GENETICS IN ENDOCRINOLOGY

Gain and loss of function mutations of the calcium-sensing receptor and associated proteins: current treatment concepts

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Abstract

The calcium-sensing receptor (CASR) is the main calcium sensor in the maintenance of calcium metabolism. Mutations of the CASR, the G protein alpha 11 (GNA11) and the adaptor-related protein complex 2 sigma 1 subunit (AP2S1) genes can shift the set point for calcium sensing causing hyper- or hypo-calcemic disorders. Therapeutic concepts for these rare diseases range from general therapies of hyper- and hypo-calcemic conditions to more pathophysiology oriented approaches such as parathyroid hormone (PTH) substitution and allosteric CASR modulators. Cinacalcet is a calcimimetic that enhances receptor function and has gained approval for the treatment of hyperparathyroidism. Calcilytics in turn attenuate CASR activity and are currently under investigation for the treatment of various diseases. We conducted a literature search for reports about treatment of patients harboring inactivating or activating CASR, GNA11 or AP2S1 mutants and about in vitro effects of allosteric CASR modulators on mutated CASR. The therapeutic concepts for patients with familial hypocalciuric hypercalcemia (FHH), neonatal hyperparathyroidism (NHPT), neonatal severe hyperparathyroidism (NSHPT) and autosomal dominant hypocalcemia (ADH) are reviewed. FHH is usually benign, but symptomatic patients benefit from cinacalcet. In NSHPT patients pamidronate effectively lowers serum calcium, but most patients require parathyroidectomy. In some patients cinacalcet can obviate the need for surgery, particularly in heterozygous NHPT. Symptomatic ADH patients respond to vitamin D and calcium supplementation but this may increase calciuria and renal complications. PTH treatment can reduce relative hypercalciuria. None of the currently available therapies for ADH, however, prevent tissue calcifications and complications, which may become possible with calcilytics that correct the underlying pathophysiologic defect.

Introduction

The calcium-sensing receptor (CASR) serves as the key calcium sensor in the maintenance of systemic calcium homeostasis. Its main physiologic functions are the regulation of parathyroid hormone (PTH) release from the parathyroid gland and calcium handling in the kidney. The CASR is a class 3 G-protein coupled receptor (GPCR) that regulates the cytosolic calcium, MAP-kinase and cAMP signaling pathways via the small G-proteins Gq/11, G12/13, and Gi (1, 2). The activity of the CASR itself is regulated by trafficking to and from the membrane, a process that involves β-arrestin, the adaptor-related protein complex 2 (AP2) and other membrane associated proteins (3, 4, 5).
Mutations causing loss of CASR function

Inactivating mutations of the CASR lead to a right-shifted dose–response curve for extracellular calcium with a higher ‘setpoint’ resulting in hypercalcemic diseases (6, 7, 8). The phenotype depends on the degree of overall functional impairment of extracellular calcium sensing by CASR-dependent mechanisms. On one end of the spectrum there is a mostly benign and asymptomatic autosomal dominant disease called familial hypocalciuric hypercalcemia (FHH), which is often discovered incidentally in otherwise healthy subjects. The laboratory pattern of elevated serum calcium, high normal or elevated PTH combined with low renal calcium excretion allows presumptive diagnosis of FHH (6). On the other end there is neonatal severe hyperparathyroidism (NSHPT), a life threatening disease with severe hypercalcemia and high PTH in newborns, which requires immediate therapeutic intervention (9, 10, 11). In general, FHH is caused by heterozygous and NSHPT by homozygous inactivating CASR mutations (6). There are, however, exceptions from this rule. Homozygous CASR mutations leading to only a mild overall impairment of extracellular calcium sensing may cause a FHH-like phenotype (8, 12, 13, 14), whereas heterozygous CASR mutations causing more pronounced functional impairment can lead to neonatal hyperparathyroidism (NHPT) (15, 16, 17). Recently it has been found that inactivating mutations in the CASR associated G protein alpha 11 (GNA11) and in the adaptor-related protein complex 2 sigma 1 subunit (AP2S1) can also cause familial hypocalciuric hypercalcemia (FHH types 2 and 3) (18, 19, 20).

Mutations causing gain of CASR function

Gain of function mutations that cause CASR activation by lower extracellular calcium levels lead to a hypocalcemic disease called autosomal dominant hypocalcemia (ADH). ADH patients have mild to moderate hypocalcemia with low to normal PTH levels and an inappropriately normal or high calcium excretion (21). Patients suffer from symptoms of hypocalcemia, develop tissue calcifications especially in the brain and kidney (22, 23, 24) and sometimes show defects in bone mineralization (25). A single mutated CASR allele suffices to produce ADH and there is only one case in the literature with a homozygous activating CASR mutation (26). An almost identical phenotype is caused by activating mutations of the small G protein alpha 11 (ADH type 2) (18, 27, 28).

There is a subgroup of five activating mutants of the CASR that in addition to hypocalcemia and relative hypercalciuria lead to renal loss of sodium, chloride, and magnesium, which results in hyperreninemia, hyperaldosteronism, hypokalemia, and metabolic alkalosis, a disease called Bartter’s syndrome type 5 (BS type 5) (29, 30, 31, 32, 33, 34). These patients usually are already symptomatic as infants or children, but some patients continue into adulthood with relatively little symptoms (31). Most patients require continuous medical care and may experience rapid changes of blood electrolyte levels in stress situations that may require intravenous treatment (35). The molecular basis for these distinct clinical phenotypes caused by gain of function CASR mutants is unknown.

CASR as a drug target for allosteric modulators

Even before the CASR was cloned in 1993 it was identified as a potential drug target (36, 37). The CASR is one of the first GPCRs for which an allosteric activator, the calcimimetic cinacalcet, has been developed and approved for therapeutic use in humans (37, 38, 39, 40) to treat primary and secondary hyperparathyroidism (41, 42). In addition, cinacalcet has been used for treatment of hyperparathyroidism related to hereditary vitamin D-resistant rickets (43) and X-linked hypophosphatemia (44). Allosteric CASR inhibitors called calcilytics (45, 46, 47) are currently investigated as a way to stimulate endogenous PTH secretion for the treatment of osteoporosis (48, 49, 50) and they have been proposed as a novel therapeutic strategy to treat allergic asthma (51), pulmonary hypertension (52) and Alzheimer’s disease (53).

Allosteric modulators (54) do not directly activate or inhibit their target receptors or abolish physiologic regulation through endogenous ligands but rather enhance or attenuate the receptor’s response to its endogenous ligand. Soon it was, therefore, proposed that these compounds could correct the molecular defect caused by inactivating or activating CASR mutations and thus might be useful in the treatment of associated diseases (41, 42, 55, 56).

In this review we compile current treatment concepts of diseases caused by inactivating and activating mutations of the CASR, GNA11, and AP2S1 genes with special emphasis on allosteric CASR modulators.

Therapy of patients with mutations causing loss of CASR function

Familial hypocalciuric hypercalcemia

FHH is generally considered to be a benign disease. Although, the CASR is expressed in many tissues, most
patients are asymptomatic and only show changes in laboratory parameters indicating that calcium homeostasis equilibrates at higher levels of serum Ca and PTH. It is therefore general consensus, that FHH patients do not require treatment if the only abnormalities are mild elevations of serum calcium and PTH. As FHH may be confused with primary hyperparathyroidism, it is of prime clinical concern to correctly diagnose FHH in order to avoid futile parathyroid surgery. Correct diagnosis, however, may be masked by severe vitamin D deficiency (8). In any case the presence of a CASR, GNA11, or AP2S1 mutation confirmed by sequencing of the respective gene and with proven functional impairment is required for making a definitive diagnosis (57, 58).

Some FHH patients are symptomatic and may suffer, for example, from recurrent episodes of pancreatitis that improve upon treatment (59). Soon after the calcimimetic cinacalcet was available, it was speculated that symptomatic FHH patients could benefit from cinacalcet therapy (Table 1). Currently there are 12 FHH type 1 patients and four patients with FHH type 3 reported who were treated with cinacalcet (Table 1). There are no reports about cinacalcet treatment of FHH type 2 patients (inactivating GNA11 mutations).

