Published in final edited form as:

Biomed Pharmacother. 2017 August ; 92: 843–848. doi:10.1016/j.biopha.2017.05.028.

Specifying the molecular pattern of sporadic parathyroid tumorigenesis—The Y282D variant of the *GCM2* gene

Elena Marchiori[a,b,*], **Maria Rosa Pelizzo**[a], **Monika Herten**[b], **Danyelle M. Townsend**[c], **Domenico Rubello**[d], and **Isabella Merante Boschin**[a]

[a]Department of Oncological Surgical and Gastroenterological Sciences (DiSCOG), University of Padova, Padova, Italy

[b]Department of Vascular and Endovascular Surgery, University Hospital Munster, Munster, Germany

[c]Department of Drug Discovery and Pharmaceutical Sciences, Medical University of South Carolina, USA

[d]Department of Nuclear Medicine, Radiology and Clinical Pathology, Medicine, Santa Maria della Misericordia Hospital, Rovigo, Italy

Abstract

Objective: Sporadic carcinoma of the parathyroid glands is a rare malignant neoplasia. The *GCM2* gene encodes a transcription factor that is crucial to embryonic parathyroid development. The Y282D variant of GCM2 exhibits increased transcriptional activity, and the presence of this variant is significantly associated with a higher prevalence of primitive hyperparathyroidism. The present study investigated the prevalence of the Y282D variant of the *GCM2* gene and its association with clinical parameters in patients with a definitive histological diagnosis of sporadic parathyroid carcinoma (SPC) or atypical adenoma (AA).

1. Introduction

Sporadic carcinoma of the parathyroid glands is an extremely rare malignancy, accounting for an estimated 0.005% of all cancer cases, with two new cases per 10,000,000 person-years [1]. The rarity of this disease presents a challenge in studying its underlying pathophysiological and molecular features.

The Glial Cell Missing 2 gene (*GCM2*, previously referred to as *GCMB*, location 6p23; HGNC gene ID:9247; NM_004752.3) was found to be responsible for several forms of congenital hypopara-thyroidism [2,3], but its role in the development of primitive hyperparathyroidism and its association with parathyroid carcinoma have only recently been

*Corresponding author at: Department of Vascular and Endovascular Surgery, University Hospital Münster, Albert-Schweitzer-Campus 1, 48149 Münster, Germany. elena.marchiori@gmail.com (E. Marchiori).

Conflict of interest

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Disclosure statement

The authors have nothing to disclose.

considered [4,5] (Fig. 1). GCM2, a transcription factor that is crucial for homeostasis, acts as a master regulator of embryonic development in the parathyroid glands [6,7]. Both phylogenetic analysis and knockout mouse model studies have demonstrated the key role of GCM2 in embryonic parathyroid development [6,8]. In fact, in GCM2-deficient mice the animals fail to develop parathyroid glands.

GCM2 has been shown to be expressed in the adult human parathyroid, and mutations in exons 2 and 5 of *GCM2* are associated with autosomal hypoparathyroidism [2,6,9,10].

In addition to its role in hypoparathyroidism, a specific variant of the *GCM2* gene leads to hyperparathyroidism by increasing its transcriptional activity by 1.4-fold relative to the wild type gene. The Y282D variant NM_004752.3c.844T>G/p. (Tyr282Asp) rs61734277 was significantly associated with a higher prevalence of primitive hyperparathyroidism in three Italian cohorts [4]. Based on these observations and considering the increased transcriptional activity of Y282D mutant GCM2 as well as its increased frequency in patients with primitive hyperparathyroidism, we hypothesized that Y282D contributes to the pathogenesis of sporadic parathyroid tumors.

The present study investigated the prevalence of the Y282D variant of the *GCM2* gene in patients with a definitive histological diagnosis of sporadic parathyroid carcinoma (SPC) or atypical adenoma (AA) using germ-line DNA samples. These findings were compared with previously described cohorts of hyperparathyroidism patients and healthy controls. Additionally, the associations of the Y282D variant with the following clinical parameters were assessed: serum calcium, PTH, and vitamin D levels.

