Recurrent FXYD2 p.Gly41Arg mutation in patients with isolated dominant hypomagnesaemia

Jeroen H.F. de Baaij1,*, Eiske M. Dorresteijn2,*, Eric A.M. Hennekam3, Erik-Jan Kamsteeg4, Rowdy Meijer4, Karin Dahan5, Michelle Muller6, Marinus A. van den Dorpel7, René J.M. Bindels1, Joost G.J. Hoenderop1, Olivier Devuyst8 and Nine V.A.M. Knoers3

1Department of Physiology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands, 2Pediatric Nephrology, Erasmus MC, Sophia Childrens Hospital, Rotterdam, The Netherlands, 3Department of Medical Genetics, University Medical Centre Utrecht, Utrecht 3508 AB, The Netherlands, 4Department of Human Genetics, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands, 5Institut de Génétique et de Pathologie, IPG, Gosselies, Belgium, 6Centre Hospitalier Peltzer-La Tourelle, Verviers, Belgium, 7Department of Internal Medicine, Maasstad Hospital, Rotterdam, The Netherlands and 8Institute of Physiology, ZIHP, University of Zurich, Zürich, Switzerland

Correspondence and offprint requests to: Nine V.A.M. Knoers; E-mail: V.V.A.Knoers@umcutrecht.nl

*These authors contributed equally to this work.

ABSTRACT

Background. Magnesium (Mg²⁺) is an essential ion for cell growth, neuroplasticity and muscle contraction. Blood Mg²⁺ levels <0.7 mmol/L may cause a heterogeneous clinical phenotype, including muscle cramps and epilepsy and disturbances in K⁺ and Ca²⁺ homeostasis. Over the last decade, the genetic origin of several familial forms of hypomagnesaemia has been found. In 2000, mutations in FXYD2, encoding the γ-subunit of the Na⁺–K⁺-ATPase, were identified to cause isolated dominant hypomagnesaemia (IDH) in a large Dutch family suffering from hypomagnesaemia, hypocalciuria and chondrocalcinosis. However, no additional patients have been identified since then.

Methods. Here, two families with hypomagnesaemia and hypocalciuria were screened for mutations in the FXYD2 gene. Moreover, the patients were clinically and genetically characterized.

Results. We report a p.Gly41Arg FXYD2 mutation in two families with hypomagnesaemia and hypocalciuria. Interestingly, this is the same mutation as was described in the original study. As in the initial family, several patients suffered from muscle cramps, chondrocalcinosis and epilepsy. Haplotype analysis revealed an overlapping haplotype in all families, suggesting a founder effect.

Conclusions. The recurrent p.Gly41Arg FXYD2 mutation in two new families with IDH confirms that FXYD2 mutation causes hypomagnesaemia. Until now, no other FXYD2 mutations have been reported which could indicate that other FXYD2 mutations will not cause hypomagnesaemia or are embryonically lethal.

Keywords: distal convoluted tubule, FXYD2, kidney, magnesium, Na⁺–K⁺‐ATPase

INTRODUCTION

Hypomagnesaemia is a common electrolyte imbalance resulting in muscle cramps, muscle weakness and cardiac arrhythmias [1]. The use of certain types of drugs including calcineurin inhibitors, proton-pump inhibitors and diuretics may cause low serum magnesium (Mg²⁺) levels [2]. In a small percentage of patients, the Mg²⁺ disturbance is of genetic origin [3]. Hypomagnesaemia-causing mutations often affect Mg²⁺ transport processes operating in the renal distal convoluted tubule (DCT). The final urinary Mg²⁺ excretion is determined in the DCT, since Mg²⁺ reabsorption does not take place beyond that nephron segment [3]. As a consequence,
patients with reduced Mg$^{2+}$ uptake in DCT have renal Mg$^{2+}$ wasting.