As shown in Table 1 the reasons to initiate cinacalcet were hypercalcemia (n = 6), presumed pHPT (n = 3), pancreatitis (n = 2), poor wound healing and calcium deposits after tympanoplasty (n = 1), psychosis and osteoporosis (n = 1), muscle cramps, aches and poor memory (n = 1), muscle cramps, aches, poor memory, paraesthesia and osteoporosis (n = 1), dental tartar, vertigo, and constipation (n = 1). Symptoms resolved or significantly improved in ten out of 12 patients with FHH type 1 and in all four patients with FHH type 3 (Table 1). In the ten responsive FHH type 1 patients the final daily doses of cinacalcet were between 30 and 90 mg, and between 30 and 60 mg in the FHH type 3 patients. The weight adjusted daily cinacalcet dose in one responsive adult patient was 0.45 mg/kg body weight. Patients were treated with cinacalcet for up to 3 years and therapy was generally well tolerated. Adverse effects were reported in two responsive patients (paresthesia and eye palpitations) and in an unresponsive one (hypotension and nausea). The adverse effects subsided after dose reduction or discontinuation of therapy respectively (Table 1) and they were within the spectrum of adverse drug reactions known from patients treated for primary hyperparathyroidism (60, 61). According to these published cases, cinacalcet appears to be an effective and well-tolerated medical treatment option in symptomatic patients with FHH type 1 and 3. Whether cinacalcet is also efficacious in FHH type 2 patients is currently unknown.

Treatment of FHH type 1 patients with cinacalcet will be only effective if the calcimimetic is able to enhance the function of the mutated CASR protein. Currently about one third of all inactivating CASR mutants have been tested for in vitro sensitivity to calcimimetics and the majority of mutants appear sensitive (see below). The three known mutants of AP2S1 all seem to be sensitive to upstream augmentation of the WT CASR with cinacalcet (Table 1). Usually there is no need for urgent therapy in FHH patients. Even if the sensitivity of a particular patient or mutant is unknown there is enough time for a therapeutic trial to test whether patients respond to treatment. Two reports studied the dynamics of calcium and PTH after a single oral dose of cinacalcet in FHH type 1 patients. A significant decrease of PTH was detected as early as 2 h and effects on serum calcium were seen 8–48 h after the administration of cinacalcet (59, 62, 63). If clinically relevant the rapid effect of cinacalcet on PTH may be used to quickly assess the effectiveness of cinacalcet in individual patients (see below).

In summary, we suggest treatment of FHH patients with a calcimimetic as first choice, if they suffer from symptoms, which are likely to be related to hypercalcemia or dysfunction of CASR, GNA11, or AP2S1. Remarkably, the range of symptoms reported in the literature cover a broad range (Table 1). Therefore, a therapeutic trial may be considered in ambiguous cases. At present cinacalcet is the only available calcimimetic. Like in other indications cinacalcet should be started with a low dose and up-titrated according to its effects on serum calcium, PTH, and on symptomatology. In one case recurrent pancreatitis only resolved after serum calcium had reached levels in the lower end of the reference range (64). At the beginning of therapy and after dose adjustments serum calcium should be monitored at short intervals until a steady state level for serum calcium has been reached. This is important as cinacalcet seems to have a more protracted and cumulative effect on serum calcium despite its rather short-lived effect on PTH levels. Long-term treatment appears to be safe. If despite, normalization of calcium metabolism, symptoms do not resolve, the link has to be questioned and therapy should be abandoned.

**Neonatal hyperparathyroidism**

Patients with NHPT are typically symptomatic already as neonates. Hypercalcemia leads to poor feeding, dehydration, and lethargy, while PTH excess causes skeletal...
Table 1  Clinical and biochemical data of FHH1 and FHH3 patients treated with cinacalcet.

<table>
<thead>
<tr>
<th>Phenotype Genotype</th>
<th>Reason to treat</th>
<th>Diagnosis</th>
<th>Cinacalcet therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Age at 1st symptoms</td>
<td>S-Ca (mM)</td>
</tr>
<tr>
<td>FHH1 (CASR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R220W/wt</td>
<td>Recurrent pancreatitis</td>
<td>37y</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Muscle cramps and aches, poor memory, osteoporosis</td>
<td>51y</td>
<td>3.0</td>
</tr>
<tr>
<td>R220W/wt</td>
<td>Muscle cramps and aches, poor memory osteoporosis</td>
<td>57y</td>
<td>3.1</td>
</tr>
<tr>
<td>R220W/wt</td>
<td>Hypercalcemia</td>
<td>35y</td>
<td>3.0</td>
</tr>
<tr>
<td>R220Q/wt</td>
<td>Poor wound healing and calcium deposits after tympanoplasty</td>
<td>6y</td>
<td>3.2</td>
</tr>
<tr>
<td>R465Q/wt</td>
<td>Presumed pHPT</td>
<td>44y</td>
<td>2.8</td>
</tr>
<tr>
<td>C568Y/wt</td>
<td>Pancreatitis</td>
<td>22y</td>
<td>2.8</td>
</tr>
<tr>
<td>C582N/wt</td>
<td>Presumed pHPT</td>
<td>44y</td>
<td>3.1</td>
</tr>
<tr>
<td>G613R/wt</td>
<td>Tartar, vertigo and constipation</td>
<td>53y</td>
<td>3.1</td>
</tr>
<tr>
<td>F809L/wt</td>
<td>Psychosis, osteoporosis</td>
<td>26y</td>
<td>3.0</td>
</tr>
<tr>
<td>T972M/wt</td>
<td>Presumed pHPT</td>
<td>68y</td>
<td>2.9</td>
</tr>
<tr>
<td>FHH3 (AP2S1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R15C/wt</td>
<td>Hypercalcemia</td>
<td>NR</td>
<td>EL</td>
</tr>
<tr>
<td>R15N/wt</td>
<td>Hypercalcemia</td>
<td>NR</td>
<td>EL</td>
</tr>
<tr>
<td>R15L/wt</td>
<td>Hypercalcemia</td>
<td>NR</td>
<td>EL</td>
</tr>
<tr>
<td>R15S/wt</td>
<td>Hypercalcemia</td>
<td>2y</td>
<td>2.9</td>
</tr>
</tbody>
</table>

FHH1, familial hypocalciuric hypercalcaemia type 1; FHH3, familial hypocalciuric hypercalcaemia type 3; CASR, calcium-sensing receptor; AP2S1, adaptor-related protein complex 2 sigma 1 subunit; pHPT, primary hyperparathyroidism; diagnosis, parameters at the time when the diagnosis was made; age at first symptoms, age at which specific disease manifestations of FHH were documented; y, years; m, months; w, weeks; d, days; S-Ca, total serum calcium; (ion) denotes ionized serum calcium; S-PTH, serum parathyroid hormone; S-PTH min, minimal S-PTH after therapy; decrease of S-Ca; Y, yes, N, no; *, mutants tested sensitive in vitro (see Table 4); minimal effective dose, minimal dose at which a decrease of serum calcium or PTH was reported; †, maximum dose used in patients without response; EL, elevated; NR, not reported.
demineralization, instability of the rib cage and respiratory problems (6). In NSHPT patients with a homozygous CASR mutation hypercalcemia is severe (>4 mM) and newborns are critically ill. Heterozygous NHPT patients, by contrast, usually have lower serum calcium levels of approximately 3 mM and may only be detected during investigation of developmental deficits in the 1st weeks of life. In homozygous patients both parents usually have FHH, while heterozygous mutations are inherited from one parent or arise de novo. Thus, measurement of serum calcium and PTH in both parents may rapidly give important cues about the mode of inheritance.

The clinical need to treat PTH excess and severe hypercalcemia in homozygous NSHPT patients is obvious; although, in rare cases spontaneous transition into a FHH phenotype has been reported (65). Parathyroidectomy is generally considered the definitive treatment of choice but serum calcium should be lowered prior to surgery. General measures, however, like hydration or forced diuresis and calcitonin do not yield clinically sufficient responses in both variants of neonatal hyperparathyroidism (15, 66, 67, 68, 69). Restriction of calcium intake is also mostly ineffective and further aggravates total body calcium deficiency in NSHPT and NHPT patients (70, 71, 72, 73).

**Bisphosphonates**

Bisphosphonates are well established for the treatment of hypercalcemia. There are reports about eight NSHPT and three NHPT patients who received pamidronate to lower serum calcium. Nine patients were treated preoperatively (Table 2). In all patients serum calcium could be lowered and improvement of the overall clinical situation of the patients was reported in four patients (Table 2). The pamidronate doses used were 0.5–1 mg/kg body weight or 20 mg/m² body surface area. The minimal time until a reduction in serum calcium was observed was 12 h and the maximum effect was achieved after 2–3 days. Pamidronate was well tolerated but hypocalcemia developed in one patient (15). Overall pamidronate appears well suited to lower serum calcium and to stabilize NSHPT patients prior to parathyroidectomy.

