CASE REPORT

Case Report: Investigation and molecular genetic diagnosis of familial hypomagnesaemia: a case report [version 1; peer review: awaiting peer review]

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Abstract
Genetic mutations causing familial hypomagnesaemia syndromes are well-recognised. Affected patients can present with severe symptoms of hypomagnesaemia, such as seizures or cardiac arrhythmia. We report an affected child, from a consanguineous family, who presented in the first weeks of life with seizures secondary to hypomagnesaemia, without other associated clinical features. We performed whole exome sequencing in the affected child and segregation analysis within the family, which revealed a novel homozygous missense mutation in TRPM6, which was confirmed as a heterozygous allele in both parents and two younger siblings who had transient hypomagnesaemia. Using in silico modelling, we provide evidence that the missense variant p.(K1098E) in TRPM6 is pathogenic, as it disrupts stabilising TRP domain interactions. Management of familial hypomagnesaemia relies on prompt recognition, early magnesium replacement and lifelong monitoring.

Keywords
hypomagnesaemia, with secondary hypocalcaemia, TRPM6, molecular genetics
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Introduction
Homeostasis of the serum magnesium level is essential for human cellular function, and levels are maintained in the normal range by tight control of magnesium reabsorption by the kidney tubules. Hypomagnesaemia can manifest with a range of symptoms, from tremor, muscle spasms or nystagmus through to seizures, arrhythmias and cardiac arrest. Early identification of the electrolyte abnormality is vital, as treatment with magnesium replacement is efficacious and inexpensive. Common causes of hypomagnesaemia in adults include refeeding syndrome, diarrhoea, malabsorption, alcohol abuse and medications such as proton pump inhibitors. Renal magnesium wasting is indicated by an inappropriately high fractional excretion of magnesium in urine despite hypomagnesaemia, and is seen in post-obstructive diuresis, the recovery phase of acute tubular necrosis, hypercalcaemia and in response to certain diuretics. However, genetically inherited mutations that cause renal hypomagnesaemia are well-recognised, and typically present in childhood if they are secondary to autosomal recessive disorders. Genetic forms of hypomagnesaemia should also be considered in certain clinical scenarios, such as in the presence of a positive family history of related disorders, consanguinity, or fulminant presentation.

Once a genetic cause of hypomagnesaemia is suspected, work-up can be guided by associated features and age at presentation. Though obtaining a genetic diagnosis will not alter the treatment of magnesium replacement therapy, it is vital for identifying others at risk and family counselling, and may help to guide the clinician to screen for associated phenotypic features.

Case report
We report a child from a consanguineous family (parents were second degree cousins) from Oman, who presented with seizures and hypomagnesaemia. The affected individual, a female child, presented at 20 days of age with tonic-clonic seizures. There was no history of fever or diarrhoea, and after an uncomplicated pregnancy she had been born healthy at term, without syndromic features. Serum magnesium was severely low at 0.35 mmol/L and was associated with a mild hypocalcaemia and suppressed parathyroid hormone (PTH) (Table 1). The urinary fractional excretion of magnesium was inappropriately high in the normal range given the severe degree of hypomagnesaemia present, suggesting contributory renal magnesium wasting. There were no other specific clinical or biochemical features; of note peripheral oxygen saturations and capillary blood glucose levels were within normal limits. Renal ultrasound scan was normal, with no nephrocalcinosis. She was initially treated with intravenous magnesium (20% MgCl$_2$ 0.1 mmol /kg every 6 hours p.r.n.) and calcium replacement (10% Calcium Gluconate 0.11 mmol/kg). At 4 years of age she is now supported with high-dose oral magnesium supplements (magnesium sulphate 500mg qds) alone, and remains well with no further seizures, though she maintains a low serum magnesium level between 0.4-0.6 mmol/L.

