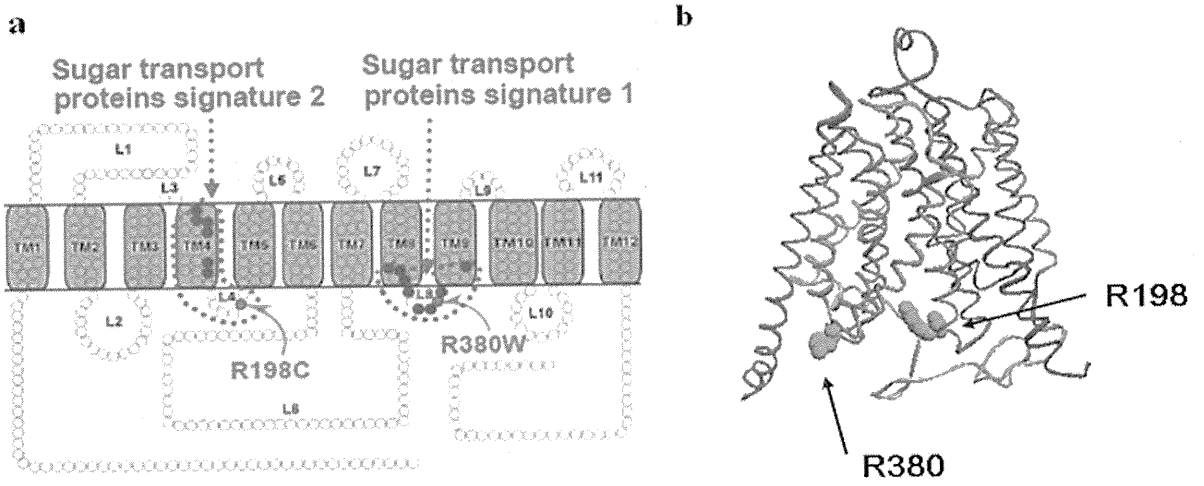

FIGURE 1 Pathogenic mutation sites of GLUT9 (Color figure available online).

R198C for GLUT9L have been identified in Japanese patients with renal hypouricemia. Both mutations are missense mutations from basic amino acid arginine to neutral amino acids, and are at equivalent positions within the cytoplasmic loops, which cause a loss of positive charge. These pathogenic mutation sites in two-dimensional and three-dimensional models are shown in Figure 1. No hypouricemia patient with the R198C mutation was identified among these 50 patients. However, we identified a novel male patient with heterozygous mutation R380W (Figure 2). SUA of this hypouricemia patient was 2.6 mg/dL (154.6 μmol/l), which is similar to that of our previous report (SUA: 2.7 mg/dL (160.6 μmol/l)).

DISCUSSION

GLUT9 mutations in renal hypouricemia patients may change its topology.

We have previously identified loss-of-function mutations of *GLUT9* in renal hypouricemic patients having no *URAT1* mutations.[4] Mutation sites in *GLUT9* (R380W and R198C for GLUT9L, corresponding to R351W and R169C for GLUT9S) locate in highly conserved amino acid motifs called "sugar transport proteins signatures," which is observed in GLUT family transporters. The corresponding mutations in *GLUT1* (R333W and R153C) are known to cause *GLUT1* deficiency syndrome.[8] Arginine residues in this motif are reported to be an important determinant of membrane topology of human GLUT1,[9] and the same may be true in GLUT9 on the basis of membrane topology.

Physiological Importance of GLUT9 in Human Urate Transport

The urate metabolism in humans is quite different from that in mice due to the lack of uricase.[10] In addition, hypouricemia is one of relatively rare

