

A Case Report of Calcium-Sensing Receptor Gene Variant *CASR* (c.659G>A; p.R220Q) and Primary Hyperparathyroidism

Keywords: Primary hyperparathyroidism; Familial hypocalciuric hypercalcemia; Calcium metabolism; Genetics

Abstract

Background: Primary hyperparathyroidism (PHPT) results from excessive parathyroid hormone from one or more overactive parathyroid gland(s). An estimated 90% of PHPT cases are sporadic, and up to 10% are inherited, comprising hereditary hyperparathyroidism (HHPT). Genetic testing can aid in diagnosis and management and influence testing of other family members.

Case Report: A 42-year-old female with hypercalcemia (diagnosed at 23 years) and nephrolithiasis due to PHPT was referred to endocrinology for further management and evaluation following 3-gland parathyroidectomy. Pre-operative workup showed calcium of 11.1 mg/dL and PTH of 177 pg/mL. Sestamibi showed persistent activity in the mid-to-inferior aspect of right thyroid lobe. Post-operative pathology showed mildly hypercellular parathyroid in left superior and right superior gland, normocellular left inferior gland. iPTH levels normalized post-surgery. Genetic evaluation was performed, given her early-onset hypercalcemia, multi-gland involvement, and notable family history (mother and daughter with primary hyperparathyroidism, maternal grandfather with parathyroidectomy, and maternal aunt with multiple bone fractures). Invitae hyperparathyroidism panel revealed a likely pathogenic variant in *CASR* (c.659G>A; p.R220Q).

Discussion: Our case is the second report of this likely pathogenic variant, previously reported in a 29-year-old proband diagnosed with familial hypocalciuric hypercalcemia (FHH) after remaining hypercalcemic following subtotal thyroidectomy. Despite the marked phenotypic heterogeneity (clinical presentation and response to surgery), both our case and this previous patient shared a personal history and family history of hypercalcemia, suggesting contributions to both causes of hypercalcemia from the same variant.

Conclusion: We interpret the structural and functional change to the CaSR to be a predisposition for both FHH and PHPT. Our case adds to the limited existing data about the variable expressivity of genes implicated in the pathogenesis of both FHH and HHPT.

Introduction

The calcium sensing receptor (CaSR) is a key mediator of serum calcium homeostasis. This G_q-protein coupled receptor is predominantly expressed in the parathyroid glands and the renal tubules [1]. In the parathyroid glands, the CaSR senses extracellular calcium concentrations, and beyond a threshold extracellular calcium concentration, it initiates the signaling pathway to decrease the release of parathyroid hormone (PTH) from the parathyroid glands and calcium reabsorption from the renal tubules [1-3]. Disorders of calcium metabolism can result from any disturbance in the regulators of calcium homeostasis, including CaSR-mediated signaling pathway defects and PTH-mediated hormonal signaling dysregulation [3].

The leading cause of hypercalcemia is primary



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hyperparathyroidism (PHPT), an endocrinopathy marked by autonomous activity of one or more parathyroid gland(s), most commonly due to parathyroid adenomas, followed by hyperplasia and rarely carcinomas [4]. While an estimated 90-95% of PHPT cases are sporadic, 5-10% are hereditary, comprising hereditary hyperparathyroidism (HHPT) [2], which includes syndromic and non-syndromic forms of hyperparathyroidism. Another disease entity considered in the differential diagnosis with PHPT is Familial hypocalciuric hypercalcemia (FHH), a rare autosomal dominantly inherited cause of mild hypercalcemia with clinical manifestations ranging from mostly asymptomatic to mimicking PHPT [5-8].

There is not a clear consensus on whether FHH is a distinct entity or if it is encompassed in the spectrum of HHPT [5]. Regardless, accurate diagnosis is critical in guiding proper management: with parathyroidectomy as the standard treatment for PHPT [7], in contrast to FHH for which parathyroidectomy is inappropriate, with persistent hypercalcemia post-parathyroidectomy [5,7].

The rapid advances in genetic sequencing technologies have facilitated the discovery and characterization of the genetic contributions to FHH and HHPT [9]. There exists a range of options for genetic testing, including gene panels which encompass genes that are functionally impacted in FHH or HHPT.

Here, we describe the second case of a likely pathogenic variant c.659G>A;p.R220Q in the calcium sensing receptor (*CASR*) gene. While this variant has been previously reported in a patient with FHH [10], our case is the first to describe this variant in association with PHPT.

Case

A 42-year-old female with PHPT with hypercalcemia and recurrent nephrolithiasis, status-post 3-gland parathyroidectomy, was referred to endocrinology for further management and evaluation of her PHPT.