**Calcimimetics (cinacalcet)**

To date there are reports about 4 NSHPT and 4 NHPT patients with five distinct genotypes who had been treated with cinacalcet (Table 3). Symptomatic hypercalcemia was the reason to commence therapy in all cases. Cinacalcet was effective in one NSHPT and all four NHPT patients.

All four NHPT patients had a heterozygous R185Q mutation and as in FHH patients a rapid fall in PTH preceding the decrease in serum calcium was observed after cinacalcet (15, 16). One patient showed a significant but temporary drop of ionized serum calcium by 0.4 mmol/l 12 h after a single dose of 20 mg/m² cinacalcet (15) and (Table 3). The other patients had gradual but constant decreases in total serum calcium after daily doses of 0.4–1 mg/kg per day of cinacalcet (Table 3). In one patient total serum calcium fell to 1.9 mmol/l after 3 days of cinacalcet, which had to be paused to prevent further hypocalcemia (16). None of the four heterozygous NHPT patients treated with cinacalcet required parathyroid surgery (Table 3). Under cinacalcet treatment all patients showed normal or catch-up growth and development for up to 32 months (Table 3), amongst them one patient who failed to thrive under previous pamidronate therapy (15 and Table 2).

One NSHPT patient with a homozygous missense mutation (R69H) suffered from recurrent hypercalcemia despite multiple attempts to remove all parathyroid tissue and treatment with pamidronate (74 and Table 2). At the age of 6 years cinacalcet 30 mg/day was initiated and a rapid drop in serum PTH and calcium like in FHH and NHPT patients was observed (74). At a final dose of 90 mg/day at the age of 10 serum calcium remained somewhat above the upper limit of normal and growth and general development were appropriate (Table 3). Cinacalcet was well tolerated and there were no signs for loss of effectiveness over time.

Two NSHPT patients with homozygous deletions leading to frameshifts in the CASR gene were unresponsive to cinacalcet and underwent parathyroidectomy. Finally, a patient from North Africa with severe developmental deficits and hypercalcemia at birth was diagnosed with a homozygous missense mutation (R680H) at the age of 3 years (75). At that time cinacalcet of up to 2.5 mg/kg per day had no effect on PTH or serum calcium. The patient was, therefore, treated with pamidronate and underwent total parathyroidectomy (Table 3).

The final doses of cinacalcet in responsive infants and children covered a wide range and were between 0.4 and 2 mg/kg per day. This is higher than the 0.25 mg/kg per day used to treat secondary hyperparathyroidism in children with end-stage renal disease, but is in line with doses given in adults (30–90 mg/day correspond to 0.4–1.3 mg/kg per day for an adult weighing 70 kg) (76). Most likely the cinacalcet dosages needed to control hypercalcemia differ for each CASR mutant. The right dose of cinacalcet is given, if serum calcium and PTH are close to or within the normal range. The rapid pharmacokinetic profile suggests that taking cinacalcet in divided
### Table 2: Clinical and biochemical data of NSHPT and NHPT patients treated with pamidronate.

<table>
<thead>
<tr>
<th>Phenotype Genotype</th>
<th>Reason to treat</th>
<th>Diagnosis</th>
<th>Pamidronate therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSHPT (CASR) R69H</td>
<td>Persistent HPT after multiple PTx</td>
<td>7d</td>
<td>8.3</td>
</tr>
<tr>
<td>c.222_226delGATAT del/frameshift R680H</td>
<td>Hypercalcemia, pre PTx</td>
<td>2d</td>
<td>4.8</td>
</tr>
<tr>
<td>R227X Hypercalcemia, pre PTx</td>
<td>7d</td>
<td>6.0</td>
<td>573</td>
</tr>
<tr>
<td>Q164X Hypercalcemia, pre PTx</td>
<td>14d</td>
<td>8.1</td>
<td>263</td>
</tr>
<tr>
<td>R680C/G509F Hypercalcemia, pre PTx</td>
<td>3d</td>
<td>5.1</td>
<td>155</td>
</tr>
<tr>
<td>NSHPT (CASR) G509R/R554X Hypercalcemia, pre PTx</td>
<td>14d</td>
<td>3.4</td>
<td>1046</td>
</tr>
<tr>
<td>NHPT (CASR) R185Q/wt Hypercalcemia</td>
<td>7d</td>
<td>3.1</td>
<td>663</td>
</tr>
<tr>
<td>R185Q/wt Hypercalcemia, pre PTx</td>
<td>1d</td>
<td>3.3–3.6</td>
<td>563</td>
</tr>
<tr>
<td>R220W/wt Hypercalcemia, pre PTx</td>
<td>1d</td>
<td>3.1</td>
<td>2330</td>
</tr>
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</table>

NSHPT, neonatal severe hyperparathyroidism; NHPT, neonatal hyperparathyroidism; CASR, calcium-sensing receptor; pre PTx, before parathyroidectomy; diagnosis, parameters at the time when the diagnosis was made; age at first symptoms; age at which specific disease manifestations of NSHPT or NHPT were documented; y, years; m, months; w, weeks; d, days; S-Ca, total serum calcium; (ion), denotes ionized serum calcium; S-PTH, serum parathyroid hormone; minimal effective dose, dose at which a significant drop in serum calcium or PTH was reported; NR, not reported.
doses might be advantageous. Consistently, most patients receiving more than 30 mg/day were given cinacalcet in two or three daily doses (Tables 1 and 3).

As mentioned above, cinacalcet may have a delayed and prolonged effect on serum calcium. Therefore, serum calcium should be monitored at short intervals at the beginning of therapy and after dose adjustments until a steady state level for serum calcium has been reached. Hypocalcemia was the only adverse effect reported in the five patients successfully treated with cinacalcet (Table 3). In the other three patients, who were unresponsive to cinacalcet, no side effects have been reported (Table 3). Recently, pediatric clinical trials of cinacalcet were suspended after the death of a 14-year-old patient (77). No specific information as to the circumstances of this case were disclosed, but the FDA advised health care professionals to pay specific attention to hypocalcemia and the most common side effects of cinacalcet in adults like nausea, vomiting, and diarrhea (77). As most patients with NHPT suffer from under-mineralized bone and total body calcium deficiency a sufficient supply of calcium and vitamin D should be ensured when serum calcium is under control.

For many future cases of NSHPT or NHPT, the particular genotype will not be known in time, not to mention the unknown sensitivity of that particular mutant to calcimetics in vivo or in vitro (see below). However, the rapid and thorough PTH levels seem to coincide between 4 and 6 h after administration of PTH or PHPT levels (15, 59, 76, 28, 79). Therefore, a cinacalcet test with measurement of PTH before and 6 h after a 2 mg/kg dose of 2 mg/kg cinacalcet may be a good predictor of its effectiveness on serum calcium in an individual patient.