In 2000, we identified a c.115G>A (p.Gly41Arg) mutation in the FXYD domain containing ion transport regulator 2 gene (FXYD2) causative for isolated dominant hypomagnesaemia (IDH, [MIM 154020]) in a large Dutch family [4–6]. Although originally described as two families, haplotype analysis revealed a 10.5 cM overlapping region suggesting a founder effect [4]. Patients in this family had normal urinary Mg$^{2+}$ values, but showed lowered urinary Ca$^{2+}$ excretion and suffered from episodes of convulsions [7]. Some patients developed symptoms of chondrocalcinosis, related to chronic hypomagnesaemia [8].

FXYD2 encodes the γ-subunit of the Na$^+$/K$^+$-ATPase and is involved in stabilization of the α-subunit of that complex [9]. Moreover, the γ-subunit decreases the affinity of the Na$^+$/K$^+$-ATPase for Na$^+$ and K$^+$ and increases the affinity for ATP [10]. The p.Gly41Arg mutation causes a trafficking defect preventing the γ-subunit to reach the basolateral membrane [5, 11]. In the kidney, two splice variants of FXYD2 are expressed. FXYD2a is expressed strongly in the thick ascending limb and proximal tubule and only discretely in the DCT [12]. In contrast, FXYD2b expression is restricted to the basolateral membrane of the DCT and connecting tubule. In addition, HNF-1β can activate FXYD2a transcription, and mutations in the HNF-1β transcription factor gene (HNF1B) cause hypomagnesaemia [13–15]. Given that each FXYD2 splice variant differentially modifies Na$^+$/K$^+$-ATPase activity, it has been hypothesized that the ratio between FXYD2a and FXYD2b expression may determine Na$^+$/K$^+$-ATPase activity. However, the mechanism by which changes in basolateral FXYD2 expression affects renal Mg$^{2+}$ reabsorption remains to be elucidated.

Since the report of the original family in 2000, new families with FXYD2 mutations have not been described. As a result, the relevance of FXYD2 mutations for hypomagnesaemia has been questioned over the years. For the first time since the initial report, we now present two new families harbouring the same p.Glu41Arg mutation in FXYD2. To provide further insight into the role of this mutation in hypomagnesaemia, full molecular and genealogical analyses were performed. Furthermore, we aimed to increase the current phenotypic knowledge and describe the clinical presentations and treatment of patients with FXYD2 mutations.

### MATERIALS AND METHODS

#### Patients

All patients in this study were diagnosed with hypomagnesaemia and were screened by the DNA diagnostics department of the Radboudumc Nijmegen for hypomagnesaemia-causing mutations. Informed consent for participation in research was obtained in accordance with Institutional Review Board guidelines. Serum and urine electrolytes were determined by general laboratory screenings. Laboratory findings of all patients are presented in Table 1.

#### Mutation analysis

Extraction of DNA from whole blood was performed using standard protocols. FXYD2 mutation screening was performed using polymerase chain reaction (PCR) primers designed on the coding sequence of the human FXYD2 gene (NM_001680.4, NM_021603.3) including exon–intron boundaries [5]. Amplified sequences were separated on agarose gel by electrophoresis and directly Sanger sequenced according to standard methods on a 3730 Sequence Analyzer (Applied Biosystems). Mutation nomenclature is according to HGVS using NM_001680.4 as a reference sequence.

#### Haplotype analysis

Microsatellite markers D11S4092, D11S4127, D11S939, D11S1356 and D11S4195 encompassing the FXYD2 gene on chromosome 11q23 were amplified by PCR using fluorescently labelled primers. PCR products are denatured and analysed on a 3730 Sequence Analyzer (Applied Biosystems).

#### Genealogy study

In-depth genealogical analysis was performed to identify common ancestors of FXYD2 patients. Lists of descendants from index patients were compiled and pedigrees were generated using Dutch and Belgian civil registers and church books.

### RESULTS

#### Recurrent FXYD2 mutation in patients with hypomagnesaemia

In two families with hypomagnesaemia, a c.115G>A mutation in the FXYD2 gene was identified by mutation analysis.