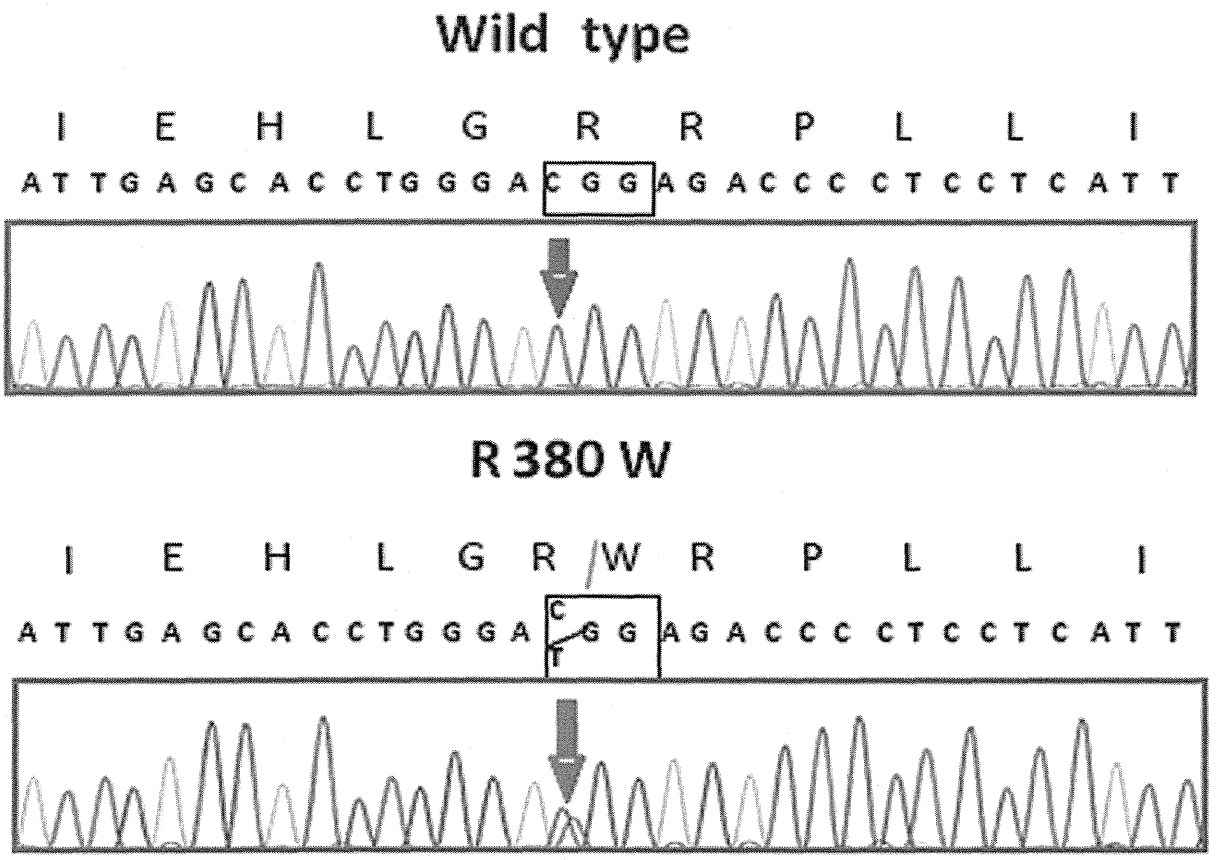

FIGURE 2 Heterozygous mutation (R380W) in a newly-identified renal hypouricemia patient (Color figure available online).

diseases compared with common diseases including hyperuricemia and gout. Therefore, it is of great significance to identify the dysfunctional *GLUT9* mutations in humans through a large population.

In MDCK cells, GLUT9L and GLUT9S show basolateral and apical localization, respectively. Since dysfunctional mutations of either GLUT9L or GLUT9S dramatically reduced the urate transport activity, renal hypouricemia caused by these mutations could be ascribed to the decreased urate reabsorption on both sides of the renal proximal tubules, where GLUT9 expresses. In the present study, we confirmed the importance of *GLUT9* as a causative gene for renal hypouricemia, which encodes a renal urate reabsorption transporter.

Identification of a Novel RHUC2 Patient

This is the second report indicating a RHUC2 patient due to *GLUT9* mutation R380W. Screening of large genome cohort samples revealed the second hypouricemia patient with *GLUT9* R380W, a pathogenic mutation of RHUC2. Our results confirm that GLUT9 can be a promising therapeutic target for hyperuricemia, gout, and related cardiovascular diseases. This finding may help to expand the understanding of RHUC pathogenesis.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to all the patients involved in this study. They are indebted to J. Abe, K. Gotanda, C. Okada, H. Inoue, and Y. Utsumi for genetic analysis. This study was supported by grants from the Ministry of Health, Labour and Welfare of Japan, the Ministry of Education, Culture, Sports, Science and Technology of Japan, the Japan Society for the Promotion of Science, the Ministry of Defense of Japan, the Kawano Masanori Memorial Foundation for Promotion of Pediatrics, Takeda Science Foundation, and the Gout Research Foundation of Japan.