Parathyroidectomy. Parathyroidectomy is highly successful in acutely lowering PTH and serum calcium. Both total and subtotal parathyroidectomy has been performed in NSHPT patients (11). After subtotal parathyroidectomy, recurrence of hyperparathyroidism is common (11). After subtotal parathyroidectomy and a review of 36 NSHPT cases in 1991 reported a high frequency of recurrence of hyperparathyroidism (14) and a reviewing of 36 NSHPT cases in 1991, reported a high frequency of recurrence of hyperparathyroidism (11). In some cases total parathyroidectomy has been combined with autotransplantation of parathyroid tissue. This, however, also carries a high risk of recurrence of hyperparathyroidism, and for many future cases of NSHPT or NHPT, the particular genotype will not be known in time, not to mention the unknown sensitivity of that particular mutant to calcimetics in vivo or in vitro (see below). However, the rapid and thorough PTH levels seem to coincide between 4 and 6 h after administration of PTH or PHPT levels (15, 59, 76, 28, 79). Therefore, a cinacalcet test with measurement of PTH before and 6 h after a 2 mg/kg dose of 2 mg/kg cinacalcet may be a good predictor of its effectiveness on serum calcium in an individual patient.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Diagnosis</th>
<th>Reason to treat</th>
<th>Age at 1st symptoms</th>
<th>S-Ca (mM)</th>
<th>S-PTH (pg/ml)</th>
<th>Decrease of S-Ca</th>
<th>Age at therapy</th>
<th>Minimal effective dose</th>
<th>S-Ca before (mg/day)</th>
<th>S-Ca min after (mg/day)</th>
<th>S-PTH before (pg/ml)</th>
<th>S-PTH min after (pg/ml)</th>
<th>Treatment duration</th>
<th>Adverse effects</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>NSHPT (CASR)</td>
<td>Hypercalcemia</td>
<td>7d</td>
<td>8.3</td>
<td>900.9</td>
<td>Y</td>
<td>6y</td>
<td>30 mg/day</td>
<td>3.2</td>
<td>3.0</td>
<td>84</td>
<td>55.7</td>
<td>4y</td>
<td>None</td>
<td>(74)</td>
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<tr>
<td>R69H c.222,226 GTAT</td>
<td>Hypercalcemia</td>
<td>2d</td>
<td>4.8</td>
<td>1096.0</td>
<td>N</td>
<td>28d</td>
<td>90 mg/m² per day*</td>
<td>2.5</td>
<td>5.0</td>
<td>80</td>
<td>55.7</td>
<td>17d</td>
<td>None</td>
<td>(66)</td>
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<td>R680H c.1392,1404 delTAT</td>
<td>Hypercalcemia</td>
<td>23d</td>
<td>5.8</td>
<td>518.0</td>
<td>N</td>
<td>2m</td>
<td>20 mg/m² per day*</td>
<td>2.6</td>
<td>2.9</td>
<td>282</td>
<td>257</td>
<td>7d</td>
<td>None</td>
<td>(67)</td>
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<td>NHPT (CASR)</td>
<td>Hypercalcemia</td>
<td>After birth</td>
<td>6.2</td>
<td>1042.0</td>
<td>N*</td>
<td>3y</td>
<td>2.5 mg/kg per day*</td>
<td>1.6</td>
<td>1.0</td>
<td>1091</td>
<td>98</td>
<td>17m</td>
<td>None</td>
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<td>R185Q/wt</td>
<td>Hypercalcemia</td>
<td>7d</td>
<td>3.1</td>
<td>663.0</td>
<td>Y*</td>
<td>23d</td>
<td>4 mg/day</td>
<td>3.2</td>
<td>1.9</td>
<td>700</td>
<td>80</td>
<td>81m</td>
<td>Hypocalcemia</td>
<td>(16)</td>
<td></td>
</tr>
<tr>
<td>R185Q/wt</td>
<td>Hypercalcemia</td>
<td>2d</td>
<td>3.2</td>
<td>1154.0</td>
<td>Y*</td>
<td>10d</td>
<td>0.4 mg/kg per day</td>
<td>3.2</td>
<td>1.9</td>
<td>700</td>
<td>80</td>
<td>81m</td>
<td>Hypocalcemia</td>
<td>(16)</td>
<td></td>
</tr>
<tr>
<td>R185Q/wt</td>
<td>Hypercalcemia</td>
<td>After birth</td>
<td>4w</td>
<td>3.3</td>
<td>76</td>
<td>Y*</td>
<td>4m</td>
<td>1–2.3 mg/kg per day</td>
<td>3.2</td>
<td>2.6–3.0</td>
<td>76</td>
<td>80</td>
<td>32m</td>
<td>Hypocalcemia</td>
<td>(73)</td>
</tr>
<tr>
<td>R185Q/wt</td>
<td>Hypercalcemia</td>
<td>After birth</td>
<td>3.2</td>
<td>152</td>
<td>84</td>
<td>Y*</td>
<td>13m</td>
<td>Decreased</td>
<td>1–2.3 mg/kg per day</td>
<td>3.2</td>
<td>2.6–3.0</td>
<td>76</td>
<td>80</td>
<td>32m</td>
<td>Hypocalcemia</td>
</tr>
</tbody>
</table>

NSHPT, neonatal severe hyperparathyroidism; NHPT, neonatal hyperparathyroidism; CASR, calcium-sensing receptor; AP2S1, adaptor-related protein complex 2 sigma 1 subunit; diagnosis parameters at the time when the diagnosis was made; age at 1st symptoms; age at which specific disease manifestations of NSHPT or NHPT were documented; y, years; m, months; w, weeks; d, days; S-Ca, total serum calcium; (ion) denotes ionized serum calcium; S-PTH, serum parathyroid hormone; S-PTH min, minimal S-PTH after therapy; decrease of S-Ca; Y, yes; N, no; *, mutants tested sensitive in vitro (see Table 4); minimal effective dose, minimal dose at which a decrease of serum calcium or PTH was reported; *, maximum dose used in patients without response; NR, not reported.
parathyroidectomy is the preferred approach which however results in hypoparathyroidism.

**Management of hypocalcemia after parathyroidectomy** ► Vitamin D and calcium supplementation is well established to treat hypocalcemia in patients with postoperative hypoparathyroidism. This also works in patients with NSHPT after surgery. In the early postoperative period very high doses of calcium might be required to maintain appropriate serum calcium levels. This is presumably because of total body calcium deficiency and of a shift of large amounts of calcium into under-mineralized bone after correction of severe hyperparathyroidism. On the long term there are no reports about difficult to treat hypocalcemia after parathyroidectomy for NSHPT. The available information, however, is scarce and in most reports there is rather little detail about the vitamin D compounds and their doses used. This may be taken as an indication that inactive as well as active vitamin D compounds may work equally well, although we would prefer calcitriol or alfacalcidol. In NSHPT patients calcium sensing is severely impaired in all tissues that physiologically express CASR. Hypocalciuria caused by mutant CASR may actually facilitate the treatment of postoperative hypoparathyroidism in NSHPT due to a lower net-loss of calcium via the urine and may protect from renal complications often observed in patients with WT CASR treated for the same condition (83).

An unresolved issue is the serum calcium level one should aim for in these patients with a ubiquitously and severely impaired CASR function. As patients’ compliance appears rather low, the patients’ own therapeutic goal seems to be the absence of hypocalcemic symptoms (82). Studies of NSHPT kindred frequently report mental impairment in adults (13, 75, 82, 84). This may reflect detrimental effects of mutated CASR itself on intrauterine and postnatal development and on body functions. Severe absolute or relative hypocalcemia after parathyroidectomy may also contribute to the impairment of physical and mental development and function (13, 75, 82, 84). Whether serum calcium levels within or even above the normal range would be more advantageous is an open question. Long-term outcome data beyond anecdotal reports from parathyroidectomized NSHPT patients are lacking, which could provide clues to answer this question.

**Therapeutic strategy and summary** ► The ultimate therapeutic goal is normalization of calcium homeostasis, which involves not only lowering serum calcium levels, but also correcting the general calcium deficiency indicated by skeletal demineralisation. Ideally, this is achieved by correcting the molecular CASR defect throughout the organism which appears possible in a subset of NSHPT and NHPT patients by medical treatment with the calcimimetic cinacalcet. In many NSHPT patients, however, parathyroidectomy is necessary to control disease manifestations. Depending on the surgical approach postoperative hypoparathyroidism or recurrent or persistent HPT may develop. If the clinical situation allows for a preoperative trial of cinacalcet, this may help to determine the optimal surgical strategy. Whenever cinacalcet fails to lower serum calcium, we suggest a total parathyroidectomy and postoperative calcitriol or alfacalcidol and calcium supplementation. If, however, hypercalcemia and hyperparathyroidism can be controlled by cinacalcet, this may allow for a less aggressive approach with subtotal parathyroidectomy or even offers a non-surgical therapeutic option, particularly in NHPT patients with only moderately elevated serum calcium levels. Nevertheless, it has to be taken into account that cinacalcet is not approved for use in children under the age of 18. Therefore, the decision to initiate cinacalcet therapy in infants and children has to be made on an individual basis and with strict clinical monitoring for potential adverse effects is mandatory (77).

**Effect of calcimimetics in vitro on mutations causing loss of CASR function**

Currently the CASR database (www.casrdb.mcgill.ca) lists 138 distinct inactivating CASR mutations causing FHH, NSHPT or familial isolated hyperparathyroidism (FIHP) (85). Forty-nine, about one third of all naturally occurring inactivating CASR mutations have been tested for in vitro sensitivity to calcimimetics (Table 4). Nine artificially engineered inactivating CASR mutations have been tested as well and were included in Table 4 for reference, in case one of these mutants will occur in a future patient. The vast majority (84%) of the naturally occurring inactivating CASR mutants were sensitive to calcimimetics in vitro. The remaining eight insensitive CASR mutants comprise six missense and two nonsense mutations with premature stops codons. For five amino acids (L159, Y218, R227, G670, R680) more than one naturally occurring amino acid exchanges were described (Table 4). This, however, affected the sensitivity to calcimimetics only for the amino acid L159 (Table 4). Several CASR mutants were tested by different research groups for their signaling activity with concordant results.