<table>
<thead>
<tr>
<th>Patient</th>
<th>NL II.1</th>
<th>NL III.1</th>
<th>NL III.2</th>
<th>B II.2</th>
<th>B III.2</th>
<th>B IV.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum magnesium (mmol/L, nl 0.7–0.95)</td>
<td>0.38</td>
<td>0.45</td>
<td>0.45</td>
<td>0.56</td>
<td>0.35</td>
<td>0.5</td>
</tr>
<tr>
<td>Serum potassium (mmol/L, nl 3.5–5.1)</td>
<td>3.3</td>
<td>4.2</td>
<td>3.8</td>
<td>4.0</td>
<td>3.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Serum bicarbonate (mmol/L, nl 21–27)</td>
<td>29.6</td>
<td>24</td>
<td>22</td>
<td>26</td>
<td>27</td>
<td>31</td>
</tr>
<tr>
<td>Serum sodium (mmol/L, nl 135–145)</td>
<td>138</td>
<td>142</td>
<td>142</td>
<td>137</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>eGFR (ml/1.73 ml/min, nl &gt;90)</td>
<td>123</td>
<td>133</td>
<td>124</td>
<td>43</td>
<td>125</td>
<td>85</td>
</tr>
<tr>
<td>Urinary magnesium excretion/day (mmol/day, nl 3.0–5.0)</td>
<td>8.9</td>
<td>8.1</td>
<td>10.6</td>
<td>8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fractional magnesium excretion (%), nl &lt;4%</td>
<td>8.3</td>
<td>11.9</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary calcium excretion/day (mmol/day, nl 1.5–7.5)</td>
<td>1.3</td>
<td>0.8</td>
<td>1.52</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary calc/creat (mmol/mmol, nl &lt;0.7)</td>
<td>0.08</td>
<td>0.04</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary sodium excretion/day (mmol/day, nl 40–220)</td>
<td>119</td>
<td>358</td>
<td>218</td>
<td>147</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 meq Mg = 0.5 mmol Mg = 12.3 mg Mg. 1 meq Ca = 0.5 mmol Ca = 20.5 mg Ca (1 mmol = 41 mg). nl = normal (reference value).

Note: Values in subject B II.2 are those recorded under supplementation, and not a presentation.
The mutation results in a p.Gly41Arg change at amino acid level. Interestingly, the c.115G>A mutation has previously been reported in a large Dutch family in 2000 and no other FXYD2 mutations have been found since [5].

**FXYD2 mutation in a Dutch family**

The first family is a Dutch family with three affected family members (Figure 1). The proband (Figure 1, NL II.1) was discovered suffering from hypokalaemia and hypomagnesaemia during admission for lung embolism at the age of 34 (Table 1). He had a chronic history of muscle cramps and fatigue and reported a spastic fluid intake. He is an only child and his parents are deceased. He reported no relevant symptoms of his father (Figure 1, NL I.1) besides a spastic fluid intake and recurrent miscarriages of his mother (Figure 1, NL I.2). The proband is currently treated with Mg2+ supplements stabilizing the serum Mg2+ and K+ levels at ±0.45 and ±3.4 mmol/L, respectively.

Hereafter, his two children were admitted to the outpatient clinic for evaluation because of muscle cramps. They both showed hypomagnesaemia. The daughter (Figure 1, NL III.1), 9 years old, reported muscle cramps and uncertainty about keeping balance, without falling. His son (Figure 1, NL III.2), 7 years old, reported an increased fluid intake and nightly muscle cramps and is known with an attention deficit disorder. Both children had a normal growth pattern, normal blood pressure, no dysmorphic features and unremarkable renal ultrasound and X-ray of the hand. Laboratory evaluation revealed isolated hypomagnesaemia with hypermagnesuria and hypercalciuria (with normal serum creatinine) (Table 1). Because the dominant inheritance of hypomagnesaemia in this family was not compatible with Gitelman syndrome, genetic analysis was performed for diagnostic purposes. No mutations were found in the HNF1B and SLC12A3 genes. A heterozygous missense mutation in the FXYD2 gene [c.121G>A (p.Gly41Arg)] was found in all three affected individuals. The proband’s daughter took Mg2+ supplements, as well as the proband’s daughter (B III.2) and granddaughter (B IV.1).

