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Review Article: Special Edition

Clinical and genetic approach to renal hypomagnesemia

Dr. Shih-Hua Lin

Min-Hua Tseng [a,b,f], *Martin Konrad* [c], *Jhao-Jhuang Ding* [d], *Shih-Hua Lin* [e,*]

[a] *Division of Nephrology, Department of Pediatrics, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan*
[b] *Department of Pediatrics, Xiamen Chang Gung Hospital, China*
[c] *Department of General Pediatrics, University Children's Hospital Münster, Münster, Germany*
[d] *Department of Pediatrics, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan*
[e] *Division of Nephrology, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan*
[f] *College of Medicine, Chang Gung University, Taoyuan, Taiwan*

ARTICLE INFO

Article history:
Received 16 September 2021
Received in revised form
1 November 2021
Accepted 3 November 2021
Available online 10 November 2021

Keywords:
Inherited disorders
Hypomagnesemia
Genetic diagnosis

ABSTRACT

Magnesium (Mg^{2+}) is an important intracellular cation and essential to maintain cell function including cell proliferation, immunity, cellular energy metabolism, protein and nucleic acid synthesis, and regulation of ion channels. Consequences of hypomagnesemia affecting multiple organs can be in overt or subtle presentations. Besides detailed history and complete physical examination, the assessment of urinary Mg^{2+} excretion is help to differentiate renal from extra-renal (gastrointestinal, tissue sequestration, and shifting) causes of hypomagnesemia. Renal hypomagnesemia can be caused by an increased glomerular filtration and impaired reabsorption in proximal tubular cells, thick ascending limb of the loop of Henle or distal convoluted tubules. A combination of renal Mg^{2+} wasting, familial history, age of onset, associated features, and exclusion of acquired etiologies point to inherited forms of renal hypomagnesemia. Based on clinical phenotypes, its definite genetic diagnosis can be simply grouped into specific, uncertain, and unknown gene mutations with a priority of genetic approach methods. An unequivocal molecular diagnosis could allow for prediction of clinical outcome, providing genetic counseling, avoiding unnecessary studies or interventions, and possibly uncovering the pathogenic mechanism. Given numerous identified genes responsible for Mg^{2+} transport in renal hypomagnesemia over the past two decades, several potential and specific molecular and cellular therapeutic strategies to correct hypomagnesemia are promising.

* *Corresponding author*. Division of Nephrology, Department of Medicine, Tri-Service General Hospital, 325, Section 2, Cheng-Kung Rd., Neihu 114, Taipei, Taiwan.
E-mail address: l521116@ndmctsgh.edu.tw (S.-H. Lin).
Peer review under responsibility of Chang Gung University.
https://doi.org/10.1016/j.bj.2021.11.002

Magnesium (Mg^{2+}), the second-most abundant intracellular cation, is pivotal as a cofactor in the maintenance of numerous cell functions. Deficiency of Mg^{2+} can lead to muscle weakness, tremor, seizure, arrhythmia, coma, and even death [1,2]. Up to 15% of hospitalized patients and 60% of patients admitted to intensive care unit have hypomagnesemia [3–5]. Gastrointestinal and renal losses are the two major causes of hypomagnesaemia. The acquired renal hypomagnesemia commonly results from medications, alcohol, and osmotic diuresis. Inherited renal hypomagnesemia is defined as hypomagnesemia of renal origin caused by defects of genes responsible for renal Mg^{2+} handling. Although the inherited renal hypomagnesemia is less common than acquired forms, the inherited renal hypomagnesemia features more variable in characteristics, more refractory in treatment and more severe in consequence. Without timely recognition and appropriate treatment, it can lead to severe complications such as cardiac arrhythmias and death.

Numerous genes encoding proteins involved in Mg^{2+} transport in the renal tubules have been identified over the last two decades. Such newfound knowledge can potentially enable us to modify and personalize therapeutic strategies. Our aim is to summarize the molecular advances that are linked to renal hypomagnesemia and propose a comprehensive molecular approach for diagnosis and management. Recent advances in molecular and cellular therapies of inherited disorders are also discussed.

Magnesium homeostasis

Magnesium (Mg^{2+}) is a crucial cation involved in a number of biological processes, including cell proliferation, immunity, cellular energy metabolism, protein and nucleic acid synthesis, and regulation of ion channels [1,2]. The serum Mg^{2+} is tightly maintained within a narrow range. In the body, around 53% are distributed and stored in bones, 27% in muscles, 19% in soft tissues, and only 1% in blood. Around 65–70% of the circulating Mg^{2+} is in the ionized form which is the active form critical for physiological function. The rest of Mg^{2+} binds with either proteins or anion such as citrate and phosphate. The extracellular Mg^{2+} concentration is tightly regulated by gut and kidney, and the serum Mg^{2+} level reflects the equilibrium between absorption in intestine and urinary excretion.

Gastrointestinal absorption of Mg^{2+}

Mg^{2+} is acquired through daily food intake, where nearly 35%–80% of the ingested Mg^{2+} is absorbed [2]. Specifically, around 30–50% of dietary Mg^{2+} intake is absorbed in the jejunum and ileum by way of passive paracellular route and in the colon through active transcellular route by transient receptor potential melastatin type 6 and 7 (TRPM6 and 7). The absorbed Mg^{2+} enters the bloodstream by CNNM4 and Na^+-Mg^{2+} exchanger at the basolateral side of cells [6].