In most in vitro studies mutant CASR was tested in the absence of WT CASR. This resembles the homozygous
Table 4  *In vitro* sensitivity of inactivating mutants of the CASR, GNA11, and AP2S1 to calcimimetics. The cells used to perform these functional assays were HEK 293 cells for every report except (109) where the Flp-In TREx HEK 293 variant was used. The concentration given is the minimal effective concentration at which an increase in signaling activity was reported. In unresponsive mutants the maximum test concentration used is given.

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Sensitivity</th>
<th>Calcimimetic</th>
<th>Concentration</th>
<th>Assay</th>
<th>References</th>
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<td>ERK1/2-P</td>
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<td>R66C</td>
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<td>(107)</td>
</tr>
<tr>
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<td>(110)</td>
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</tr>
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<td>(88)</td>
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<td>R227Q</td>
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<td>[Ca$^{2+}$];</td>
<td>(109)</td>
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<td>ERK1/2-P</td>
<td>(107)</td>
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<td>R680C</td>
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<td>(75)</td>
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<td>V689M</td>
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<td>M734R</td>
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<td>M734R</td>
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<td>(109)</td>
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<td>G778D</td>
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<td>(109)</td>
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<td>R795W</td>
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<td>[Ca$^{2+}$];</td>
<td>(123)</td>
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<tr>
<td>R795W</td>
<td>Y</td>
<td>NPS R568</td>
<td>10 μM</td>
<td>ERK1/2-P</td>
<td>(107)</td>
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<td>P798T</td>
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<td>NPS R568</td>
<td>10 μM</td>
<td>ERK1/2-P</td>
<td>(88)</td>
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</table>
situation usually encountered in NSHPT patients. FHH patients, by contrast, are mostly heterozygous and express both WT and mutant CASR. So far, only six CASR mutants have been tested \textit{in vitro} when co-expressed with WT CASR and all were sensitive to calcimimetic treatment including the truncation mutant R648X. This supports the proposed notion that even if the CASR mutant itself may not be responsive to calcimimetics, the remaining normal CASR allele in FHH or in heterozygous NHPT could confer sensitivity to calcimimetic treatment \textit{in vivo} (86). In addition, calcimimetics may act as pharmacological chaperones to rescue misfolded inactivating mutants (87, 88, 89). The polymorphic variants A986S and R990G occur in about 24% and 4% of healthy adults respectively and lead to slight changes in ionized serum calcium levels (90). Due to their high prevalence, they can occur as compound heterozygous or homozygous variants together with CASR mutations causing FHH, NHPT or NSHPT (91). \textit{In vitro} data indicate that the presence of these polymorphisms should not preclude sensitivity to calcimimetics in such situations (92 and unpublished results). Taken together, based on \textit{in vitro} data, the majority of CASR mutants appear responsive to calcimimetics and hence these compounds may offer a therapeutic option for the majority of FHH and NSHPT patients.

### Correlation between \textit{in vitro} and \textit{in vivo} sensitivity of inactivating CASR mutants to calcimimetics

Five of the 13 CASR mutants from patients with FHH or NSHPT, who were treated with cinacalcet, have been tested \textit{in vitro}. Three CASR mutants were from FHH patients and all mutants were responsive to calcimimetics \textit{in vitro}. One CASR mutant (CS82R), however, showed no \textit{in vivo} response to cinacalcet treatment despite positive \textit{in vitro} results (Tables 1 and 4). Likewise, the two CASR mutants from NSHPT patients, a heterozygous R185Q and a homozygous R680H one, were reported to be sensitive to

<table>
<thead>
<tr>
<th>Mutant</th>
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<th>Calcimimetic</th>
<th>Concentration</th>
<th>Assay</th>
<th>References</th>
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<td>([Ca^{2+}]); IP3</td>
<td>(122)</td>
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<td>V817I</td>
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<td>NPS R568</td>
<td>10 (\mu M)</td>
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<td>(126)</td>
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<td>([Ca^{2+}])</td>
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**Table 4 Continued.**

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<th>Calcimimetic</th>
<th>Concentration</th>
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<td>NPS R568</td>
<td>1 (\mu M)</td>
<td>IP3</td>
<td>(127)</td>
</tr>
<tr>
<td>H766A</td>
<td>Y</td>
<td>NPS R568</td>
<td>1 (\mu M)</td>
<td>IP3</td>
<td>(127)</td>
</tr>
<tr>
<td>P823A</td>
<td>Y</td>
<td>NPS R568</td>
<td>1 (\mu M)</td>
<td>IP3</td>
<td>(108)</td>
</tr>
<tr>
<td>EB37A</td>
<td>N</td>
<td>NPS R568</td>
<td>1 (\mu M)</td>
<td>IP3</td>
<td>(127)</td>
</tr>
<tr>
<td>A877Stop</td>
<td>Y</td>
<td>NPS R467</td>
<td>1 (\mu M)</td>
<td>([Ca^{2+}])</td>
<td>(123)</td>
</tr>
</tbody>
</table>

**Artificial inactivating CASR variants**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Sensitivity</th>
<th>Calcimimetic</th>
<th>Concentration</th>
<th>Assay</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>L135Q</td>
<td>Y</td>
<td>Cinacalcet</td>
<td>20 nM</td>
<td>([Ca^{2+}])</td>
<td>(93)</td>
</tr>
<tr>
<td>I200del</td>
<td>Y</td>
<td>Cinacalcet</td>
<td>40 nM</td>
<td>([Ca^{2+}])</td>
<td>(93)</td>
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</table>

**Naturally occurring inactivating GNA11 mutants**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Sensitivity</th>
<th>Calcimimetic</th>
<th>Concentration</th>
<th>Assay</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>R15C</td>
<td>Y</td>
<td>Cinacalcet</td>
<td>10 nM</td>
<td>([Ca^{2+}])</td>
<td>(94)</td>
</tr>
<tr>
<td>R15H</td>
<td>Y</td>
<td>Cinacalcet</td>
<td>10 nM</td>
<td>([Ca^{2+}])</td>
<td>(94)</td>
</tr>
<tr>
<td>R15L</td>
<td>Y</td>
<td>Cinacalcet</td>
<td>10 nM</td>
<td>([Ca^{2+}])</td>
<td>(94)</td>
</tr>
</tbody>
</table>

### CASR, calcium-sensing receptor; GNA11, G protein alpha 11; AP2S1, adaptor-related protein complex 2 sigma 1 subunit; sensitivity, increase in signaling activity; Y, yes; N, no; \([Ca^{2+}]\), measurement of intracellular free calcium; IP3, inositol 1,4,5-trisphosphate accumulation assay; ERK1/2-P, anti-extracellular-signal-regulated kinase 1 and 2 phosphorylation specific western blot; SRE-Luc, serum response element luciferase reporter assay; PI, phosphoinositide hydrolysis assay.
calcimimetics in vitro, but only the patients with the heterozygous R185Q CASR mutation responded to cinacalcet with a decrease in serum calcium in vivo. All three AP2S1 mutations causing FHH type 3 were responsive in vitro and in vivo (Tables 1 and 4). Discrepancies between in vivo and in vitro results may have multiple reasons ranging from genetic to environmental factors. Explanations could be that the ambient calcium concentration required to confer an effect of calcimimetic treatment on mutated CASR function, is too high to be of clinical relevance or that the cinacalcet concentrations used for in vitro testing are not achievable in patients in vivo. For example, pharmacokinetic studies show that after single oral doses of 25–100 mg of cinacalcet peak plasma concentrations range between 14 and 70 nM (5–25 ng/ml) in patients with chronic renal failure (78) and between 34 and 120 nM (12–43 ng/ml) in male healthy subjects (79). Most in vitro experiments, however, used cinacalcet concentrations well in excess. Overall the proportion of FHH patients responsive to cinacalcet in vivo (83%) and of CASR mutants in vitro (84%) is high and strikingly similar. As publication bias, however, cannot be ruled out with negative results (84%) is high and strikingly similar. As publication bias, however, cannot be ruled out with negative results, the true proportion of FHH and/or NSHPT patients treatable with cinacalcet may be lower.