The patient reported that his mother (Figure 2, B I.2, deceased at age 62) was known for Mg2+ problems. She suffered from chronic fatigue and articulation problems. In addition, she took Mg2+ supplements, as well as the proband’s daughter (B III.2) and granddaughter (B IV.1). The daughter of the proband (Figure 2, B III.2), aged 41 years, complained of fatigue since childhood, with limited physical activity. She had cramps and dysesthesia of the face and hands. She was diagnosed with hypomagnesaemia as a young adult and has taken Mg2+ supplements since then. Plasma analyses revealed persistent hypomagnesaemia and hypokalaemia, with normal HCO3− and normal renal function. The urinary Mg2+ loss was inappropriately high (Table 1).

The granddaughter of the proband (B IV.1), aged 16 years, experienced two epileptic crises at 5 and 15 years of age and complained of chronic fatigue. The neurological work-up was negative but hypomagnesaemia and hypokalaemia were noticed, with inappropriate urinary Mg2+ loss and hypercalciuria (Table 1). Renal function was normal.

Over the years, plasma Mg2+ remained, in the Belgian family, below or just at the low-normal values despite
supplementation. Follow-up K⁺ levels were improved, with levels of 4.5 mmol/L (B II.2), 3.75 mmol/L (B III.2) and 3.64 mmol/L (B IV.1) observed 1 year after first contact (Table 1). These values, which were all within normal limits, were however variable and not always confirmed during follow-up in other institutions.

Common haplotype bearing the FXYD2 mutation

To examine whether the newly identified families share the same haplotype as the original family, marker analysis was initiated. Five markers encompassing the FXYD2 gene were selected: D11S4092, D11S4127, D11S939, D11S1356 and D11S4195 (Figure 3). For this analysis, relatives from the original family were included. Indeed, patients from all three families share the same haplotype, suggesting a founder effect. Although the genetic cause was only found in 2000 [4], the first patients with mutations in the FXYD2 gene were clinically evaluated in 1987 [16, 17]. Twenty-two individuals harbouring FXYD2 mutations showed IDH, hypocalciuria and occasionally also hypomagnesaemia-associated chondrocalcinosis was detected. Since the first identification of the FXYD2 p.Gly41Arg mutation in 2000 [15], no other FXYD2 mutations have been reported in the literature or detected in our diagnostic testing facility. Therefore, the role of FXYD2 in hypomagnesaemia has been questioned. The identification of the new Dutch and Belgian families with FXYD2 mutations confirms