Of note, a younger sibling of the proband, also female, presented at 18 days old with abnormal eye movements in association with a complex partial seizure. Her serum magnesium was below normal limits (0.53 mmol/L), with serum calcium and PTH within the normal range (Table 1). The fractional excretion of magnesium was inappropriately high, and again renal ultrasound scan was normal and no other clinical features were noted. She was treated with intravenous magnesium replacement (20% MgCl$_2$ 0.1 mmol /kg every 6 hours p.r.n.), followed by a period of maintenance oral magnesium replacement (magnesium sulphate 300 mg b.d.). At 2 years of age she remains well with no further seizures, and she maintains magnesium levels within the normal range without additional supplementation. A younger asymptomatic male sibling was screened with serum biochemistry tests at 1 week of age. Serum magnesium was low at 0.6 mmol/L, with normal serum calcium and PTH levels (Table 1). Supplementation was not started, and by 1 year of age serum magnesium was within the normal range.

Genetic investigations
Detailed information on the techniques described below is given in the Methods section. Following informed consent, whole exome sequencing (WES) was performed in the eldest sibling, II:1 (Figure 1). Analysis using a combination of homozygosity

| Table 1. Clinical and biochemical features of siblings. |
|-----------------|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Child**       | **Age at**        | **Clinical**    | **Serum Magnesium at** | **Serum**       | **Parathyroid**  | **Fractional**  | **Current**     | **Maintenance** |
|                 | **diagnosis /**    | **Presentation**| **presentation (NR**  | **calcium**     | **hormone**     | **Excretion of**| **serum**       | **Magnesium**   |
|                 | **screening**      |                 | **0.65-1.1 mmol/L)**  | (corrected) at  | (NR** 1.6-6.9**| **Magnesium**   | **magnesium**   | **supplements** |
|                 |                   |                 |                          | presentation (NR | pmol/L)**       | (NR 2-4%)**     | (NR 0.65-1.1)   | (magnesium sulphate)** |
| II:1            | 20 days           | Generalised      | 0.35                      | 1.38             | 1.11            | 2.1%           | 0.4-06 age 5    | 500 mg qds      |
|                 |                   | seizure          |                           |                  |                |                | years          |                  |
| II:2            | 18 days           | Complex          | 0.53                      | 2.62             | 5.6             | 5.5%           | 0.7-0.9 aged 3  | 300 mg bd       |
|                 |                   | partial seizure  |                           |                  |                |                | years          | reduced to none |
| II:3            | 7 days            | Asymptomatic     | 0.6                       | 2.60             | N/A             | N/A            | 0.7-1.0 aged 1  | None            |

NR, normal range; Fractional excretion of magnesium (%) = Urine Magnesium x Plasma Creatinine / (0.7 x Plasma Creatinine x Urine creatinine) x 100.
mapping and variant calling revealed a homozygous missense mutation c.3292A>G, p.(K1098E) in TRPM6 within a large region of homozygosity by descent (Figure 1). The missense variant was confirmed by Sanger sequencing, and cascade screening confirmed this variant was in its heterozygous state in both parents and both mildly affected siblings. In silico tools confirmed evolutionary conservation (Figure 1) as well as the rarity and predicted pathogenicity of the variant (Table 2). Using predictive modelling of the protein structure we were able to show that the lysine residue at position 1098 is predicted to form a stabilising interaction within the TRP domain, and that the missense mutation of TRPM6 K1098 to glutamate is predicted to disrupt this interaction (Figure 2).

Discussion
As the second most abundant intracellular cation, magnesium is vital for normal cell function. The majority of ingested magnesium load is absorbed in the distal small bowel via paracellular mechanisms, and the remainder is absorbed in the colon by transient receptor potential melastatin type 6 (TRPM6) ion channels in gut epithelium. Serum magnesium homeostasis is therefore tightly regulated by reabsorption in the kidney; the majority is reabsorbed in the thick ascending limb of the loop of Henle via a paracellular route, and the ‘fine-tuning’ is performed in the distal convoluted tubule (DCT) via apically located TRPM6 channels.

