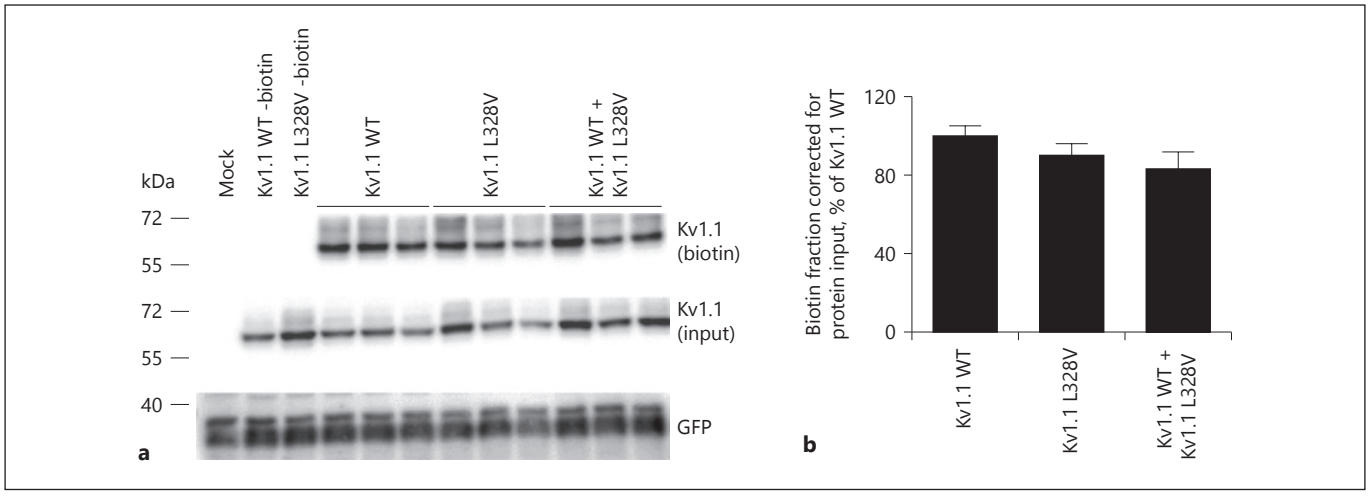

Fig. 3. Expression of wild-type and mutant Kv1.1 channels. **a** Cell surface biotinylation of HEK293 cells expressing either mock, Kv1.1 wild type, Kv1.1-p.Leu328Val, or co-expressing Kv1.1 wild type and Kv1.1-p.Leu328Val. Kv1.1 expression was analyzed by immunoblotting for plasma membrane fraction and input from the total cell lysates. Representative immunoblot of 4 independent experiments is shown. **b** Bar graph representing the quantification of the cell surface biotinylation experiments. It depicts the relative plasma membrane expression compared to input, and is shown as percentage of Kv1.1 wild type. Mean ± SEM is shown.

were performed. Figure 3a shows a representative immunoblot of the biotinylation demonstrating an equal amount of Kv1.1 wild type and Kv1.1-p.Leu328Val channels at the plasma membrane (upper panel). Furthermore, cells co-expressing Kv1.1 wild type and Kv1.1-p.Leu328Val showed similar plasma membrane abundance as Kv1.1 wild type or Kv1.1-p.Leu328Val alone (Fig. 3a). Quantification of 3 independent experiments confirmed no significant differences between the conditions (Fig. 3b), suggesting that similar amounts of either homotetrameric (wild type or mutant) channels or heterotetrameric (wild type plus mutant) channels were located at the plasma membrane. Of note, no difference in Kv1.1 expression was observed in the total cell lysates (Fig. 3a, middle panel). green fluorescent protein (GFP) expression was used as transfection control and was equal in all conditions tested (Fig. 3a, lower panel).

Discussion

Here, we describe the first new *KCNA1* mutation that is causative for hypomagnesemia since the identification of the initial family in 2009. A de novo c.982C>G mutation in *KCNA1* was identified in a patient with hypomagnesemia and tetany. Electrophysiological and biochemical analyses show that the resulting Kv1.1-p.Leu328Val amino acid change causes loss of function, despite normal plasma membrane expression.