2. Subjects and methods

2.1. Study population

The cohort consisted of 62 patients with a definitive histological diagnosis of SPC (n = 23) or AA (n = 39) who underwent surgery between January 1990 and December 2014. The study was conducted in agreement with the International Conference on Harmonization (ICH) Guidelines, Good Clinical Practice (GCP), the Declaration of Helsinki and ethics committee requirements. All patients provided written informed consent before inclusion in the study. The inclusion criteria were positivity for hyperparathyroidism based on anamnesis, a history of parathyroid mass formation after and in the context of hyperparathyroidism, surgical treatment of the parathyroid mass, and a definitive histological diagnosis of atypical adenoma or parathyroid carcinoma. Patients with a family history of multiple endocrine neoplasias or with positivity for multiple endocrine neoplasia or other possible forms of hereditary hyperparathyroidism based on anamnesis were excluded.

2.2. Surgery

The surgical treatment consisted of removal of the pathological tumor tissue with the intention of radical excision (Table 1). Preoperative localization was assessed using imaging techniques including high resolution neck ultrasound, CT magnetic resonance imaging, scintigraphy, a single-session 99mTc-sestamibi scan and positron emission tomography/

computed tomography (PET/CT) with 18F-fluorodeoxyglucose (18F-FDG) as described previously [11–13]. All surgical procedures were performed by the same experienced surgeon. To confirm successful parathyroidectomy, the intraoperative PTH level was measured by assessing the decrease in serum PTH levels from baseline to 10 min after removal of the pathological tissue [14].

2.3. Histopathological characterization

The diagnosis of sporadic parathyroid carcinoma or atypical adenoma was determined via histological examination of the surgical findings (Table 2). Histological exams were conducted at the Department of Pathological Anatomy of the University of Padua by pathologists experienced in parathyroid pathology, guaranteeing consistent and reliable results.

The criteria of malignancy included: i) a high proliferation index, ii) abundant fibrous peritumoral tissue (fibrous capsule) and fibrous tissue within the tumor; iii) a lack of adipocytes, cells typical of healthy parathyroid tissue; iv) presence of necrosis.

These properties are common to both sporadic parathyroid carcinoma and atypical adenoma. A diagnosis of SPC was made in cases of unequivocal invasion of the adjacent tissues or metastasis. SPC is characterized by the presence of vascular, parenchymal, or perineural invasion of tumor cells, as well as by the rupture of fibrous capsules with dissemination to adjoining tissues. This invasion is absent in AA. Distant or lymph node metastasis was considered definitive evidence of malignancy [15–18].

2.4. Genetic analysis

Patient germ-line DNA was obtained from whole blood through buffy coat and/or buccal swabs

2.5. DNA extraction

DNA extraction was performed with the QIAamp DNA Blood Kit according to the manufacturer’s protocol (Qiagen, Hilden, Germany). The DNA concentration was determined with a spectrophotometer (Nanodrop 1000 UV-vis, Thermo Scientific, Waltham, MA, USA).

2.6. Amplification and purification of the PCR product

The coding region of exon 5 from c.717 to c.1023 of the *GCM2* gene was amplified according to the manufacturer’s instructions using the appropriate primers (GCM2ex5F: CCCTTCCTTCCCAAAGTCTGA; GCM2ex5R: CCATGGTTAGTCCTTTCCACAAG) and AmpliTaq Gold® 360 Master Mix (Life Technologies, Carlsbad, CA, USA) in a thermocycler (Veriti, Applied Biosystems, Thermo Scientific, Waltham, MA, USA). The PCR protocol was 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s followed by a final extension step of 72 °C for 10 min. The PCR product was purified from unused primers and nucleotides with ExoSAP-IT® PCR Product Cleanup reagent (Affymetrix Inc., Santa Clara, CA, USA).