Hypomagnesaemia is a common electrolyte disturbance, with a prevalence of 20% in hospitalised patients. Causes in adults include inadequate intake, refeeding syndrome, renal losses, gastrointestinal losses in diarrhoea, gastrointestinal malabsorption, and medications such as proton pump inhibitors (PPIs). Serum magnesium levels may be requested as part of an extended biochemical panel if there is clinical concern about these risk factors, if symptoms or cardiac arrhythmia are present, or if other disturbances such as hypokalaemia or hypocalcaemia prompt the consideration of magnesium depletion. Measurement of urinary magnesium may help distinguish between gastrointestinal and renal losses. Urinary magnesium levels will be low if hypomagnesaemia is secondary to gastrointestinal losses, as the kidneys appropriately work to maximally reabsorb filtered magnesium, but raised or inappropriately normal despite low serum magnesium levels in renal magnesium wasting conditions. The majority of
Table 2. TRPM6 variant and in silico analysis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide variant</th>
<th>Predicted amino acid change</th>
<th>ExAC frequency</th>
<th>gnomAD</th>
<th>MutationTaster</th>
<th>SIFT</th>
<th>POLYPHEN2</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPM6</td>
<td>c.3292A&gt;G</td>
<td>p.(K1098E)</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Disease causing</td>
<td>Deleterious</td>
<td>Possibly damaging</td>
</tr>
</tbody>
</table>

Reference sequence NM_017662.5

Figure 2. The TRPM6 K1098E variant affects a conserved basic residue in the TRP domain. (A) Sequence alignments of TRP domain residues from human TRPM1-TRPM8. Fully-conserved residues are highlighted in black and semi-conserved residues in grey. TRPM6 K1098 is outlined in red. (B) Cryo-EM structure of Ca²⁺- and Na⁺-bound TRPM2 (PDB 6C07). One monomer of the homotetrameric channel is highlighted in orange and the co-ordinated Ca²⁺ and Na⁺ ions are shown as green and purple spheres, respectively. (C) Superposition of the TRPM2 monomer (orange) and TRPM6 homology model (green). The TRP domain region (box and inset), wherein the TRPM6 K1098E variant lies, is shown to highlight the predicted structural homology between TRPM6 and TRPM2. The Ca²⁺ ion (green sphere) is shown for orientation. (D) Close up view of TRPM2 Q896 and E1110 involved in co-ordination of the Ca²⁺ ion (green sphere) and R1114 (homologous to TRPM6 K1098, shown in (E)) predicted to form a stabilising interaction within the TRP domain. (F) Mutation of TRPM6 K1098 to glutamate (magenta) is predicted to disrupt stabilising TRP domain interactions. The relative position of the Ca²⁺ ion (green sphere) in TRPM2 is shown in (E) and (F) for orientation.
renal causes of hypomagnesaemia are not genetic, such as renal losses induced by post-obstructive diuresis, the recovery phase of acute tubular necrosis, hypercalcaemia, or drugs such as loop and thiazide diuretics, cisplatin, tacrolimus and aminoglycosides.

Magnesium wasting disorders found in families have been shown to be associated with over a dozen genes\(^1\). Similar to other monogenic diseases causing renal tubule phenotypes, the study of these diseases has greatly contributed to our knowledge of the renal tubular transport proteins responsible for homeostatic and physiological functioning. Familial hypomagnesaemic renal disorders may be inherited in both autosomal dominant and recessive patterns, and the underlying genes uncovered so far all encode proteins found in the thick ascending limb of the loop of Henle or DCT. Familial hypomagnesaemias may be categorised into four groups. These include hypercalciuric hypomagnesaemias (secondary to mutations in CLCNKB (Bartter syndrome type 3), CLDN16, CLDN19, CASR); Gitelman-like hypomagnesaemias (secondary to mutations in SLC12A3 (Gitelman syndrome), BSNB (Bartter syndrome type 4), KCNJ10, FYXZ2, HNF1B, PCBD1); mitochondrial hypomagnesaemias; (mutations in SARS2, MT-TI and Kearns–Sayre syndrome) and other hypomagnesaemias (secondary to mutations in TRPM6, CNM2, EGF, EGFR, KCNA1, FAM111A)\(^2\).