Renal regulation of Mg^{2+} reabsorption

Serum Mg^{2+} is subsequently excreted in the urine after storage in organs and cellular utilization. The kidney determines the final urinary Mg^{2+} excretion, and hence plays a major role in Mg^{2+} homeostasis. In the kidney, only 3–5% of filtered Mg^{2+} is excreted after reabsorption in renal tubules. As shown in Fig. 1, around 15–25% of filtered Mg^{2+} is absorbed in the PCT via paracellular pathway. The increasing intratubular Mg^{2+} concentration triggers the passive reabsorption via claudin 1 and 2 in the late PCT [7,8]. Schlingmann et al. identified children with biallelic variants in *KCNJ16*, which encodes basolateral potassium channel (Kir5.1) of PCT and DCT, have hypokalemic tubulopathy, salt wasting, disturbed acid-base homeostasis, sensorineural deafness, and hypomagnesemia [9]. In the TALH, about 60–70% of filtered Mg^{2+} is reclaimed passively by paracellular pathway via claudin 16 and 19 triggered by positive luminal transepithelial voltage. This positive intraluminal voltage is generated by apical Na^+-K^+-Cl^- cotransporter (NKCC2)-mediated Na^+, K^+, Cl^- reabsorption, and apical renal outer medullary potassium (ROMK)-mediated parallel K^+ excretion [10–13]. Calcium-sensing receptor (CaSR) expressed abundantly in basolateral side of TALH regulates paracellular Mg^{2+} reabsorption by involving the regulation of salt reabsorption in TAL and the expression of claudin-14 [14,15]. Recent study has shown that children with heterozygous variants in *RRAGD*, which encodes GTPase RagD of TALH and DCT, has hypomagnesemia, salt wasting, nephrocalcinosis, and dilated cardiomyopathy [16]. The RagD variants leads to constitutive activation of mTOR signaling in vitro and is supposed to interfere with the handling of Mg^{2+} and other electrolytes in TALH and DCT.

Although the amount of reabsorbed Mg^{2+} in distal convoluted tubule (DCT) is lower than that in PCT and TALH, the DCT plays a crucial role of determining final urinary Mg^{2+} excretion. The active reabsorption of 5–10% of filtered Mg^{2+} is tightly regulated in the DCT through transcellular TRPM6 [Fig. 1]. Genetic defects of *TRPM6* encoding TRPM6 result in renal Mg^{2+} wasting and consecutive hypoparathyroidism and hypocalcemia (HSH). The mutations of *SLC12A3* encoding thiazide-sensitive sodium chloride cotransporter (NCC) in DCT lead to Na^+, K^+, and Cl^- wasting, and reduced expression of TRPM6 [17]. Kv1.1, the apical voltage-gated potassium channel, has been demonstrated to be involved in Mg^{2+} reabsorption, by the finding that non-functional Kv1.1 results in isolated dominant hypomagnesemia [18]. Kv1.1 facilitates Mg^{2+} reabsorption by generation of intraluminal positive voltage through apical secretion of potassium in DCT. The binding of epidermal growth factor (EGF) and EGF receptor in the basolateral side of DCT regulates the apical shuttling of TRPM6, and defects in *EGF* gene have been reported to cause isolated recessive hypomagnesemia, where apical TRPM6 expression is reduced [14,19]. The γ-subunit of the basolateral Na^+-K^+ ATPase encoded by *FXYD2* stabilizes Na^+-K^+ ATPase. Mutations in *FXYD2* have been demonstrated to cause diminished NCC activity and reduced driving force of Mg^{2+} reabsorption via TRPM6 [20]. The α1-subunit encoded by

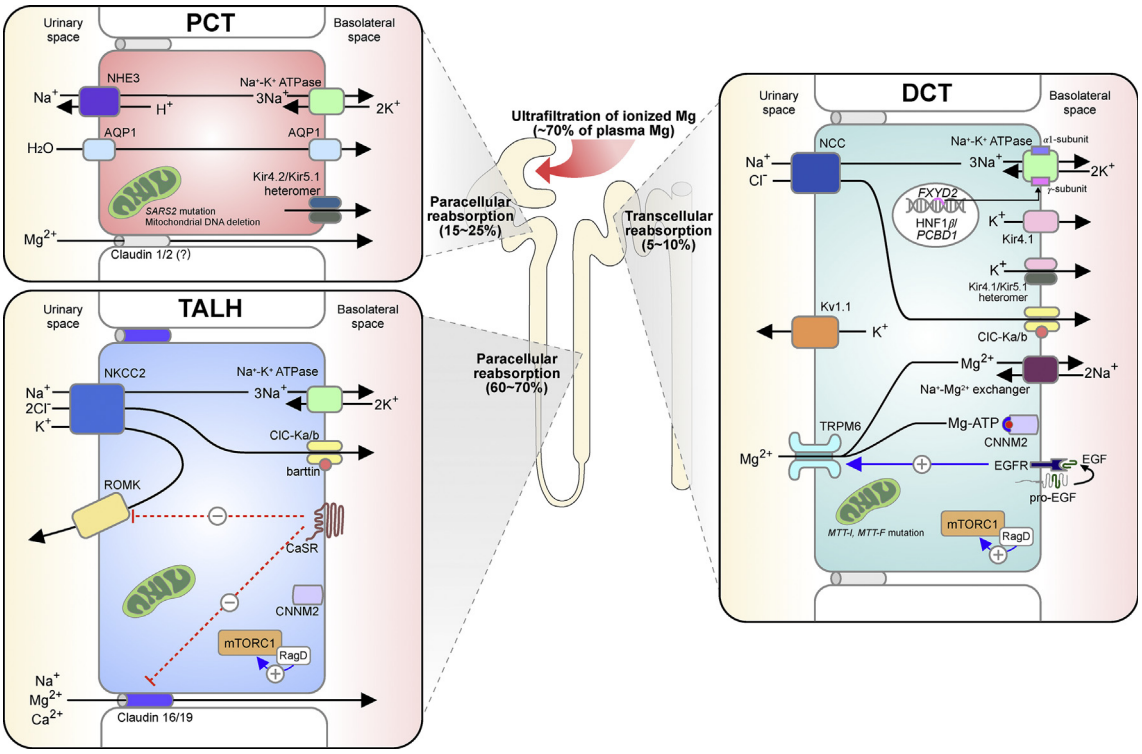

Fig. 1 **Magnesium reabsorption in proximal convoluted tubule, cortical thick ascending limb of Henle's loop, and distal convoluted tubule.** Around 15–25% of filtered Mg^{2+} is reabsorbed passively via paracellular pathway (probable claudin 1 and 2) in proximal convoluted tubule. The positive luminal voltage generated by apical NKCC2 and ROMK provides the driving force for paracellular reabsorption of 60–70% filtered Mg^{2+} in thick ascending limb of Henle's loop. The basolateral Na–K-ATPase in provides the driving force of NKCC2. The basolateral CaSR exerts inhibitory effects on paracellular claudin and ROMK. The Mg^{2+} is reabsorbed actively through TRPM6 in DCT. The apical Kv1.1 provide apical membrane potential by apical excretion of K^+. The basolateral Na–K-ATPase establishes the transapical membrane gradient critical for activity of TRPM6. The transcriptional factors including HNF1β and PCBD1 of γ-subunit of Na–K-ATPase, encoded by FXYD2, regulate Mg^{2+} reabsorption via alteration expression of γ-subunit of Na–K-ATPase. Kv4.1 located on basolateral side recycles the imported by Na–K-ATPase via conducting outward K^+ currents. The paracrine action of EGF regulates the activity of TRPM6. The Mg^{2+} efflux is conducted in Na^+-Mg^{2+} exchanger and possibly also in CNNM2.