2.7. Single nucleotide polymorphism (SNP) analysis

The single nucleotide polymorphisms were amplified using the fluorescent dideoxy chain termination method of cycle sequencing with color-tagged dNTPs and antisense primers according to the manufacturers instructions (Big Dye® Terminator, Applied Biosystems). The amplification protocol consisted of an initial denaturation of 96°C for 1 min and 25 cycles of 96 °C for 10 s, 50°C for 5 s, and 60 °C for 10 s. The amplified product was purified with an AutoSeq G50 column (GE Healthcare, Little Chalfont, UK), sequenced (ABI PRISM® 3130xl Genetic Analyzer, Applied Biosystems), and analyzed (SeqScape software, Applied Biosystems).

2.8. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Software release 23.0 (2013, Chicago, USA). Continuous variables were presented as means ± SD, whereas categorical variables were presented as frequencies and percentages. Analysis of normality of the distribution of each continuous variable was performed using the Kolmogorov-Smirnov test prior to further statistical testing. The Mann-Whitney-U-test was used for comparison of non-parametric values between two study groups. Proportions were assessed using Chi-Squared and Fisher-Exact tests as appropriate. A P value < 0.05 was considered significant.

3. Results

3.1. Patient characteristics

The patient cohort consisted of 7 women and 16 men with sporadic parathyroid carcinomas and 28 women and 11 men with atypical adenomas, resulting in female to male ratios of 0.4:1 and 2.5:1, respectively. The median age at surgery for patients with SPC and AA was 60.1 and 59.0 years, respectively. All patients had primary hyperparathyroidism (PHPT). Patient clinical data did not significantly differ between the SPC and AA groups (Table 3).

3.2. Distribution of single nucleotide polymorphisms (SNPs)

Among the 62 index cases, 9 (14.5%) patients were heterozygous for a heterozygotic SNP in the *GCM2* gene (Table 4). The numbers of patients harboring an SNP in the *GCM2* gene in the SPC and AA groups were 4 (17.4%) and 5 (12.8%), respectively. The percentage of patients with SNPs in the *GCM2* gene was not significantly different between the two groups ($p = 0.715$). The OR for patients from both groups to have a variant of the *GCM2* gene was 1.36 (0.41–4.55). Patients in the SPC group exhibited a 1.43-fold higher risk for a *GCM2* gene variant than patients in the AA group (OR 1.43 [0.234–5.99]).

Six patients (9.7%) were heterozygous for the Y282D variant (c.844 T>G Y282D) of the *GCM2* gene (Table 5). The Y282D gene variant was detected in 4 of 23 patients (17.4%) in the SPC group and 2 of 39 patients (5.1%) in the AA group. The percentage of patients harboring the Y282D gene variant was not significantly different between groups ($p = 0.153$) (Table 4).

Three patients (4.8%) in the patient cohort, all of which were in the AA group (7.7%), harbored one of two additional SNPs in the *GCM2* gene, c.900 T > C p.Asn300 orc.905 C >

T p.Thr302Ile, neither of which are known to modify the transcriptional activity of the protein (Tables 4 and 5). The percentages of patients in the SPC group without an SNP in the *GCM2* gene, with the Y282D gene variant, or with other variants did not significantly differ from those in the AA group (p = 0.173) (Tables 4 and 5).