The proband presented with hypomagnesemia, tetany, muscle weakness, and tremor, which is highly comparable to the Brazilian family described previously [3]. In line with this family, the proband showed inappropriately normal urinary Mg^{2+} excretion despite hypomagnesemia indicating a renal leak [3]. Treatment with Mg^{2+} supplements was only partially successful, as it restored the serum Mg^{2+} concentration to the low-normal range but did not completely relieve clinical symptoms. Interestingly, the mutation identified here occurred de novo, as its presence was excluded in all other family members tested including the parents. Furthermore, it was not transmitted to the unaffected daughter of the patient. These findings confirm the causality of *KCNA1* mutations for hypomagnesemia and demonstrate that this aspect is not mutation-specific for p.Asn255Asp.

KCNA1 mutations have been widely described as cause for EA1, with thus far 38 different mutations involved [13, 14]. The majority of the mutations result in reduced channel function, weakened surface expression, or changes in the biophysical characteristics that lead to a dominant-negative effect upon association with wild-type subunits [14]. The latter is also observed in our study. The phenotype of EA1 patients is highly heterogeneous and may include myokymia, cataplexy, epilepsy, neuromyotonia, paroxysmal dyspnea, and also skeletal defects [13, 15–20]. Of note, episodic ataxia is not present in all patients. Despite the identification of the p.Asn255Asp mu-

tation as cause for hypomagnesemia in 2009 [3], hypomagnesemia has not been reported in (other) patients affected by EA1 due to mutations in *KCNA1*. Therefore, it is still difficult to assess the penetrance of the hypomagnesemia phenotype in patients with *KCNA1* mutations.

The novel identified *KCNA1-p.Leu328Val* mutation has never been described before. However, closely located mutations p.Glu325Asp and p.Leu329Ile have been reported in EA1 patients, in which serum Mg^{2+} levels are unknown [19–21]. The Leu328, as well as these latter 2 residues, are located on the cytoplasmic side of the transmembrane helix S5. The KCNA1-p.Glu325Asp, p.Leu328Val, and p.Leu329Ile mutations result only in small changes of glutamic acid to aspartic acid and leucine to valine or isoleucine, respectively, which are amino acids that are similar in size, hydrophobicity, and charge. Based on our homology model, they are likely to interact with the lipids in the plasma membrane and provide stability of the protein complex. Moreover, the amino acid changes might disrupt the S5 helix, which then disturbs the channel pore structure and results in reduced channel function as apparent in our study. Likewise, the KCNA1 p.Glu325Asp was shown to significantly alter channel function characteristics [22]. Interestingly, the other hypomagnesemia-causing p.Asn255Asp mutation was also located at the cytoplasmic interface of the plasma membrane, and demonstrated a similar dominant-negative loss of channel function observed in the present study [3]. Despite recognition of the wide distribution throughout the protein and altered effects on channel properties, there is still no clear association reported between the broad spectrum of clinical phenotypes and specific mutations. Detailed analysis will be necessary to elucidate how functional consequences could explain the disease diversity.

Although expression levels of Kv1.1 in the kidney are low, immunohistochemical stainings have shown that Kv1.1 is exclusively expressed at the apical membrane of the DCT [3, 5]. In the DCT, intracellular Mg^{2+} levels are comparable to the Mg^{2+} concentration in the pro-urine and therefore a chemical gradient for Mg^{2+} reabsorption is suggested to be virtually absent. It has therefore been hypothesized that K^+ secretion via Kv1.1 provides an electrical gradient that drives Mg^{2+} reabsorption via TRPM6 [3, 4]. Indeed, our previous studies showed that Kv1.1 activity determined the membrane potential in HEK293 cells [3]. This is in line with studies in rabbit distal tubule cells showing that the apical K^+ conductance sets the membrane potential but has been recently contested by experiments in mouse DCT segments that did not show apical K^+ transport [23, 24]. Recent expression studies suggest that expression of Kv1.1 and other K^+ channels, like ROMK, depend on the specific conditions including K^+ and Mg^{2+} availability [25, 26]. Future studies should aim at resolving the inconsistencies on the exact nature of the apical K^+ channel in the DCT.

In conclusion, this study reports a patient with hypomagnesemia and tetany caused by a de novo *KCNA1* mutation, unequivocally showing that *KCNA1* mutations are causative for hypomagnesemia.

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Ethics Statement

The study was performed in accordance with the guidelines of the Declaration of Helsinki. The local Ethics Committee approved the study. Informed consent was obtained from the patient and her relatives.

Disclosure Statement

The authors declare that there are no conflicts of interest to disclose.

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