ATP1A1 represents the exclusive of α-subunits of basolateral Na^+-K^+ ATPase in kidney [21]. Heterozygous mutations in *ATP1A1* recently have been reported to cause renal hypomagnesemia, seizure, and mental retardation [22]. The hepatocyte nuclear factor 1 (HNF1β) and pterin-4a-carinolamine dehydratase (PCBD1), the transcriptional regulatory proteins of γ-subunit of the basolateral Na^+-K^+ ATPase, have been reported to be associated with renal Mg^{2+} wasting, and mutations in *HNF1B* and *PCBD1* lead to inherited dominant hypomagnesemia [15,23]. The basolateral Kir4.1 encoded by *KCNJ10* (potassium voltage-gated channel subfamily J member 10) has been found to be involved in Mg^{2+} reabsorption by maintaining the function of Na^+-K^+ ATPase [24,25]. The genetic defects of *KCNJ10* can result in compromised NCC activity and consequent impairment of Mg^{2+} reabsorption [24]. As far, Na^+-Mg^{2+} exchanger and cyclin M2 are the two reported basolateral proteins that are proposed to act as Mg^{2+} efflux channels [10,26]. Cyclin M2, encoded by *CNNM2*, is primarily expressed in both TALH and DCT and is thought to be a basolateral Mg^{2+} transporter and also act as intracellular Mg^{2+} sensor by inducing a conformational change upon binding of Mg^{2+}-ATP [27]. Mutations of *CNNM2* have been identified to be the causative gene for patients with hypomagnesemia, seizure, intellectual disability, a clinical entity referred to as HSMR syndrome [10].

Clinical manifestations of hypomagnesemia

Hypomagnesemia is defined as total serum Mg^{2+} concentration less than 1.7 mg/dL (0.7 mM), and the clinical symptoms of hypomagnesemia may not be significant unless it reaches profound hypomagnesemia below 1.2 mg/dL (0.5 mM). Since Mg^{2+} is involved in many vital physiological functions hypomagnesemia may cause a myriad of manifestations involving multiple organs [Fig. 2]. Neuronal Mg^{2+} involved in the regulation of *N*-methyl-$_D$-aspartate (NDMA) and γ-aminobutyric acid (GABA) receptor, and deficiency of Mg^{2+} leads to hyperexcitability of NMDA and decreased stimulation of GABA [28,29]. In the central nervous system, as shown in Fig. 2, hypomagnesemia can cause tremor and convulsion from neuromuscular hyperexcitabiliy and it is also associated with

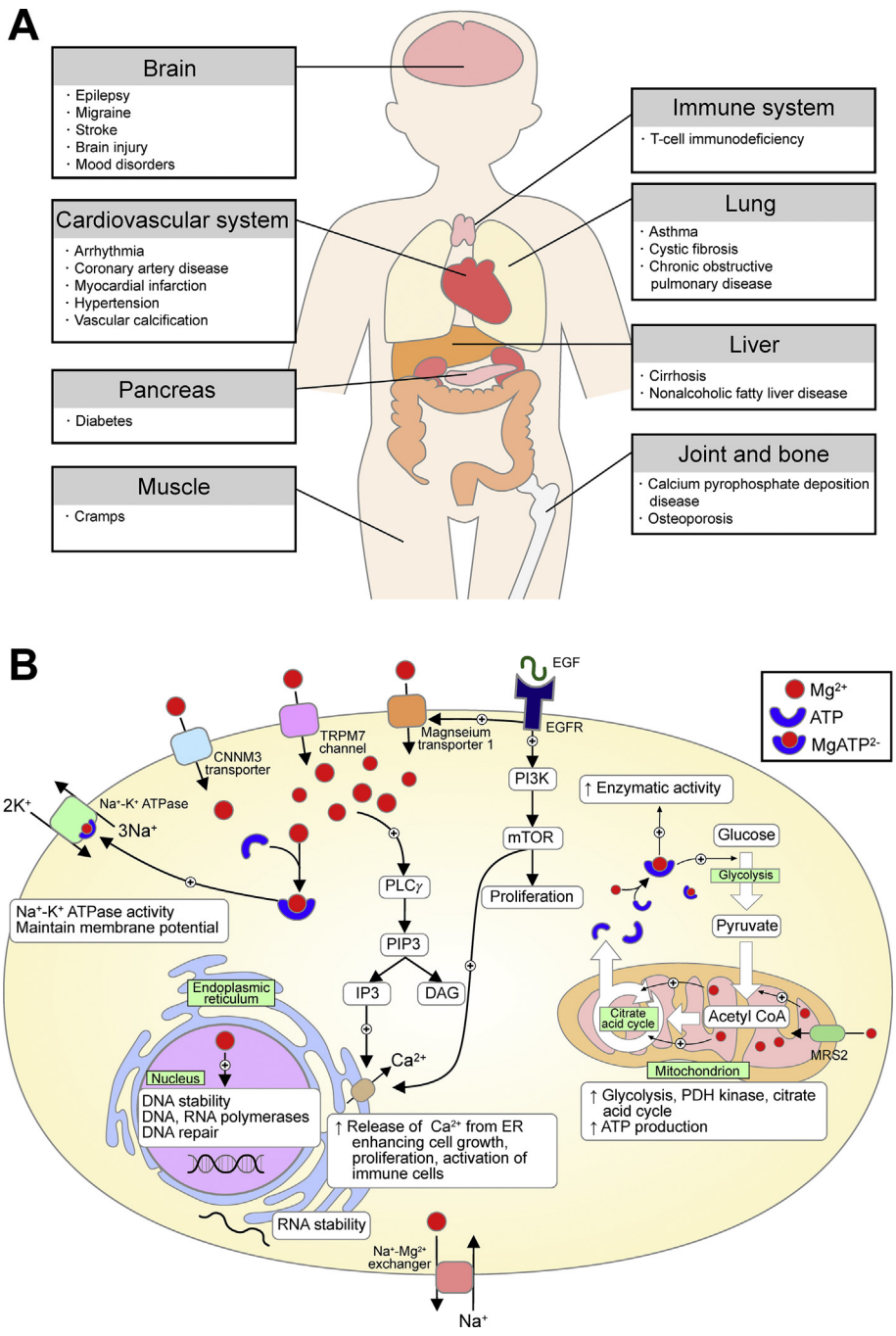

Fig. 2 **Pathophysiology of hypomagnesemia.** (A) Clinical manifestations and organ-specific consequences of hypomagnesemia. (B) Cellular physiology of Mg^{2+}. Several transporters are responsible for the cellular Mg^{2+} homeostasis. Mg^{2+} stabilizes the structures of DNA and RNA, DNA and RNA polymerases, and their repair in the nucleus. Additionally, Mg^{2+} also regulated the cell growth and proliferation. In cytosol, Mg^{2+} is involved in many enzymatic reactions and regulates the glycolysis and ATP synthesis.