This is a proceeding of the poster presentation made at the 15th International Symposium on Purine and Pyrimidine Metabolism in Man, PP13, in Madrid, Spain (June 9–13, 2013).

REFERENCES

1. Diamond, H.S.; Paolino, J.S. Evidence for a postsecretory reabsorptive site for uric acid in man. *J. Clin. Invest.* **1973**, 52, 1491–1499.
2. Kikuchi, Y.; Koga, H.; Yasutomo, Y.; Kawabata, Y.; Shimizu, E.; Naruse, M.; Kiyama, S.; Nonoguchi, H.; Tomita, K.; Sasatomi, Y.; Takebayashi, S. Patients with renal hypouricemia with exercise-induced acute renal failure and chronic renal dysfunction. *Clin. Nephrol.* **2000**, 53, 467–472.
3. Enomoto, A.; Kimura, H.; Chairoungdua, A.; Shigeta, Y.; Jutabha, P.; Cha, S.H.; Hosoyamada, M.; Takeda, M.; Sekine, T.; Igarashi, T.; Matsuo, H.; Kikuchi, Y.; Oda, T.; Ichida, K.; Hosoya, T.; Shimokata, K.; Niwa, T.; Kanai, Y.; Endou, H. Molecular identification of a renal urate anion exchanger that regulates blood urate levels. *Nature.* **2002**, 417, 447–452.
4. Matsuo, H.; Chiba, T.; Nagamori, S.; Nakayama, A.; Domoto, H.; Phetdee, K.; Wiriyasermkul, P.; Kikuchi, Y.; Oda, T.; Nishiyama, J.; Nakamura, T.; Morimoto, Y.; Kamakura, K.; Sakurai, Y.; Nonoyama, S.; Kanai, Y.; Shinomiya, N. Mutations in glucose transporter 9 gene SLC2A9 cause renal hypouricemia. *Am. J. Hum. Genet.* **2008**, 83, 744–751.
5. Kawamura, Y.; Matsuo, H.; Chiba, T.; Nagamori, S.; Nakayama, A.; Inoue, H.; Utsumi, Y.; Oda, T.; Nishiyama, J.; Kanai, Y.; Shinomiya, N. Pathogenic GLUT9 mutations causing renal hypouricemia type 2 (RHUC2). *Nucleosides Nucleotides Nucleic Acids.* **2011**, 30, 1105–1111.
6. Matsuo, H.; Kamakura, K.; Saito, M.; Okano, M.; Nagase, T.; Tadano, Y.; Kaida, K.; Hirata, A.; Miyamoto, N.; Masaki, T.; Nakamura, R.; Motoyoshi, K.; Tanaka, H.; Tsuji, S. Familial paroxysmal dystonic choreoathetosis: clinical findings in a large Japanese family and genetic linkage to 2q. *Arch. Neurol.* **1999**, 56, 721–726.
7. Matsuo, H.; Takada, T.; Ichida, K.; Nakamura, T.; Nakayama, A.; Ikebuchi, Y.; Ito, K.; Kusanagi, Y.; Chiba, T.; Tadokoro, S.; Takada, Y.; Oikawa, Y.; Inoue, H.; Suzuki, K.; Okada, R.; Nishiyama, J.; Domoto, H.; Watanabe, S.; Fujita, M.; Morimoto, Y.; Naito, M.; Nishio, K.; Hishida, A.; Wakai, K.; Asai, Y.; Niwa, K.; Kamakura, K.; Nonoyama, S.; Sakurai, Y.; Hosoya, T.; Kanai, Y.; Suzuki, H.; Hamajima, N.; Shinomiya, N. Common defects of ABCG2, a high-capacity urate exporter, cause gout: a function-based genetic analysis in a Japanese population. *Sci. Transl. Med.* **2009**, 1, 5ra11.
8. Pascual, J.M.; Wang, D.; Yang, R.; Shi, L.; Yang, H.; De Vivo, D.C. Structural signatures and membrane helix 4 in GLUT1: inferences from human blood-brain glucose transport mutants. *J. Biol. Chem.* **2008**, 283, 16732–16742.
9. Sato, M.; Mueckler, M. A conserved amino acid motif (R-X-G-R-R) in the Glut1 glucose transporter is an important determinant of membrane topology. *J. Biol. Chem.* **1999**, 274, 24721–24725.
10. Wu, X.W.; Lee, C.C.; Muzny, D.M.; Caskey, C.T. Urate oxidase: primary structure and evolutionary implications. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, 86, 9412–9416.