3.3. Association of the *GCM2* gene variant with clinical parameters

Between the SCP and AA groups, there was no difference in the clinical parameters of pre-surgical vitamin D levels and IOPTH (intra-surgical PTH level decrease after removal of the pathological tissue) in patients with the Y282D variant or other variants of the *GCM2* gene (Table 6). SNP of the *GCM2* gene was not associated with these parameters. The PTH level in patients without any SNP (group 1, 631 ±761.6 ng/l) was higher than in that in the groups with SNPs (groups 2, 3 and 4; 436 ±220.3, 476 ±284 and 382 ± 131 ng/l, respectively), but these differences were not statistically significant. However, the blood calcium levels were significantly elevated in patients with a *GCM2* gene variant relative to those without any *GCM2* gene variant (Table 6; group 1 vs. group 2; p = 0.025 and p = 0.007 for pre- and post-surgical treatment, respectively). This difference in calcium levels was even more pronounced in patients with the Y282D variant (group 1 vs. group 3; p = 0.005 and p = 0.010 for pre- and post-surgical treatment, respectively) relative to those without any SNP of the *GCM2* gene (Table 6).

4. Discussion

The present study demonstrated a 17.4% prevalence of the Y282D variant of the *GCM2* gene in germ-line DNA of patients with a definitive histological diagnosis of sporadic parathyroid carcinoma and a 5.1% prevalence in patients with a definitive histological diagnosis of atypical adenoma. Our study provides the first evaluation of the potential relationship between the Y282D variant of the *GCM2* gene and parathyroid carcinoma.

Multiple studies using phylogenetic analysis and knock-out mouse models have revealed that the *GCM2* gene is the master regulator of parathyroid gland development [6,8,19–21]. Studies of chicken embryos and mice have shown that *GCM2* is expressed at the pharyngeal pouch of developing parathyroids in mammals [8,21]. Studies using mouse models have revealed that the transcription factor encoded by *GCM2* plays a key role in regulating embryonic development of the parathyroids; its expression is limited to the parathyroid glands, and loss-of-function mutations in GCM2 compromise parathyroid formation [8,21].

GCM2 is expressed at the initial stages of embryonic development, but it is essential at later stages when it coordinates differentiation and survival of parathyroid cells during parathyroid formation. In humans, loss-of-function mutations in GCM2 lead to pediatric-onset congenital hypoparathyroidism [2,3]. The detailed molecular mechanisms of the transcription factor encoded by *GCM2* are not known. However, its biological function is known to be downstream of every other transcription factor involved in embryonic parathyroid development and upstream of the production and secretion of PTH [20,22].

In 2014, D'Agruma et al. studied the Y282D variant of GCM2 in hyperparathyroidism [4]. They analyzed the frequency of the Y282D variant in more than 500 patients with primary

hyperparathyroidism (PHPT) and in a control population of more than 600 people. The prevalence of the variant was found to be significantly higher in PHPT patients than in controls (11.1% vs. 3.1%). Using an *in vitro* GCM promoter-luciferase reporter assay in HEK293 cells, they showed that the Y282D variant had significantly higher transcriptional activity (by 1.4-fold) than the wild-type form. They suggested that this overactive variant could be a cause of the pathogenesis of parathyroid hyperactivity.

Based on these observations, we hypothesized that the Y282D variant of GCM2 contributes to the pathogenesis of sporadic parathyroid tumors. Our study demonstrated the prevalence of the Y282D variant of the *GCM2* gene in patients with a definitive histological diagnosis of sporadic parathyroid carcinoma or atypical adenoma, implicating this SNP as a factor contributing to parathyroid tumorigenesis. This was the first analysis of germ-line DNA from patients with sporadic parathyroid carcinoma.