migraine, brain injury, stroke, and mood disorders [30–32]. In lung, Mg^{2+} has bronchodilatory and anti-inflammatory effects on airway [33,34]. Hypomagnesaemia has been shown to be associated with asthma and chronic obstructive pulmonary disorder, therefore, Mg^{2+} is supposed to have therapeutic roles on these disorders. In cardiovascular system, Mg^{2+} regulates the myocardial contractility and has effects of anti-inflammation, vasodilatation, and inhibitory crystallization of calcium-phosphate [35,36]. Hence, hypomagnesemia may cause arrhythmia, myocardial infarction, vascular calcification, hypertension, and coronary artery diseases. In muscle, Mg^{2+} acts as Ca^{2+} antagonist to compete the binding sites of proteins responsible for muscle contraction [37]. Hence, Mg^{2+} deficiency may lead to hypercontractibility and muscle cramps. In bone, Mg^{2+} participates in bone formation by stimulating the proliferation of osteoblast and increasing the

solubility of minerals. Mg^{2+} deficiency has been reported to be associated with osteoporosis [38]. Mg^{2+} may have effects on the secretion of insulin by the findings that patients with Mg^{2+} deficiency have a decreased insulin secretion [39]. Additionally, patients with diabetes also have low serum Mg^{2+} levels [40]. In liver, patients with liver cirrhosis and fatty liver are Mg^{2+} depleted. This may be due to enzymatic reactions in liver that are Mg^{2+}- dependent [41]. Mg^{2+} has been reported to regulate the proliferation and development of T lymphocytes, and it is associated with X-linked T-cell immunodeficiency [1,42].

Approach to hypomagnesemia

Apart from inadequate dietary intake and malabsorption in gut, defects of renal reabsorption are largely responsible for the dysregulation of Mg^{2+} homeostasis. In general, causes of hypomagnesemia can be divided into two categories: high renal Mg^{2+} excretion and low renal Mg^{2+} excretion including gastrointestinal, tissue sequestration, and shifting origins. Besides detailed medical and family histories, complete physical examination, and urine and serum electrolytes measurement, fractional excretion rate of Mg^{2+} (FEMg) can provide a rapid differentiation of these two categories [Fig. 3]. In the setting of hypomagnesemia, FEMg >4% indicates renal Mg^{2+} wasting, while FEMg <2% suggests appropriate renal Mg^{2+} conservation and Mg^{2+} wasting of extra-renal origin. Of note, a low glomerular filtration rate may reduce the filtered load of Mg^{2+}, and therefore lowers the cutoff values of FEMg indicating renal wasting. Common causes of gastrointestinal Mg^{2+} losses include dietary deprivation, diarrhea, malabsorption, use of proton pump inhibitor, and rare primary familial hypomagnesemia caused by molecular defects in *TRPM6*. Acute pancreatitis, massive blood transfusion, hungry bone syndrome, refeeding syndrome, and cardiopulmonary bypass are common causes of hypomagnesemia secondary to cellular shift and tissue sequestration. Rarely, in hypervolemia and/or overall reduction of serum anion, FEMg may still appear appropriate, despite an overall increase in renal Mg^{2+} excretion. Renal hypomagnesemia can be caused by an increased glomerular filtration and defective transport in proximal tubular segments, thickening ascending limb of the loop of Henle or distal convoluted tubules.

Identify genetic forms of renal hypomagnesemia

Before establishing the diagnosis of inherited renal hypomagnesemia, the acquired etiologies of renal Mg^{2+} wasting such as medications should be carefully excluded. Historical clues such as prior hypomagnesemia, associated organ abnormalities in family members, use of drugs affecting hypomagnesemia-loop diuretics, proton pump inhibitor, calcineurin inhibitor, aminoglycoside, foscanet, cisplatin and cetuximab, age of onset, and nephrolithiasis or nephrocalcinosis must be carefully obtained. The diagnosis of inherited renal hypomagnesemia would be made after exclusion the acquired etiologies above-mentioned.

Differential diagnosis of inherited renal hypomagnesemia

The mode of inheritance, assessment of urine and blood biochemistry studies, presence of extra-renal symptoms all