She initially sought evaluation when she was 23-years-old due to chronic joint pain and at that time, was found to have serum

calcium of 14mg/dL. She had a history of recurrent abdominal pain, gastric ulcers, cholecystectomy and recurrent nephrolithiasis, with prior imaging revealing multiple non-obstructive <2mm bilateral renal stones. She also noted anxiety, depression, and memory and concentration difficulties. At age 41 years, she was diagnosed with primary hyperparathyroidism. Family history was significant for a mother and daughter with primary hyperparathyroidism, maternal grandfather with parathyroidectomy, and maternal aunt with multiple bone fractures (Figure 1). Preoperative laboratory evaluation revealed serum calcium of 11.1mg/dL, albumin 4.2g/dL, intact parathyroid hormone (iPTH) of 177pg/mL, creatinine 0.92mg/dL, and 24-hour urinary calcium levels were not obtained (Table 1). No parathyroid adenoma was visualized on CT soft tissue neck. Parathyroid nuclear scan with Sestamibi showed persistent activity in the mid to inferior aspect of right thyroid lobe suspicious for parathyroid adenoma. At the age of 41 years, she underwent 3-gland parathyroidectomy, and surgical pathology revealed mildly hypercellular parathyroid in the left superior and right superior glands and a normocellular left inferior gland, consistent with primary hyperparathyroidism.

Post-operatively, her iPTH levels decreased appropriately to <4pg/mL, DEXA scan had Z-scores within normal range, and calcium level was well maintained with Citracal Calcium 650 mg and Vitamin D 1000 units daily (Table 1). Calcitriol had to be discontinued later as the patient could not tolerate it. Despite her largely uncomplicated post-operative course, further evaluation for the etiology of her PHPT was pursued, given her early-onset hypercalcemia, multi-gland involvement, and notable family history, which suggested a genetic etiology. Genetic work up with the Invitae Hyperparathyroidism Gene

Panel, which contains seven genes (*AP2S1, CASR, CDC73, CDKN1B, GNA11, MEN1, RET*) associated with HHPT, revealed a heterozygous likely pathogenic variant in *CASR* (NM_000388.4:c.659G>A; p.R220Q) (Table 2).

Table 1: Preoperative and postoperative lab values.

	2017	2019	Post-op 2020*
Calcium (mg/dL)	11.1	11.1	8.7
iPTH (pg/ml)	61	77	2
Albumin (g/dL)	4.2	4.0	4.6
25OH vit D (ng/ml)	18	-	37
eGFR	77	78	81

*on Citracal 650mg/vitamin D 1000U PO BID

Table 2: Genetic variant information.

Gene	Variant	Zygoty	Variant interpretation	Allele frequency (gnomAD)	ACMG Criteria*
<i>CASR</i>	c.659G>A p.R220Q	Heterozygous	Likely Pathogenic	0.00000399	PM1 PM5, PP5 PP2, PP3

*American College of Medical Genetics and Genomics (ACMG) variant classification criteria [15]:

PM1: Variant is located in a mutational hotspot and/or well-established functional domain

PM5: Novel missense change at an amino acid residue where a different pathogenic missense variant has been previously established

PP5: Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

PP2: Missense variant in a gene that has low rate of benign missense variation and in which missense variants are a common mechanism of disease

PP3: Multiple lines of computational evidence support a deleterious effect on the gene or gene product

Key: PM= Pathogenic moderate, PP= Pathogenic supporting.