DISCUSSION

In this study, a c.115G>A (p.Gly41Arg) mutation in FXYD2 was identified in two families with hereditary hypomagnesaemia. Importantly, these are the first new patients reported since 2000 confirming that FXYD2 mutations cause hypomagnesaemia. In both patient families, the same c.115G>A mutation was detected that was previously described in a large Dutch family. All three families shared the same haplotype, suggesting a founder effect.
its importance in renal Mg\(^{2+}\) handling. Moreover, several regulatory factors of \(FXYD2\) have additionally been implicated in Mg\(^{2+}\) homeostasis. The transcription of \(FXYD2\) is induced by HNF-1\(\beta\) [14] and patients with \(HNF1B\) mutations also develop hypomagnesaemia (Renal Cyst and Diabetes syndrome, [MIM 137920]) [13]. Recently, pterin-4-\(\alpha\)-carbinolamine dehydratase (encoded by \(PCBD1\)) was identified as an additional transcriptional regulator of \(FXYD2\) and again patients with \(PCBD1\) mutations showed hypomagnesaemia [MIM 126090] [15]. Although the exact molecular mechanism by which the \(PCBD1–HNF1B–FXYD2\) axis regulates renal Mg\(^{2+}\) reabsorption is still unclear, the effects of \(FXYD2\)-encoded \(\gamma\)-subunit on Na\(^+-K^+\)-ATPase activity have been well established. The \(\gamma\)-subunit increases the affinity of the Na\(^+-K^+\)\(-\)ATPase for ATP and decreases the affinity for Na\(^+\) and K\(^+\) [10]. The p.Gly41 residue is located central in the \(\alpha\)-helix that forms the \(\gamma\)-subunit (PDB: 2mkv). Mutation of the small glycine residue in the larger arginine residue may disrupt the structure of the \(\gamma\)-subunit. To date, other \(FXYD2\) mutations have not been identified, suggesting that other \(FXYD2\) mutations may not cause hypomagnesaemia. Alternatively, the importance of \(\gamma\)-subunit for Na\(^+-K^+\)-ATPase activity could suggest that more severe mutations in \(FXYD2\) render a dysfunctional Na\(^+-K^+\)-ATPase and therefore may be embryonic lethal.

In the new families from the Netherlands and Belgium, both hypomagnesaemia and hypocalciuria could be confirmed in all patients. Of note, hypomagnesaemia was associated with moderate hypokalaemia in the two patients with lowest serum Mg\(^{2+}\) values. Hypokalaemia is often secondary to hypomagnesaemia [5]. Hypomagnesaemia results in decreased intracellular Mg\(^{2+}\) levels in the distal part of the nephron, possibly due to the fact the Mg\(^{2+}\) normally inhibits the renal outer medullary K\(^+\) channel minimizing renal K\(^+\) secretion. In addition, several patients reported high fluid intake and thirst. These manifestations are typically encountered in patients suffering from chronic hypokalaemia, as described for instance in Gitelman syndrome [18, 19]. Hypokalaemia has been shown to induce a down-regulation of AQP2 water channels in the collecting duct, impairing the urinary concentration [20]. In turn, this could stimulate compensatory thirst, in order to maintain the plasma osmolality and sodium levels.

Other clinical manifestations of the patients harboring \(FXYD2\) mutations include renal failure (chronic kidney disease stage 3) (BE II.2), but other factors in the development of renal failure cannot be excluded (e.g. analgesics and NSAID). For chronic pain due to chondrocalcinosis, all affected family members reported muscle cramps. In addition, one patient suffered from episodes of epilepsy (B IV.1). Moreover, chondrocalcinosis was detected in the proband of the Belgian family (B II.2). Both epilepsy and chondrocalcinosis were observed in the other patients from the original family [13, 14], and are classical complications of hypomagnesaemia. In the Dutch family, daily supplementation using Mg(OH)\(_2\) achieved plasma Mg\(^{2+}\) levels in the low-normal range.

Genealogical analysis, going back to 1700 of all three patients’ families with the similar \(FXYD2\) mutation did not result in the identification of a common ancestor. Interestingly, the ancestry of two families could be traced back to the same geographical area near Liège in Belgium. If indeed the common ancestor lived before 1700, the dominant inheritance of IDH suggests that more, still unidentified, descendants of this common ancestor could live in the Netherlands and Belgium. However, given the variety of this phenotype and the non-specificity of the symptoms these patients often remain undetected. Increasing the awareness of this syndrome may help identifying more patients with \(FXYD2\) mutations and allow proper treatment by Mg\(^{2+}\) supplementation.

In conclusion, this study reports two new families with IDH caused by \(FXYD2\) mutations. Further insight in the clinical spectrum associated with genetic Mg\(^{2+}\) disorders can provide useful information for clinicians to make an early differential diagnosis. \(FXYD2\) mutations should be considered in patients with hypomagnesaemia and hypocalciuria.

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CONFLICT OF INTEREST STATEMENT

None declared.

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