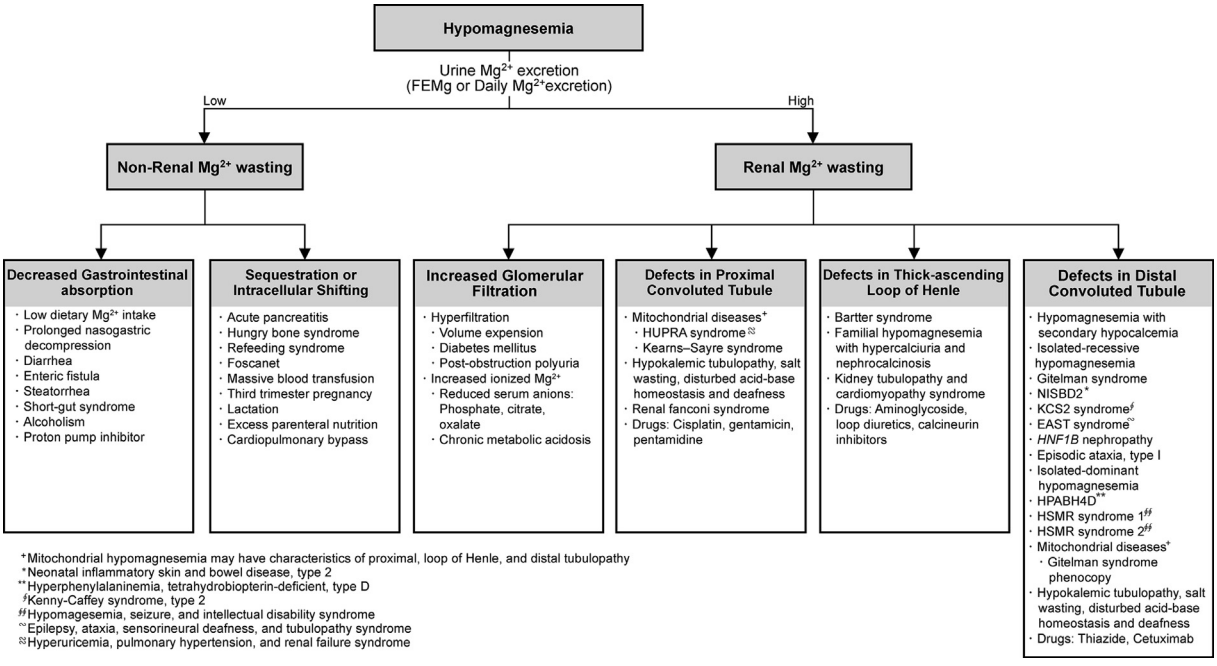

Fig. 3 Congenital and acquired causes of hypomagnesemia.

aid in determination of further genetic testing. The models of inheritance of inherited renal hypomagnesemia include autosomal dominant, autosomal recessive and maternal inheritance. As shown in Fig. 4, the etiology of inherited renal hypomagnesemia can be divided into lesions in TALH, DCT and PCT by renal calcium excretion. Hypercalciuria points the TALH tubulopathy such as Bartter syndrome, autosomal dominant hypocalcemia, and familial hypercalciuria hypomagnesemia nephrocalcinosis. Absence of hypercalciuria can be further divided into lesions in DCT and PCT. The presence of hypokalemia is cardinal feature for differentiating the Gitelman syndrome-like hypomagnesemia from others DCT tubulopathy [Fig. 4]. Gitelman syndrome-like hypomagnesemia can be further differentiated by the presence of extrarenal manifestations and modes of inheritance. Finally, mitochondrial hypomagnesemia is one of important causes of PCT tubulopathy.

Genetic approach to inherited renal hypomagnesemia

As shown in Fig. 5, direct Sanger sequencing can identify the disease-specific gene assessed by above-mentioned approach [Table 1 and Fig. 4]. Of note, direct Sanger sequencing will miss deep intronic mutation not rare in Gitelman syndrome and large deletion including cBS, familial hypomagnesemia hypercalciuria nephrocalcinosis, HNF1β nephropathy, hypomagnesemia secondary hypocalcemia, and EAST syndrome [43,44]. Therefore, if unable to identify the causative genetic mutation, analysis of cDNA for deep intronic mutation or MLPA (multiplex ligation-dependent probe amplification) for large deletion may be warranted. In patients of diseases with uncertain gene due to the overlapping phenotype and genetic heterogeneity, a gene panel can be considered to assess multiple causal genes simultaneously. The gene panel is also recommended for patients of suspicion of specific disease without detected variants by direct Sanger sequencing. Some patients might have no detected variants by direct Sanger sequence, MLPA, cDNA, and gene panels. In such cases, next generation sequencing (NGS) will aid in the molecular diagnosis. NGS, such as whole exome sequencing (WES) and whole genome sequencing (WGS), provide rapid screen of known genes, as well as modifier genes and epigenetic modification. The bioinformatics analysis and variants databases are essential for determining the pathogenic role of variants identified by NGS. Epigenetic analysis, including DNA methylation, siRNA regulation, and chromatin immunoprecipitation sequencing, may be the further potential tests for patients without identified variants by NGS.

Management of hypomagnesemia

Delivery of Mg^{2+} supplementation, avoidance of exacerbated renal Mg^{2+} wasting, and correction of accompanying metabolic disarrangement is the mainstay treatment in inherited renal hypomagnesemia. The route of administration and dosage of Mg^{2+} supplement dependents on the severity of hypomagnesemia. In patients with acute symptomatic hypomagnesemia, parenteral Mg^{2+} supplementation should be considered for alleviating potential complications. Oral Mg^{2+} supplementation is suitable for patients with non-acute and asymptomatic hypomagnesemia. The common formulations of Mg^{2+} supplementations and Mg^{2+}-rich food are summarized in Table 2. Although there is no upper limit of dietary Mg^{2+}, oral elemental Mg^{2+} supplementation less than 350 mg

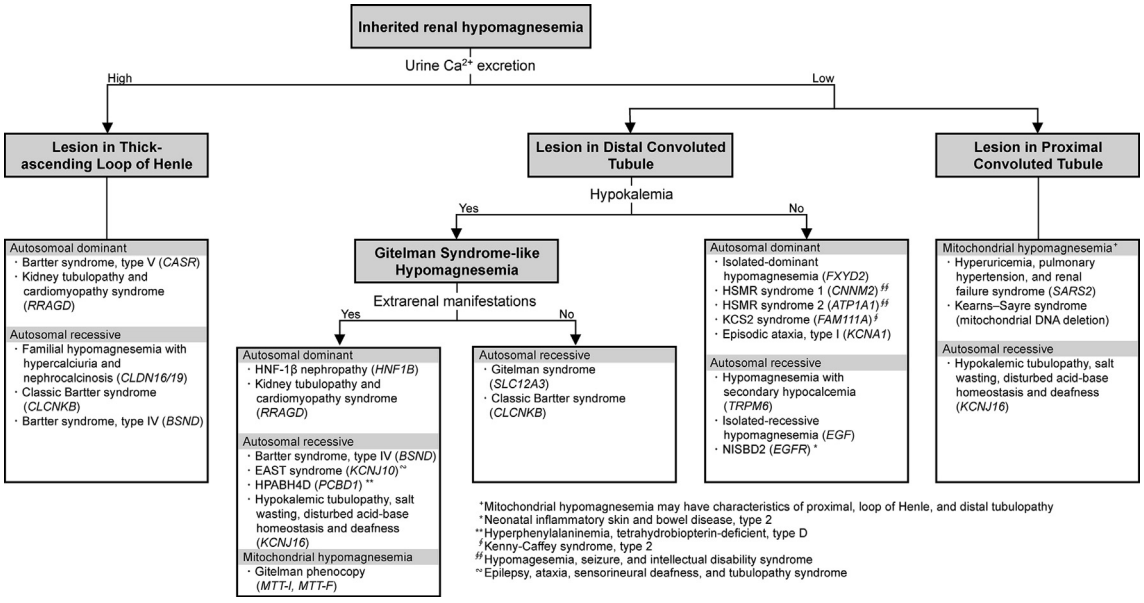

Fig. 4 Differential diagnosis of inherited renal hypomagnesemia.