PEDIATRICS INTERNATIONAL Official Journal of the Japan Pediatric Society

Pediatrics International (2014) **56**, 309–314 doi: 10.1111/ped.12357

Review Article

Branchio-oto-renal syndrome: Comprehensive review based on nationwide surveillance in Japan

Naoya Morisada, Kandai Nozu and Kazumoto Iijima

Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan

Abstract Branchio-oto-renal (BOR) syndrome is an autosomal dominant disorder characterized by branchiogenic malformation, hearing loss and renal anomalies. The prevalence of BOR syndrome is 1/40 000 in Western countries, and nationwide surveillance in 2009–2010 identified approximately 250 BOR patients in Japan. Three causative genes for BOR syndrome have been reported thus far: *EYA1*, *SIX1*, and *SIX5*, but the causative genes for approximately half of all BOR patients remain unknown. This review article discusses the epidemiology, clinical symptoms, genetic background and management of BOR syndrome.

Key words branchio-oto-renal syndrome, *EYA1*, hearing loss, renal anomaly, *SIX1*.

Branchio-oto-renal (BOR) syndrome (OMIM 113650) is an autosomal dominant disorder characterized by branchiogenic malformation, hearing loss and renal abnormalities. BOR syndrome was first described by Melnick *et al.* in 1976.[1] Patients with BOR syndrome who do not present with renal abnormalities are also said to have branchio-otic (BO) syndrome (OMIM 602588). Both BOR and BO syndrome are allelic disorders. In the OMIM database, two BOR and three BO syndromes have been registered (Table 1). Due to recent advances in genetics, several of the causative genes of BOR syndrome have been identified (*EYA1*, *SIX1*, and *SIX5*), as well as the chromosomal region, microdeletions or microduplications within which can also lead to the syndrome. The causative genes for approximately half of all BOR patients, however, have yet to be identified.

This review article discusses the epidemiology, clinical symptoms, genetic background, differential diagnosis and management of BOR syndrome.

Epidemiology

Fraser *et al.* surveyed 421 children with hearing loss attending schools for hearing-impaired children in Montreal. They noted that 19 students had auricular pits, which led them to speculate that the prevalence of BOR syndrome is approximately one in 40 000 live births.[2] In Japan the prevalence of BOR syndrome was unknown until very recently. Our group carried out research in 2009–2010 to clarify the number of Japanese BOR patients.[3] An initial questionnaire was sent to the 1715 central hospitals throughout Japan. The collection rate of the questionnaire was 47.8%, and 85 patients with BOR phenotypes were identified. A second, more detailed questionnaire was then sent to each of these 85 patients and to their doctors. The collection rate was 37.6% (32/85) and 58.8% (50/85), respectively.

From this nationwide surveillance we estimated that there were approximately 250 BOR patients (95% confidence interval: 170–320) in the period 2009–2010 who were seen in clinics in Japan. These data suggest that the prevalence of BOR syndrome in Japan is much lower than in Western countries; the reason for this is unknown, but we suggest that BOR syndrome may be underdiagnosed in Japan because it is not well known. There may be many more patients in Japan who have not yet been accurately diagnosed.

Clinical symptoms and diagnosis

Chen *et al.* produced a very comprehensive report on the clinical symptoms of BOR syndrome in the Western world.[4] That study has been very useful for increasing awareness of BOR syndrome. The present study comprising nationwide surveillance in Japan clarified the phenotype of BOR patients of Japanese descent (Table 2).

Second branchial arch anomalies

Second branchial arch anomalies include the branchial cleft sinus tract appearing as a pinpoint opening anterior to the sternocleidomastoid muscle, and the presence of a branchial cleft cyst as a palpable mass under the sternocleidomastoid muscle.[5] These symptoms were observed in approximately half of all BOR patients in both Western countries and Japan.

Hearing loss

Hearing loss is the most common symptom of BOR syndrome. Chen *et al.* reported that >90% of BOR patients have a hearing impairment.[4] According to our survey, hearing loss is also present in >90% of Japanese BOR patients. Various types of hearing loss were also observed, with mixed type being the most common cause (Table 3). In addition, two of the present patients had

Correspondence: Naoya Morisada, MD PhD, Department of Pediatrics, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. Email: morisada@med.kobe-u.ac.jp

Received 6 March 2014; revised 24 March 2014; accepted 3 April 2014.