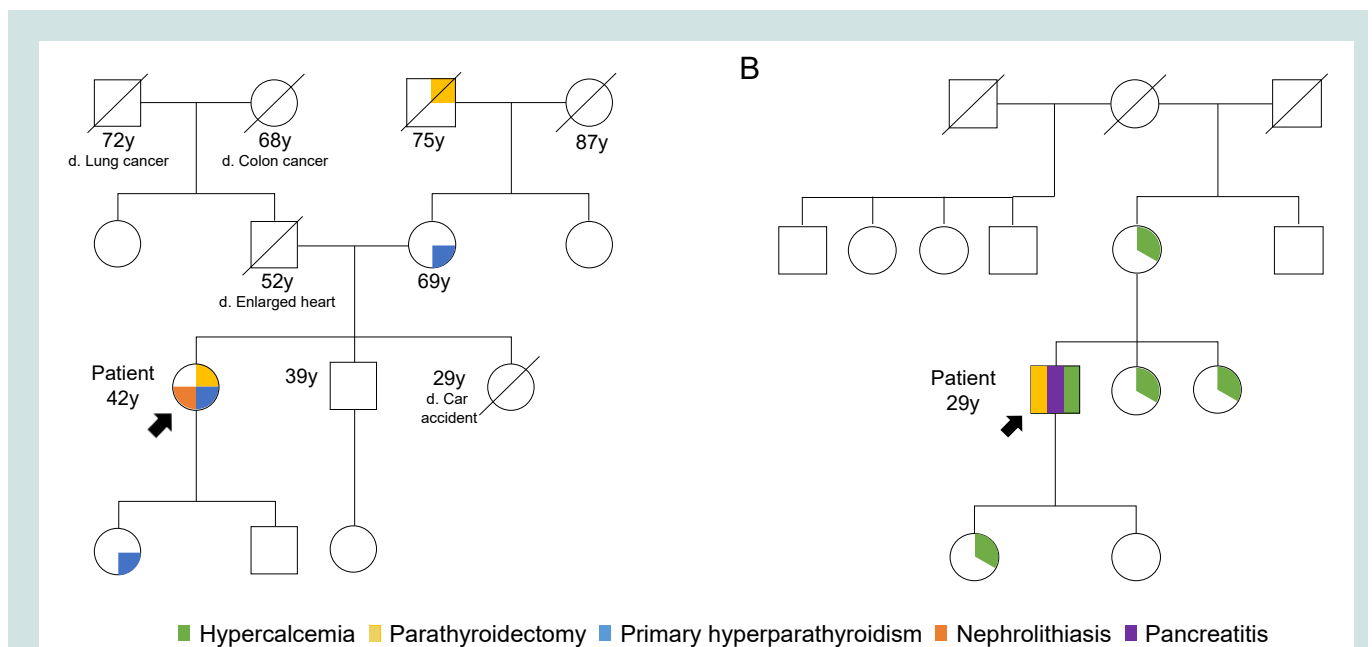


Figure 1: Pedigrees of the two families with proband and family history of hypercalcemia and the likely pathogenic variant NM_000388.4:c.659G>A; p.R220Q in *CASR*. A) Our patient with PHPT status post 3-gland parathyroidectomy and medically manageable calcium postoperatively (arrow). Family history of hypercalcemia and parathyroidectomy in the maternal grandfather, bone fractures in the maternal aunt, and hyperparathyroidism workup in the mother. B) Pedigree of patient with FHH and recurrent pancreatitis and hypercalcemia that did not resolve with parathyroidectomy. Family history of hypercalcemia without pancreatitis in daughter, two sisters, and mother. Pedigree modified from Davies et al 1981 [11].

Discussion

To date, our case is the second to report this likely pathogenic variant c.659G>A; p.R220Q in *CASR*. This variant was previously reported in a 29-year-old male with FHH and recurrent pancreatitis, a common complication of FHH, known to be associated with pathogenic variants in *CASR* [9,10]. His family history was notable for 4 relatives (mother, two sisters, daughter) with hypercalcemia, but no pancreatitis (Figure 1). He was re-admitted in February 1977 with a hematoma and pancreatitis with serum calcium 11.5mg/dL, 24-hour urine calcium 100mg/24hr, phosphate 2.6mg/dL, and PTH <100pg/mL. Surgical exploration for suspected PHPT led to 2-gland parathyroidectomy. During his 20 years follow-up, pancreatitis did not recur; however, he remained hypercalcemic (serum calcium 10.4-11.2 mg/dL), subsequently leading to the diagnosis of FHH [10,11].

While our case and the former case share the same variant that may underlie the patient and family history of hypercalcemia, the response to parathyroidectomy suggests that this variant is associated with FHH in the previous case (with persistent postoperative hypercalcemia) and with a form of HHPT in our case (medically-manageable calcium postoperatively). Unlike other variants that are known to be diagnostic, there is currently not sufficient evidence available to conclude causality, especially considering the association with both FHH and HHPT. Rather, we interpret the variant to be a genetic predisposition to both FHH and PHPT via a common mechanism that requires study, and modifier genes and environmental factors may contribute to the variable expressivity. Using the ACMG guidelines to assign classification [15], the following existing evidence suggests this variant likely contributes to the hypercalcemia in both our PHPT case and the FHH case: (1) This variant is present in only one individual in the Genome Aggregation Database (gnomAD v2.1.1), supporting that it is rare in unselected populations; (2) This variant affects an evolutionarily conserved extracellular domain residue. The extracellular domain is a mutational hotspot for loss-of-function variants; (3) Another missense variant impacting the same amino acid residue, p.R220W, is classified as pathogenic and causes FHH in the heterozygous state and neonatal hyperparathyroidism in the homozygous state, supporting the key role of this residue [13,14]; and (4) in-silico algorithms (SIFT, PolyPhen-2, Align-GVGD) have predicted that the c.659G>A; p.R220Q variant disrupts the structure and function of CaSR (Table 2). However, to date, these predictions have not been confirmed by functional studies, which is a factor that hinders the upgrading of this variant from likely pathogenic to pathogenic. Mullin, et al. identified three novel variants in *CASR* in 4 patients with presumed FHH [16]. Bioinformatics gave conflicting results but suggested the variants to be probably/possibly damaging, but in the absence of other supporting evidence by ACMG classification criteria, these variants were classified as variant of uncertain significance (VUS). Functional assessment demonstrated impaired CaSR activation, permitting the upgrading of the VUS to a likely pathogenic variant. Similar functional assessment of c.659G>A; p.R220Q could upgrade this variant to pathogenic. However, even if this variant is upgraded to pathogenic, the question of the role of this variant (causative versus predisposing) remains to be clarified.