**TRPM6** is expressed in both the colon and the DCT of the kidney, and mutations here can cause the condition known as hypomagnesaemia with secondary hypocalcaemia. There have been dozens of distinct mutations in **TRPM6** associated with this condition, and different variants can cause different effects on the function of the TRPM6 transporter\(^3\). In patients with **TRMP6** mutations magnesium absorption from the colon is decreased (primary intestinal hypomagnesaemia), and the DCT is unable to perform the ‘fine-tuning’ of magnesium reabsorption and inappropriately wastes magnesium via the urine. Due to this dual pathology, the condition can cause the most profound electrolyte wasting of the genetic hypomagnesaemias. It typically presents in the neonatal period with severe symptoms due to hypomagnesaemia and hypocalcaemia such as seizures, which are subsequently responsive to magnesium administration\(^4\). The hypocalcaemia is thought to be secondary to hypoparathyroidism, which is induced by hypomagnesaemia\(^5\). Interestingly, the observation that treatment with PPIs is associated with hypomagnesaemia has led to a proposed mechanism of PPI-induced inhibition of TRPM6 and TRPM7 channels in the gastrointestinal tract\(^6\). **TRPM6** may also be downregulated in the DCT in response to cyclosporine, resulting in renal magnesium wasting\(^7\).

Treatment of all the genetic hypomagnesaemia disorders, including those caused by **TRPM6** mutations, is with magnesium replacement therapy, either oral or intravenous depending on urgency and the tolerability of oral products. The major side-effect of oral magnesium replacement is diarrhoea, which can limit treatment compliance and paradoxically cause worsening of hypomagnesaemia due to increased gastrointestinal losses. Overall the prognosis of hypomagnesaemia with secondary hypocalcaemia is excellent, and serum calcium levels normalise as serum magnesium levels improve.

Given what is known about hypomagnesaemia with secondary hypocalcaemia, our first patient presented typically, with severe symptoms and the expected biochemical profile, including low PTH. WES confirmed a homozygous missense mutation in **TRPM6**, and clearly the family history of consanguinity was consistent with the diagnosis of an autosomal recessive disorder. In keeping with previous case reports she did not maintain magnesium concentration in the normal range, despite high dose oral replacement. Interestingly, the second child also had severe symptoms at presentation despite ultimately proving to be heterozygous for the **TRPM6** mutation. However, it can be seen that her presentation was less fulminant, without the development of tonic-clonic seizures and with milder derangement of biochemical parameters. In keeping with this less severe phenotype, she now maintains normal serum magnesium levels without supplementation. Finally, the third sibling had documented transient and asymptomatic hypomagnesaemia, which corrected by 1 year of age. These two siblings provide some evidence that a heterozygous allele in infants may lead to a transient biochemical phenotype, presumably related to the immaturity of the DCT to regulate magnesium. Adults heterozygous for **TRPM6** pathogenic variants have never been reported to have abnormal serum magnesium levels\(^8\). Heterozygous *Trpm6* knockout mice exhibit mild hypomagnesaemia under a normal diet, suggesting that a milder phenotype may be associated with the loss of one **TRPM6** allele\(^9\).