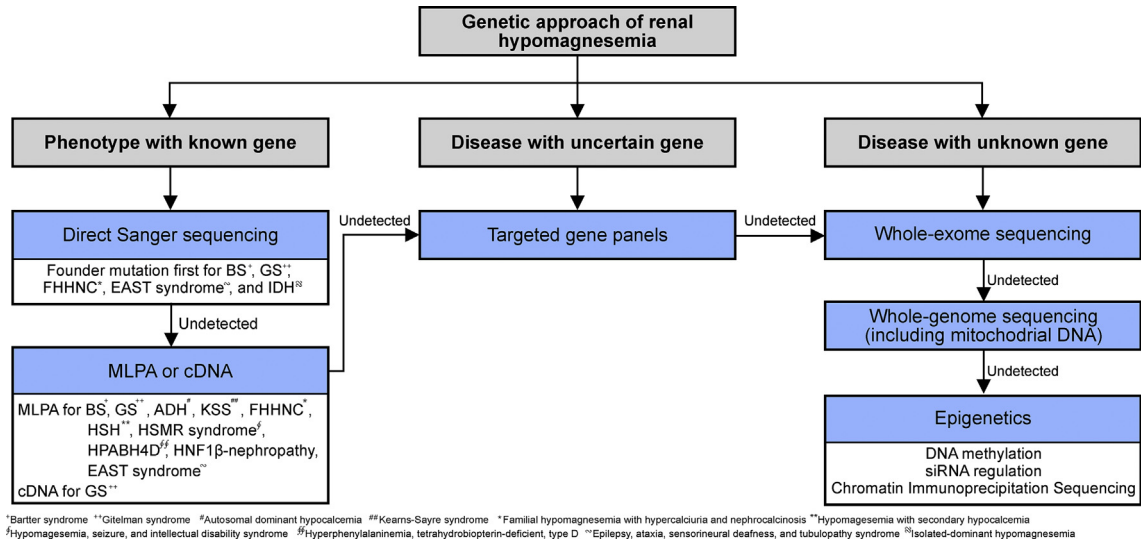

Fig. 5 **Genetic strategy for diagnosis of inherited renal hypomagnesemia.** The diagnostic paradigm for inherited hypomagnesemia diseases is determined by spectrum of specific disease, disorders localized in specific tubules, and disorders with unknown mechanism candidate gene after comprehensive evaluation of phenotypes. The following molecular methods including cDNA analysis multiplex ligation-dependent probe amplification, gene panels, and next generation is considered step by step.

per day is safe for adults and children older than 8 years [45]. The tolerable upper limit of daily elemental Mg^{2+} supplementation for children 1–3 years and 4–8 years are 65 mg and 110 mg, respectively [46]. Based on the different bioavailability of Mg^{2+} formula, organic Mg^{2+} compounds including Mg^{2+} citrate, Mg^{2+} aspartate, Mg^{2+} chloride, and Mg^{2+} glycerophosphate are superior to Mg^{2+} oxide and Mg^{2+} sulfate in correcting hypomagnesemia [47]. Conditions associated with renal hyperfiltration (osmotic diuresis, poor control of hyperglycemia, and massive fluid intake) and increased filterable Mg^{2+} (loop diuretics, chronic metabolic acidosis, low serum anion including hypoalbuminemia, hypophosphatemia) could aggravate the renal Mg^{2+} wasting and should corrected appropriately. Accordingly, angiotensin-converting-enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARB) that reduce glomerular filtration can be considered for patients who are in a glomerular hyperfiltrative state. In addition, aldosterone antagonist has been reported to ameliorate the renal Mg^{2+} wasting and maintain serum Mg^{2+} levels in patients with congestive heart failure [48]. Aldosterone has been demonstrated to induce renal Mg^{2+} wasting through increases in intracellular Mg^{2+} shifts from muscle and bone [49]. Additionally, aldosterone has also been shown to downregulate the activity of renal TRPM6 [50]. Several metabolic disarrangements including hypokalemia, hypocalcemia, hypercalciuria, and hyperglycemia caused by diabetes mellitus may also occur in patients with inherited hypomagnesemia. The condition of hyperglycemia could further exacerbate hypomagnesemia. Hypocalcemia, hypokalemia and hypercalciuria may further deteriorate renal function if left untreated. Therefore, these accompanied metabolic disarrangements should be addressed simultaneously.

Potential therapy for genetic renal hypomagnesemia

As shown in Fig. 6, molecular therapies that target modifications at the level of DNA, RNA, and proteins are currently being developed and applied on variety of inherited diseases. Successful development of personalized treatment approaches is dependent on the severity of diseases, identification of genetic defects, and understanding the pathophysiological mechanism [34–47]. DNA therapies include gene replacement therapy and genomic editing [51,52]. Gene replacement therapy works by using the therapeutic vectors to insert the normal copy of mutant gene into host cells, and this might be potential for patients with large deletion mutation. Heikkilä et al. succeeded in delivering the *COL4A5* gene by adenoviral vector to glomerular cells [53]. Genomic editing allows for precise editing of genomic DNA in vivo or ex vivo by utilizing CRISPR/Cas system and EFNs and TALENs [54,55]. Daga et al. developed two-plasmid approach to achieve a stable variant-specific correction in the X-linked *COL4A5* (p.Gly624Asp) and *COL4A3* gene (p.Gly856Glu) using CRISP/Cas9 genome editing [56]. RNA therapies are represented by splicing modulation, RNA silence, and RNA editing. The splicing modulation is conducted for correcting aberrant splicing by antisense oligonucleotides, U1 splicesomal RNA, or trans-splicing [57–59]. As shown in Table 1, splice site

Table 1 Clinical, biochemical, and genetic characteristics of inherited disorders of renal hypomagnesemia.