### Therapy of patients with mutations causing gain of CASR function

ADH patients may be asymptomatic and hypocalcemia is only discovered incidentally (22). Hypocalcemia, however, may cause muscle weakness, cramps, seizures and cardiac arrhythmias, which require immediate therapy (22, 24, 95, 96, 97, 98, 99) and chronically may lead to cataract formation and calcification of the basal ganglia (24, 98, 99, 100, 101). The severity of neurological symptoms appears to correlate with the degree of hypocalcemia (99). Relative hypercalcuria is another feature, which is present in almost all ADH patients (21, 100) and poses them at risk for nephrocalcinosis, nephrolithiasis and renal failure. Besides chronic hypocalcemia and relative hypercalcuria, low or even undetectable PTH levels may also contribute to some of the pathophysiological consequences of ADH (56). In principle, one would like to correct the underlying pathophysiological mechanism to prevent or ameliorate these risks even in patients who are seemingly asymptomatic. None of the currently available therapies, however, meets this criterion.

#### Table 5  Clinical and biochemical data of ADH and Bartter's syndrome type 5 patients treated with recombinant PTH1-34

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Diagnosis</th>
<th>Age at 1st symptoms</th>
<th>Therapy (mg/mg)</th>
<th>S-Ca before (mM)</th>
<th>S-Ca after therapy (mM)</th>
<th>S-Po4 before (mM)</th>
<th>S-Po4 after therapy (mM)</th>
<th>Adverse effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADH, CASR</td>
<td>C131S/wt</td>
<td>HC, NC</td>
<td>6y</td>
<td>3.8</td>
<td>1.1</td>
<td>2.0</td>
<td>1.5</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>ADH, CASR</td>
<td>T151M/wt</td>
<td>HC, cramps, paroxysms</td>
<td>2y</td>
<td>2.1</td>
<td>1.0</td>
<td>1.9</td>
<td>1.0</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>ADH, CASR</td>
<td>P221L/wt</td>
<td>NC</td>
<td>1y</td>
<td>2.1</td>
<td>2.0</td>
<td>1.9</td>
<td>2.0</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>ADH, CASR</td>
<td>L727Q/wt</td>
<td>HC, cramps, paroxysms</td>
<td>3y</td>
<td>2.0</td>
<td>1.8</td>
<td>1.9</td>
<td>2.0</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>ADH, CASR</td>
<td>F806S/wt</td>
<td>HC</td>
<td>1y</td>
<td>2.0</td>
<td>1.8</td>
<td>1.9</td>
<td>2.0</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>ADH, CASR</td>
<td>A843E/wt</td>
<td>HM</td>
<td>1d</td>
<td>1.6</td>
<td>2.4</td>
<td>2.0</td>
<td>2.5</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>ADH, CASR</td>
<td>F895W/wt</td>
<td>HC, NC</td>
<td>1y</td>
<td>2.1</td>
<td>2.0</td>
<td>1.9</td>
<td>2.0</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

**ADH, autosomal dominant hypocalcemia; BS type 5, Bartter’s syndrome type 5; CASR, calcium-sensing receptor; HM, hypomagnesemia; HC, hypocalcemia; NC, nephrocalcinosis; U-Ca, urinary calcium; U-Ca/Cr, ratio of urinary calcium concentration (mg/dl) to urinary creatinine concentration (mg/dl); U-CaEx, urinary calcium excretion (mg/dl); U-Ca/CrEx, ratio of urinary calcium clearance to creatinine clearance; NR, not reported.**
Table 6  *In vitro* sensitivity of activating mutants of the CASR and GNA11 to calcilytics. The cells used to perform these functional assays were HEK 293 cells for every report except (109) where the Flp-In TREx HEK 293 variant and (112) where Cos7 cells were used. The concentration given is the minimal effective concentration at which an increase in signaling activity was reported. In unresponsive mutants the maximum test concentration used is given.

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Sensitivity</th>
<th>CASR</th>
<th>Concentration</th>
<th>Assay</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>N124K</td>
<td>Y</td>
<td>NPS 2143</td>
<td>1 μM</td>
<td>Surface expression</td>
<td>(108)</td>
</tr>
<tr>
<td>F128L</td>
<td>Y</td>
<td>NPS 2143</td>
<td>10 μM</td>
<td></td>
<td>(107)</td>
</tr>
<tr>
<td>T151R</td>
<td>Y</td>
<td>NPS 2143</td>
<td>1 μM</td>
<td></td>
<td>(113)</td>
</tr>
<tr>
<td>T151R/wt</td>
<td>Y</td>
<td>ATF936; AXT914</td>
<td>0.3 μM</td>
<td></td>
<td>(111)</td>
</tr>
<tr>
<td>T151R/wt</td>
<td>Y</td>
<td>ATF936; AXT914</td>
<td>0.3 μM</td>
<td></td>
<td>(111)</td>
</tr>
<tr>
<td>E191K</td>
<td>Y</td>
<td>NPS 2143</td>
<td>10 μM</td>
<td>Surface expression</td>
<td>(107)</td>
</tr>
<tr>
<td>P221L</td>
<td>N</td>
<td>NPS 2143</td>
<td>1 μM</td>
<td></td>
<td>(113)</td>
</tr>
<tr>
<td>P221L</td>
<td>Y</td>
<td>NPS 2143</td>
<td>1 μM</td>
<td></td>
<td>(113)</td>
</tr>
<tr>
<td>P221L/wt</td>
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<td>ATP936; AXT914</td>
<td>0.3 μM</td>
<td></td>
<td>(111)</td>
</tr>
<tr>
<td>D410E</td>
<td>Y</td>
<td>AXT914</td>
<td>0.001 μM</td>
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<td>(129)</td>
</tr>
<tr>
<td>P569H</td>
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<td>NPS 2143</td>
<td>0.1 μM</td>
<td></td>
<td>(110)</td>
</tr>
<tr>
<td>P569H/wt</td>
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<td>ATP936; AXT914</td>
<td>0.1 μM</td>
<td></td>
<td>(110)</td>
</tr>
<tr>
<td>Q681H</td>
<td>Y</td>
<td>NPS 2143</td>
<td>10 μM</td>
<td>Surface expression</td>
<td>(107)</td>
</tr>
<tr>
<td>Q681H</td>
<td>Y</td>
<td>NPS 2143</td>
<td>1 μM</td>
<td></td>
<td>(109)</td>
</tr>
<tr>
<td>L773Q</td>
<td>Y</td>
<td>NPS 2143</td>
<td>3 μM</td>
<td>Surface expression</td>
<td>(109)</td>
</tr>
<tr>
<td>N802I</td>
<td>Y</td>
<td>NPS 2143</td>
<td>0.3 μM</td>
<td>Elk-1-Luc</td>
<td>(112)</td>
</tr>
<tr>
<td>F832L</td>
<td>Y</td>
<td>NPS 2143</td>
<td>3 μM</td>
<td></td>
<td>(109)</td>
</tr>
<tr>
<td>G830S</td>
<td>Y</td>
<td>NPS 2143</td>
<td>3 μM</td>
<td></td>
<td>(113)</td>
</tr>
<tr>
<td>G830S/wt</td>
<td>Y</td>
<td>ATP936; AXT914</td>
<td>0.3 μM</td>
<td></td>
<td>(111)</td>
</tr>
<tr>
<td>G830S/wt</td>
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<td>ATP936; AXT914</td>
<td>0.3 μM</td>
<td></td>
<td>(111)</td>
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<tr>
<td>F832S</td>
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<td>1 μM</td>
<td></td>
<td>(109)</td>
</tr>
<tr>
<td>A835D</td>
<td>N</td>
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<td></td>
<td>(111)</td>
</tr>
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<td>ATP936; AXT914</td>
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<td>(111)</td>
</tr>
<tr>
<td>A835D/wt</td>
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<td>1 μM</td>
<td></td>
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</tr>
<tr>
<td>A835D/wt</td>
<td>Y</td>
<td>ATP936; AXT914</td>
<td>0.3 μM</td>
<td></td>
<td>(111)</td>
</tr>
<tr>
<td>V836L</td>
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<td>1 μM</td>
<td></td>
<td>(108)</td>
</tr>
<tr>
<td>V836L</td>
<td>Y</td>
<td>NPS 2143</td>
<td>3 μM</td>
<td></td>
<td>(109)</td>
</tr>
<tr>
<td>I839T</td>
<td>Y</td>
<td>NPS 2143</td>
<td>0.1 μM</td>
<td>ERK1/2-P; SRE-Luc</td>
<td>(110)</td>
</tr>
<tr>
<td>I839T/wt</td>
<td>Y</td>
<td>NPS 2143</td>
<td>0.1 μM</td>
<td>ERK1/2-P; SRE-Luc</td>
<td>(110)</td>
</tr>
<tr>
<td>A844T</td>
<td>Y</td>
<td>NPS 2143</td>
<td>1 μM</td>
<td></td>
<td>(113)</td>
</tr>
<tr>
<td>A844T</td>
<td>Y</td>
<td>ATP936; AXT914</td>
<td>0.3 μM</td>
<td></td>
<td>(111)</td>
</tr>
<tr>
<td>A844T/wt</td>
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<td>NPS 2143</td>
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<td>(113)</td>
</tr>
<tr>
<td>A844T/wt</td>
<td>Y</td>
<td>ATP936; AXT914</td>
<td>0.3 μM</td>
<td></td>
<td>(111)</td>
</tr>
</tbody>
</table>