While in our case this variant does not lead to a definitive molecular diagnosis, in the context of the overall clinical picture

favoring PHPT, it can help narrow the differential diagnosis by ruling out established genetic causes of PHPT [9]. Moreover, genetic testing can guide management, even if the effects of the variant are not fully established, and variants can be reclassified once sufficient evidence is available. For instance, Bletsis et al. describes a 35-year-old woman with persistent hypercalcemia post-subtotal parathyroidectomy for hypothesized PHPT. Family history was significant for hypercalcemia in her mother, maternal uncle, and maternal aunt, and history of persistent postoperative hypercalcemia in the mother. The mother and maternal aunt and uncle had undergone genetic testing prior to the patient's presentation, revealing a VUS in *CASR* (c.392C>A; p.A110D). She was also found to be heterozygous for *CASR* (c.392C>A; p.A110D). While there is not currently sufficient evidence to upgrade this VUS, its co-segregation in multiple family members with hypercalcemia, persistent postoperative hypercalcemia, and low 24-hour urine calcium did influence diagnosis and management; FHH instead of HHPT was the most likely diagnosis, leading to avoidance of reoperation [18]. Likewise, it can influence cascade testing (i.e., genetic testing of other family members) and predict recurrence risk in the subsequent generation [17,18]. Pre-operative molecular diagnosis of PHPT can guide surgical management, both in planning urgency of operation (i.e., based on risk of malignancy), surgical approach (i.e., minimally-invasive versus open), as well as evaluation and potential surgical management of other organ systems associated with some PHPT syndromes [17,19]. Particularly for asymptomatic individuals or individuals with subclinical or early-stage disease, the information from genetic testing can promote prevention or early intervention and more vigilant management.

In our case, genetic testing was performed after the diagnosis of PHPT had already been established and after successful surgical management. While the genetic testing results may have not necessarily changed the diagnosis or management for our patient, it provided a likely genetic explanation for her family history and informs expectant management for at-risk relatives who may choose to undergo testing for the familial variant. Moreover, no pathogenic or likely pathogenic variants were returned in other genes that cause PHPT, especially those with concomitant involvement of other organ systems. For other patients, in the postoperative setting, genetic testing may influence management and surveillance [17].

Conclusion

Our case with PHPT is the second report of a likely pathogenic variant c.659G>A; p.R220Q in the *CASR* gene. This variant was first reported in 1981 in a 29-year-old male with FHH and recurrent pancreatitis [10,11]. We interpret the shared genetic variant as an indication that the predicted change to the structure and function of the CaSR is a predisposition for both causes of hypercalcemia, and this finding further emphasizes the question of whether FHH and HHPT are distinct entities or whether FHH is an atypical form of HHPT that is resistant to parathyroidectomy [5]. Genetic testing of this patient's family members and other patients with suspected PHPT or FHH who meet the proposed indications may reveal other patients with this variant that will expand our understanding of the role and penetrance of this variant (causative versus predisposing) [17].

There is an overlap between the genes found in families with FHH

and HHPT, similar to the previously described degree of biochemical overlap [17]. These and suggest at least a partially shared molecular pathogenesis between the two entities. Our case adds to the existing knowledgebase, not only for the specific c.659G>A; p.R220Q variant, but also, it illustrates that variants that contribute to FHH are not mutually excluded from also contributing to PHPT in another patient, and vice versa.

Furthermore, we discuss the multi-fold utility of genetic testing in the differential diagnosis of hypercalcemia: it can help rule-out other known causes, inform familial recurrence risk, predict and potentially prevent other medical problems that are also a part of an inherited syndrome, and be integrated with other findings to form the most likely diagnosis. However, it may not lead to a definitive clinical diagnosis, and it is limited by the currently existing data about the variant and variable expressivity of variants in genes implicated in the pathogenesis of both FHH and HHPT.

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