Disorders[a]	Involved tubule	Inheritance	Gene	Protein	Large deletion	Age at onset	Serum Ca	Serum K	Blood pH	Urine Mg	Urine Ca	Extrarenal manifestation	Nephrocalcinosis/ nephrolithiasis	Renal anomaly	Early ESRD[c]	Reference
ADH	TALH	AD	*CASR*	CaSR	Yes	Adolescence/ adulthood	↓	–	–	↑	↑	Hypoparathyroidism	Yes	No	No	[65]
FHHNC	TALH	AR	*CLDN16, CLDN19*	Claudin-16, Caludin-19	Yes	Childhood/ adolescence	–	–	–	↑↑	↑↑	Ocular abnormalities hyperparathyroidism	Yes	No	Yes	[66–69]
cBS	TALH DCT	AR	*CLCNKB*	ClC-Kb	Yes	Childhood	var.	↓↓	↑	- or ↑	–	No	Yes (infrequent)	No	No	[70–73]
BS, type IVa	TALH DCT	AR	*BSND*	Barttin	Yes	Infancy	var.	↓↓	↑	- or ↑	–	Sensorineural deafness	Yes (infrequent)	No	Yes	[74]
BS, type IVb	TALH DCT	AR	*CLCNKA CLCNKB*	ClC-Ka ClC-Kb	Yes	Infancy	var.	↓↓	↑	- or ↑	–	Sensorineural deafness	Yes (infrequent)	No	Yes	[75,76]
KICA syndrome	TALH, DCT	AD	*RRAGD*	RagD	No	Infancy/ Childhood	–	↓	↑	↑	↑ or ↓	Dilated Cardiomyopathy	Yes	No	No	[77]
HNF1B nephropathy	DCT	AD	*HNF1B*	HNF1beta	Yes	Adolescence/ adulthood	–	?	?	- or ↑	- or ↓	MODY5, hyperuricemia	No	Renal cysts, dysplasia, agenesis	Yes	[78–81]
EAST syndrome	DCT	AR	*KCNJ10*	Kir4.1	Yes	Neonate/ infancy	–	↓	↑	↑	↓	Epilepsy, ataxia, sensorineural deafness, intellectual disability	No	No	No	[82,83]
HPABH4D	DCT	AR	*PCBD1*	PCBD1	Yes	adolescence/ adulthood	–	–	?	↑	–	MODY	No	No	No	[23,84]
GS[d]	DCT	AR	*SLC12A3*	NCC	Yes	Adolescence/ adulthood	–	↓	↑	↑	↓	Chondrocalcinosis	No	No	No	[43,85–89]
IDH	DCT	AD	*FXYD2*	γ subunit of Na^b-K^b ATPase	No	Adolescence/ adulthood	–	–	–	- or ↑	↓	No	No	No	No	[90]
HSMR syndrome 2	DCT	AD	*ATP1A1*	α subunit of Na^b-K^b ATPase	No	Neonate/ infancy	↓	↓	↑	↑↑	- or ↑	Intellectual disability, epilepsy	Yes	No	No	[22]
HSMR syndrome 1	DCT	AD/AR	*CNNM2*	Cyclin M2	Yes	Infancy/ childhood	–	–	–	- or ↑	?	Intellectual disability, epilepsy	No	No	No	[91]
KCS2 syndrome	DCT	AD	*FAM111A*	FAM111A	No	Infancy	↓↓	?	?	?	?	Impaired skeletal development, hypocalcemia, hypoparathyroidism	No	No	No	[92,93]
EA1	DCT	AD	*KCNA1*	Kv1.1	No	Childhood	–	–	?	- or ↑	–	Episodic ataxia, myokymia, epilepsy, intellectual disability	No	No	No	[94]
HSH	DCT	AR	*TRPM6*	TRPM6	Yes	Infancy	↓	–	–	- or ↑	;= or ↑	intellectual disability, epilepsy, hypoparathyroidism	No	No	No	[95,96]
IRH	DCT	AR	*EGF*	Pro-EGF	No	Infancy	–	–	–	- or ↑	–	intellectual disability, epilepsy	No	No	No	[19]
NISBD2	DCT	AR	*EGFR*	EGFR	No	Infancy	–	–	–	- or ↑	–	Severe inflammation of skin and bowel heart abnormalities	No	Renal dysplasia	No	[97]
GS phenocopy	DCT[b]	Mt	*MT-TI, MT-TF*	Mt. $tRNA^{ile, phe}$	No	Adulthood	–	↓	- or ↑	↑	↓	No	No	No	No	[98]] [99]

(*continued on next page*)

Table 1 – (*continued*)

Disorders[a]	Involved tubule	Inheritance	Gene	Protein	Large deletion	Age at onset	Serum Ca	Serum K	Blood pH	Urine Mg	Urine Ca	Extrarenal manifestation	Nephrocalcinosis/ nephrolithiasis	Renal anomaly	Early ESRD[c]	Reference
HUPRA syndrome	PCT[b]	AR	*SARS2*	SARS2	No	Infancy	?	↓	↑	↑↑	↓?	Hyperuricemia, elevated serum lactate, pulmonary hypertension, prematurity, intellectual disability, diabetes mellitus	No	No	Yes	[100]
KSS	PCT[b]	Mt	*Mitochondrial deletion*	–	Yes	Childhood	↓↓	↓	↓ or ↑	↑↑	↑	Brain, eye, ear involvement. Muscle weakness, ataxia, intellectual disability, epilepsy, diabetes mellitus, gonadal failure, thyroid disease, hypoparathyroidism	No	No	No	[101–103]
Hypokalemic tubulopathy, salt wasting, disturbed acid-base homeostasis and deafness	PCT[a] TALH DCT	AR	*KCNJ16*	KCNJ16	No	Infancy/ Childhood	–	↓	↑	↑	↓	Sensorineural deafness	No	No	No	[9]

[a] Abbreviations: ADH: autosomal dominant hypocalcemia; BS: Bartter syndrome; cBS: classic Bartter syndrome; DCT: distal convoluted tubule; EA1: episodic ataxia, type I; EAST syndrome: epilepsy, ataxia, sensorineural deafness, and tubulopathy syndrome; ESRD: end stage renal disease; FHHNC: familial primary hypomagnesemia with hypercalciuria and nephrocalcinosis; GS: Gitelman syndrome; HHH syndrome: hypertension, hypercholesterolemia, and hypomagnesemia syndrome; HPABH4D: hyperphenylalaninemia, BH4-deficient, type D; HSH: hypomagnesemia with secondary hypocalcemia; HSMR syndrome: hypomagnesemia, seizures, and intellectual disability syndrome; HUPRA syndrome: hyperuricemia, pulmonary hypertension, and renal failure syndrome; IDH: isolated-dominant hypomagnesemia; IRH: isolated-recessive hypomagnesemia; KCS2 syndrome: Kenny-Caffey syndrome, type 2; KICA syndrome: kidney tubulopathy and cardiomyopathy; KSS: Kearns–Sayre syndrome; NISBD2: neonatal inflammatory skin and bowel disease; PCT: proximal convoluted tubule; TALH: thick-ascending loop of Henle.