Naturally occurring activating CASR mutants causing BS type 5

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Sensitivity</th>
<th>CASR</th>
<th>Concentration</th>
<th>Assay</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>K29E</td>
<td>Y</td>
<td>NPS 2143</td>
<td>0.3 μM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
<tr>
<td>K29E</td>
<td>Y</td>
<td>ATP936; AXT914</td>
<td>0.3 μM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
<tr>
<td>K29E/wt</td>
<td>Y</td>
<td>NPS 2143</td>
<td>1 μM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
<tr>
<td>K29E/wt</td>
<td>Y</td>
<td>ATP936; AXT914</td>
<td>0.3 μM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
<tr>
<td>L125P</td>
<td>Y</td>
<td>NPS 2143</td>
<td>1 μM</td>
<td>PI</td>
<td>(108)</td>
</tr>
<tr>
<td>L125P</td>
<td>Y</td>
<td>NPS 2143</td>
<td>0.3 μM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
<tr>
<td>L125P</td>
<td>Y</td>
<td>ATP936; AXT914</td>
<td>0.3 μM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
<tr>
<td>L125P/wt</td>
<td>Y</td>
<td>NPS 2143</td>
<td>1 μM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
<tr>
<td>L125P/wt</td>
<td>Y</td>
<td>ATP936; AXT914</td>
<td>0.3 μM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
<tr>
<td>C131W</td>
<td>N</td>
<td>NPS 2143</td>
<td>1 μM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
</tbody>
</table>
The specific alterations of the calcium metabolism in ADH and their therapy also apply to patients with BS type 5. The therapy of renal salt wasting in these patients, however, is beyond the scope of this review.

**Vitamin D and calcium**

Vitamin D analogues and calcium supplementation is the standard treatment of hypocalcemia and can also raise serum calcium in patients with ADH (98, 99, 100, 102) and BS type 5 (103). Several vitamin D compounds (vitamin D2, vitamin D3, calcitriol, alfacalcidol) have been used in ADH patients without any published preference (99). PTH is low or even undetectable in patients with activating CASR mutations. This leads to impaired PTH-dependent renal 1-alpha-hydroxylation of 25-OH-vitamin D3, and thus, to reduced formation of 1,25-dihydroxyvitamin D3. We, therefore, prefer the use of either calcitriol or alfacalcidol. Raising serum calcium by vitamin D3 and calcium supplementation inevitably aggravates hypercalciuria in patients with activating CASR mutations (97, 98, 100, 102). There is considerable phenotypic variation (95, 98) and tissue calcifications were similar in ADH patients with and without hypercalciuria (100) or in patients with or without vitamin D and calcium therapy in a large kindred (98). There is, however, general consensus, that therapy with vitamin D and calcium should be given cautiously and only in the amount necessary to reduce symptoms to an acceptable level and to prevent severe complications such as seizures and cardiac arrhythmias. Vitamin D and calcium have also been successfully administered to ADH patients during pregnancy (104). In any case vitamin D and calcium treatment requires close and regular monitoring of serum calcium, calciuria and renal function.

**Magnesium**

Hypomagnesemia is occasionally found in ADH (105) and is of particular concern in BS type 5 (103). Magnesium levels should be monitored and supplemented if reduced, as they can further impair PTH secretion (103).

---

### Table 6 Continued.

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Sensitivity</th>
<th>Calcium/Ca-resistant receptor (CASR) variants</th>
<th>Concentration</th>
<th>Assay</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>C131W</td>
<td>Y</td>
<td>ATF936; AXT914</td>
<td>0.3 µM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
<tr>
<td>C131W/wt</td>
<td>N</td>
<td>NPS 2143</td>
<td>1 µM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
<tr>
<td>C131W/wt</td>
<td>Y</td>
<td>ATF936; AXT914</td>
<td>0.3 µM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
<tr>
<td>A843E</td>
<td>Y</td>
<td>NPS 2143</td>
<td>10 µM</td>
<td>surface expression</td>
<td>(107)</td>
</tr>
<tr>
<td>A843E</td>
<td>N</td>
<td>NPS 2143</td>
<td>1 µM</td>
<td>PI</td>
<td>(108)</td>
</tr>
<tr>
<td>A843E</td>
<td>N</td>
<td>NPS 2143</td>
<td>3 µM</td>
<td>[Ca²⁺]</td>
<td>(109)</td>
</tr>
<tr>
<td>A843E</td>
<td>N</td>
<td>NPS 2143</td>
<td>10 µM</td>
<td>ERK1/2-P; SRE-Luc</td>
<td>(110)</td>
</tr>
<tr>
<td>A843E</td>
<td>N</td>
<td>NPS 2143</td>
<td>1 µM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
<tr>
<td>A843E</td>
<td>N</td>
<td>NPS 2143</td>
<td>0.3 µM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
<tr>
<td>A843E/wt</td>
<td>N</td>
<td>NPS 2143</td>
<td>10 µM</td>
<td>ERK1/2-P; SRE-Luc</td>
<td>(110)</td>
</tr>
<tr>
<td>A843E/wt</td>
<td>N</td>
<td>NPS 2143</td>
<td>1 µM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
<tr>
<td>A843E/wt</td>
<td>Y</td>
<td>ATF936; AXT914</td>
<td>0.3 µM</td>
<td>[Ca²⁺]</td>
<td>(111)</td>
</tr>
</tbody>
</table>

**Artificial activating CASR variants**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Sensitivity</th>
<th>Calcium/Ca-resistant receptor (CASR) variants</th>
<th>Concentration</th>
<th>Assay</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>L776A</td>
<td>Y</td>
<td>Calhex 231</td>
<td>IC50 0.07 µM</td>
<td>IP3</td>
<td>(130)</td>
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<tr>
<td>L776A</td>
<td>Y</td>
<td>Calhex 231</td>
<td>IC50 0.4 µM</td>
<td>IP3</td>
<td>(127)</td>
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<td>F821A</td>
<td>Y</td>
<td>Calhex 231</td>
<td>IC50 0.06 µM</td>
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<td>(130)</td>
</tr>
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<td>F821A</td>
<td>Y</td>
<td>Calhex 231</td>
<td>IC50 0.6 µM</td>
<td>IP3</td>
<td>(127)</td>
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<tr>
<td>E837D</td>
<td>Y</td>
<td>NPS 2143</td>
<td>1 µM</td>
<td>PI hydroysis</td>
<td>(108)</td>
</tr>
<tr>
<td>E837K</td>
<td>N</td>
<td>NPS 2143</td>
<td>1 µM</td>
<td>PI hydroysis</td>
<td>(108)</td>
</tr>
<tr>
<td>I841A</td>
<td>Y</td>
<td>Calhex 231</td>
<td>IC50 3 µM</td>
<td>IP3</td>
<td>(130)</td>
</tr>
<tr>
<td>I841A</td>
<td>Y</td>
<td>NPS 2143</td>
<td>IC50 4 µM</td>
<td>IP3</td>
<td>(127)</td>
</tr>
</tbody>
</table>

**Naturally occurring activating GNA11 mutants causing ADH2**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Sensitivity</th>
<th>Calcium/Ca-resistant receptor (CASR) variants</th>
<th>Concentration</th>
<th>Assay</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>R181Q</td>
<td>Y</td>
<td>NPS 2143</td>
<td>20 nM</td>
<td>[Ca²⁺]</td>
<td>(93)</td>
</tr>
<tr>
<td>F341L</td>
<td>Y</td>
<td>NPS 2143</td>
<td>40 nM</td>
<td>[Ca²⁺]</td>
<td>(93)</td>
</tr>
</tbody>
</table>

CASR, calcium-sensing receptor; GNA11, G protein alpha 11; ADH1; autosomal dominant hypocalcemia type 1; ADH2; autosomal dominant hypocalcemia type 2; BS type S, Bartter’s syndrome type S; sensitivity, decrease in signaling activity; Y; yes, N, no; [Ca²⁺], measurement of intracellular free calcium; IP3, inositol 1,4,5-trisphosphate accumulation assay; ERK1/2-P, anti-extracellular-signal-regulated kinase 1 and 2 phospho-specific western blot; -Luc, serum response element luciferase reporter assay; Elk-1-Luc, Elk-1 trans-reporting luciferase reporter assay; PI, phosphoinositide hydrolysis assay.
Hydrochlorothiazide

Treatment with hydrochlorothiazide (HCT) has been reported to reduce urinary calcium excretion in patients with ADH (106) and BS type 5 (29, 96, 103). The doses given ranged from 0.5 mg/kg per day in young adults (96) to 5 mg/kg per day in infants (106). HCT treatment, however, is not always successful (95). Its effectiveness seems to decline over time (29) and common side effects like hypokalemia limit its use (29). In patients, where hypercalciuria is an issue, HCT is worth a trial.