The location and predicted pathological effect of the missense mutation warrants further discussion. Previously described missense mutations in **TRPM6** include p.(S141L) and p.(P1017R), which lead to either trafficking or gating impairment of the **TRPM6** channel\(^10\). Additional missense mutations p.(I747R), p.(T354P) and p.(C707T) have also been reported\(^11\). Here we have taken advantage of recently published cryo-EM structures of **TRPM2**–**TRPM8**, **TRPM4**–**TRPM7** and **TRPM8**\(^12\), which suggest a conserved global architecture for **TRPM** family members, consistent with sequence conservation (see Extended data, Supplementary Figure 1)\(^13\). We therefore utilised all structures to interpret the likely pathogenic effect of the **TRPM6** K1098E variant (with the exception of **TRPM8**, due to low resolution in the homologous region)\(^14\). **TRP** domain sequence analysis (Figure 2A) revealed conservation of a basic residue at the homologous position to K1098 in **TRPM6**, indicating an important functional role for the positively charged side-chain. Studies of **TRPM6**–**TRPM8** suggest that positively charged residues in the **TRP** domain may interact with the negatively charged phosphate groups in phosphatidylinositol-4,5-bisphosphate (PIP\(_2\)) to mediate channel activation\(^15\). Neutralisation of the positive charge by substitution with glutamine was shown to abolish channel activity in **TRPM6** K1098Q\(^16\) and **TRPM8** R1008Q\(^17\), although surprisingly no significant effect on channel activity was seen in the homologous **TRPM7** K1125Q variant\(^18\). Moreover, the homologous **TRPM4** variant (R1072Q) exhibited normal sensitivity to PIP\(_2\), arguing against this residue being involved directly
in PIP3 binding. Based on the available TRPM structures, we predict K1098 may mediate one of several stabilising interactions in TRPM6. In the cryo-EM structure of TRPM2 (Figure 2B), and TRPM6 homology model (Figure 2C), the TRP domain lies in close proximity to the ion conduction pathway, with mutations in this domain likely to affect channel gating. Indeed, mutation of TRP domain residues in TRPM2 (E1110) and TRPM4 (E1068) were shown to impair the binding of Ca2+ necessary for priming the channel for voltage-dependent opening. In TRPM2, E1110 in the TRP domain stabilises Q896 in the S2 helix (Figure 2D), correctly orienting Q896 for Ca2+-binding. Interestingly, the glutamine residues involved in co-ordination of the Ca2+ ion in TRPM2 (Q896) and TRPM4 (Q831) are conserved in all Ca2+-dependent TRPM channels (Figure 3), but are replaced by glutamate (E889) in the Ca2+-independent TRPM6 (Figure 2E). TRPM6 modelling

![Figure 3. TRPM family members exhibit a conserved global structural architecture.](image-url)
suggests that K1098 in the TRP domain could form stabilising interactions with E889 and E885 (equivalent to R1114 and E892 in TRPM2; Figure 2D) in the S2 helix (Figure 2E), thereby priming the channel for activation in a Ca2+-independent manner. Alternatively, TRPM6 K1098 may potentially serve to stabilise the TRP domain helix itself, either through a cation-π interaction with Y1095 (Figure 2E), equivalent to that identified in the cryo-EM structure of TRPM4 between R1072 and F1069, or via interaction with the hydroxyl side-chain of T1094 (equivalent to TRPM2 E1110; Figure 2E). This latter potential interaction is analogous to that modelled between TRPM8 R1008 and E1004, whereby agonist/antagonist binding was predicted to modulate the position and intra-protein contacts of R1008 (equivalent to TRPM6 K1098), with resultant changes in the TRP domain helix effecting channel opening/closing, respectively13. Substitution of TRPM6 K1098 with the negatively charged glutamate (K1098E; Figure 2F) is predicted to be pathogenic since this change would destabilise any of the potential interactions discussed.

Conclusion

Here we provide evidence for a novel pathogenic missense mutation p.(K1098E) in TRPM6 which leads to a severe hypomagnesaemia with secondary hypocalcaemia phenotype in an affected child. In silico modelling of homologs of the TRPM channels supports an important stabilising role for this residue.

Methods

Clinical summaries were prepared and DNA samples taken from whole blood following informed and written consent. Ethical approval for this study was obtained from the National Research Ethics Service (09/H0903/36).

A DNA sample from the affected proband underwent WES, performed via GATC Biotech. A DNA library was prepared using enrichment with SureSelectXT and a human All Exon Kit. Sequencing was performed using Illumina with paired end reads of 2 x150 bp with a >30X average on target coverage. Raw data was analysed via a commercial bioinformatics pipeline (GATC Biotech) for publication of this case report and accompanying images.

Extended data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Consent

Written informed consent was obtained from the patients’ family for publication of this case report and accompanying images.

Author contributions

The project was conceived and directed by JAS. JW and JAS drafted the manuscript. All authors were involved in the revision of the draft manuscript and have agreed to the final content.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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