[b] Lesion in PCT may have characteristics of TALH and DCT tubulopathy.

[c] End stage renal disease.

[d] Deep intronic mutation.

Table 2 Magnesium formulations and magnesium-rich foods.

Supplement	Elemental Mg^{2+} content
Intravenous formulations	
Magnesium sulfate	0.10 mg/mg
Oral formulations	
Magnesium oxide	0.61 mg/mg
Magnesium hydroxide	0.42 mg/mg
Magnesium gluconate	0.059 mg/mg
Magnesium chloride	0.12 mg/mg
Magnesium carbonate	0.29 mg/mg
Magnesium lactate	0.12 mg/mg
Magnesium aspartate hydrochloride	0.10 mg/mg
Magnesium citrate	0.16 mg/mg
Food sources	
Seeds (pumpkin, chia)	400–560 mg/100 g
Almonds, dry roasted	286 mg/100 g
Whole grains	232 mg/100 g
Dark chocolate	230 mg/100 g
Peanuts, oil roasted	107 mg/100 ml
Spinach, boiled	66 mg/100 ml
Tofu	53 mg/100 g
Salmon	30 mg/100 g
Banana	27 mg/100 g
Chicken breast, roasted	26 mg/100 g
Soymilk	26 mg/100 ml
Yogurt	19 mg/100 g
Milk	10.1–11.4 mg/100 ml

mutations are identified in several genes responsible for inherited renal hypomagnesemia. Ramsbottom and his colleagues delivered antisense oligonucleotide (ASO)-induced splicing of the mutated exon (*CEP20* G1890*) to restores protein expression in cells from patient with Joubert syndrome [60]. Translation or protein targeting includes translational read-through, restoring proteostasis and pathway-specific therapy. Aminoglycoside and PTC124, the two main drugs of translational read-through, allow the translational machinery to bypass the premature termination code [61]. We had significantly increased survival rate and partially rescued $Na^+/HCO3^-$ cotransporter 1 (NBCe1) activity ex vivo by delivering PTC124 therapy in NBCe1 p.W516* knock-in mice [62]. Cell therapy allows integrating the exogenous delivery of cells and reactivation of cellular function. The induced pluripotent stem cells from somatic cells of patients or embryonic stem cells could be used and differentiated to tubular precursor cells [63]. Of note, the genome-edited cells from patients by CRISPR-engineered cell therapies can be also used for cell therapy and has been successfully applied clinically [56,64]. The above-mentioned potential therapies required appropriate cellular and/or animal models to test the efficacy and safety in the future.

Concluding remarks

The etiologies responsible for impaired Mg^{2+} homeostasis are complex and heterogeneous, therefore, a comprehensive evaluation is essential for accurate diagnosis. After the exclusion of acquired causes, genetic causes can be further classified into defects in TALH, DCT and PCT according to the specific biochemical characteristics and extra-renal manifestations. Strategy for molecular diagnosis of inherited renal hypomagnesemia can be further stratified: patients with specific disease, disease with uncertain gene, and those with unknown gene. Algorithms for genetic testing can be developed based on the knowledge of advantages and limitations of molecular methods including direct Sanger sequencing, cDNA analysis, MLPA, gene panels, and WES. Once the causative gene is detected, prediction of clinical outcome, accurate therapeutic intervention, and genetic consulting are all facets of personalized medicine that ultimately lead to improved outcome within a shorter time frame. Although increasing numbers of genetic defects involving renal Mg^{2+} homeostasis is being identified and several molecular and cellular therapy

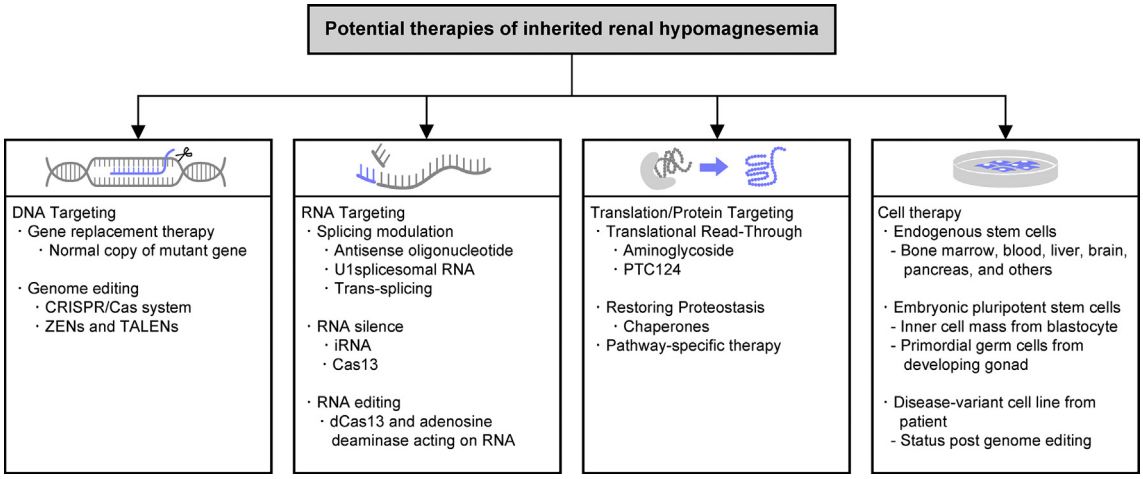

Fig. 6 Proposed molecular and cell therapies for inherited renal hypomagnesemia.

is proposed, a clear understanding of underlying biomolecular mechanisms still underpins the basis of management of inherited renal hypomagnesemia.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This work was supported by research fund of the Ministry of Science and Technology (MOST109-2314-B-182A-134-MY3).

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