Parathyroid hormone

Activating CASR mutations lead to low or even undetectable PTH levels. Replacement of PTH1-34 (teriparatide) has been reported in two adults and three children with ADH and one child with BS type 5 (Table 5). PTH1-34 was generally effective in reducing symptoms or raising serum calcium despite a reduction of other calcium elevating therapies, and it was well tolerated for up to 14 years (25). Urinary calcium excretion declined or was constant despite rising serum calcium levels in five out of six patients (Table 5). Although, a modest reduction of calcium excretion does not necessarily prevent nephrocalcinosis (25), it appears likely that reduced calciuria may postpone renal complications, which, however, remains to be proven. As CASR activation suppresses PTH signaling in the kidney, patients with different activating CASR mutants may show variable responses to PTH treatment. In the USA recombinant PTH1-84 has been approved as an adjunct to calcium and vitamin D to control hypocalcemia in patients with hypoparathyroidism. In countries of the European Union, however, PTH1-84 is not yet available for clinical use in hypoparathyroidism, but orphan designation was granted by the European Commission for this indication.

Calcilytics as a future therapeutic perspective

None of the current therapeutic strategies corrects the underlying pathophysiologic mechanism, clinically normalization of hypocalcemia is often limited by hypercalciuria and many patients develop long-term complications. An increasing body of evidence from in vitro and in vivo studies suggests that calcilytics could be a very promising novel therapeutic approach for ADH and BS type 5 patients.

Effect of calcilytics

In vitro on mutations causing gain of CASR function ▶ The CASR database (www.casrdb.mcgill.ca) lists 63 distinct

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Study population</th>
<th>Study type</th>
<th>Dose</th>
<th>Calcilytic Treatment</th>
<th>Time to effect</th>
<th>S-Ca before (mM)</th>
<th>S-Ca after (mM)</th>
<th>S-PTH before (pg/ml)</th>
<th>S-PTH min after (pg/ml)</th>
<th>U-Ca excretion</th>
<th>Adverse effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>Phase I and phase II</td>
<td>49 healthy volunteers and osteopenic postmenopausal women</td>
<td>AXT914 4–120 mg/day</td>
<td>PTH 1 h</td>
<td>Ca 8–24 h</td>
<td>Normal</td>
<td>Normal – elevated</td>
<td>Normal</td>
<td>Elevated</td>
<td>NR</td>
<td>Hypercalcemia, gastrointestinal symptoms</td>
</tr>
<tr>
<td>WT</td>
<td>Phase I</td>
<td>34 osteopenic postmenopausal women</td>
<td>Ronacaleret 100 or 400 mg/day</td>
<td>PTH 1 h</td>
<td>Ca 8–10 h</td>
<td>Normal</td>
<td>Normal – elevated</td>
<td>Normal</td>
<td>Elevated</td>
<td>NR</td>
<td>Acute administration: 1 chronic administration: 2</td>
</tr>
<tr>
<td>WT</td>
<td>Phase II</td>
<td>344 osteopenic postmenopausal women</td>
<td>Ronacaleret 100 or 400 mg/day</td>
<td>PTH 1 h</td>
<td>Ca</td>
<td>Normal</td>
<td>Normal – elevated</td>
<td>Normal</td>
<td>Elevated</td>
<td>NR</td>
<td>Hypercalcemia</td>
</tr>
<tr>
<td>WT</td>
<td>Phase II</td>
<td>54 osteopenic postmenopausal women</td>
<td>MK-5442 5–15 mg/day</td>
<td>PTH 1 h</td>
<td>Ca</td>
<td>Normal</td>
<td>Normal – elevated</td>
<td>Normal</td>
<td>Elevated</td>
<td>NR</td>
<td>Dose-dependent increase in hypercalcemia</td>
</tr>
</tbody>
</table>

S-Ca, total serum calcium; S-PTH, serum parathyroid hormone; and U-Ca excretion, urinary calcium excretion.
activating CASR mutations causing ADH and four mutations causing BS type 5 (85). Functional in vitro tests with calcilytics have been reported for 28 naturally occurring activating CASR mutations (Table 6). Five artificially engineered CASR mutants with enhanced CASR functions have been tested as well and were included in Table 6 for reference. All 24 naturally occurring activating ADH mutants and four BS type 5 mutants were sensitive to at least one calcilytic in vitro. For one amino acid (E767) two distinct naturally occurring amino acid exchanges were described, which both responded to calcilytics. Two ADH and two BS type 5 CASR mutants were independently tested by different research groups. The results were consistent except for the CASR mutant A843E where NPS-2143 was reported to decrease cell surface expression (107) but not signaling activity (108, 109, 110, 111). Very recently NPS-2143 has been demonstrated to mitigate the gain of function of activating GNA11 mutations R181Q and F341L from ADH type 2 patients (93).

There appear to be differences in the efficacy of calcilytics with different chemical structure. NPS-2143 and its close relative ronacaleret are amino-alcohols, whereas ATF936 and AXT914 are quinazolinones. These different classes of calcilytics have partly different bindings sites (48) and data from four publications show that some but not all ADH CASR mutants were sensitive to NPS-2143 (108, 111, 112, 113). In contrast all five ADH and all four BS5 mutants tested so far were sensitive to ATF936 and AXT914 (111).

In vivo treatment with calcilytics Although calcilytics are not yet approved for therapeutic use, phase I and II trials of AXT914 the NPS-2143 derivative ronacaleret and MK5442 have been performed in healthy subjects and osteopenic postmenopausal women (Table 7). While these trials failed to significantly increase bone density in osteoporosis, a significant rise in serum calcium and PTH was observed and in one study a reduction in urinary calcium excretion for up to 12 h after administration of ronacaleret was reported (Table 7). These are exactly the effects which are desired in patients with activating CASR mutations. Recent studies using knock-in mouse models of ADH and BS type 5 showed, that calcilytics could increase plasma calcium and PTH, reverse hypercalciuria and renal calcification and were more effective than PTH (56, 114). The most common adverse effects in humans reported were mild neurological and gastrointestinal symptoms like fatigue, headache, constipation, diarrhea, nausea, and dyspepsia. Overall, however, calcilytics were well tolerated (Table 7).

Therapeutic strategy and summary
Symptomatic patients need treatment to raise serum calcium to levels where symptoms disappear. The first line approach is vitamin D and calcium supplementation. If hypercalciuria gives cause for concern, addition of a thiazide diuretic could be considered. Alternatively, recombinant PTH could be tried, which may raise serum calcium while maintaining an acceptable level of urinary calcium excretion. Treatment of asymptomatic ADH patients, however, is generally not recommended. For this group of patients proven benefit of treatment has not yet been demonstrated, but worsening of hypercalciuria especially under vitamin and calcium supplementation may put them at a higher risk for renal complications. In principle, however, an improvement of this therapeutic approach appears desirable, since both treated symptomatic as well as untreated asymptomatic patients may develop long-term complications like basal ganglia calcifications, cataract formation or neuropsychological symptoms, which appear to be associated with chronic hypocalcemia independent from its cause. In that respect it is unclear what the serum calcium levels should be to ensure normal physical and mental development and function in ADH patients. PTH treatment may offer new perspectives, as higher serum calcium levels could be achievable without increasing hypercalciuria and renal risks. Whether this is actually the case and whether long-term complications could be minimized or prevented by PTH, will have to await further studies in ADH patients. In the future calcilytics, which could correct the underlying molecular defect in ADH patients, may offer a causal therapy and have the potential to reconsider the current therapeutic approach.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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