The present study corroborated recent reports on the role of the Y282D variant of the *GCM2* gene in the development of primitive hyperparathyroidism and demonstrated for the first time its association with sporadic parathyroid carcinoma. These findings were compared with previously described cohorts of patients with hyperparathyroidism and healthy controls. Since it is known that the variants of the *GCM2* gene are differentially distributed based on geography and that the frequency of Y282D exhibits a regionaltrend in Italy, we compared our data to the data from a neighboring sub-population gathered at the hospital center of Milan [4]. In the present study, the prevalence of Y282D in sporadic parathyroid carcinoma patients was 17.4%, which was higher than the previously reported 11.1% in patients with primitive hyperparathyroidism. This 17.4% prevalence is also higher than that reported for healthy controls (3.1%) [4]. We demonstrated the prevalence of Y282D in patients with atypical adenoma to be 5.1%, which is higher than that in healthy controls (3.1%) [4] but lower than that in patients with primitive hyperparathyroidism (11.1%) [4]. Atypical adenoma shows histologic characteristics of parathyroid cancer except for a lack of signs of invasion. Atypical adenoma was demonstrated to be a different entity with no progression towards carcinoma, resulting in better prognosis [23]. In the present study, we demonstrated a distinct prevalence of Y282D between the two groups (17.4% for the SPC group and 5.1% for the AA group). Since invasiveness is the most distinguishable characteristic between SPC and AA, further investigation into the contribution of the Y282D variant to invasion is warranted. Due to its increased transcriptional activity (1.4-fold higher than the wild type form) [4], the Y282D variant could confer a selective advantage in the context of parathyroid tumor development. Other *GCM2* gene variants with increased transcriptional activity may have significant biologic effects that induce tumorigenic progression. Therefore, we cannot exclude the possibility that there were other analogous variants in our patients, which we have not yet considered.

In the present study, the 282D variant was associated with elevated serum calcium levels ($p < 0.025$). The average PTH level in patients without *GCM2* SNPs was higher than in patients with *GCM2* SNPs, but these differences were not statistically significant. Since GCM2 continues to be expressed in adult parathyroid tissue, it may be essential for the expression of the PTH gene and the calcium sensing receptor (CASR) [4]. SNPs in genes such as CASR and VDR have been associated with hyperparathyroidism itself and/or

features related to the presentation of this condition, and these SNPs could be a cause of GCM2 polymorphisms [4].

A limitation of this study is the small sample size, due to the rarity of this pathology [1], yielding wide confidence intervals that hinder statistically significant results. Control data were obtained from a previous study using Italian cohorts. A cohort of control samples in this study was not available because of the high patient numbers needed (more than one thousand patients).

5. Conclusions

Our study provides the first evaluation of the potential relationship between the Y282D variant of the *GCM2* gene and parathyroid malignancy. The Y282D variant was significantly associated with increased serum calcium levels in both SPC and AA patients. These data support a potential biological role of Y282D in promoting parathyroid pathology. The relationship between the Y282D variant and tumorigenic progression warrants further investigation.

Acknowledgements

The authors thank Prof. Giuseppe Opocher and Dr. Francesca Boaretto for their support as well as Dr. Francesca Schiavi and Dr. Virginia Lanza for their excellent technical assistance (Veneto Institute of Oncology IOV-IRCCS, Padova, Italy). We are grateful to Dr. Clara Benna (Surgical Pathology Unit, Department of Surgery, Oncology and Gastroenterology Sciences, University-Hospital of Padova, Italy) for the organization of the samples. We also thank Dr. Nani Osada (Department of Biomedical Informatics, Medical Faculty, University of Munster, Germany) for the statistical analysis.

Funding

The work was funded by the University of Padova, Italy.

References

[1]. van der Zwan JM, Mallone S, van Dijk B, Bielska-Lasota M, Otter R, Foschi R, Baudin E, Links TP, Rarecare WG, Carcinoma of endocrine organs: results of the RARECARE project, Eur. J. Cancer 48 (2012) 1923–1931. [PubMed: 22361014]

[2]. Bowl MR, Mirczuk SM, Grigorieva IV, Piret SE, Cranston T, Southam L, Allgrove J, Bahl S, Brain C, Loughlin J, Mughal Z, Ryan F, Shaw N, Thakker YV, Tiosano D, Nesbit MA, Thakker RV, Identification and characterization of novel parathyroid-specific transcription factor Glial Cells Missing Homolog B (GCMB) mutations in eight families with autosomal recessive hypoparathyroidism, Hum. Mol. Genet 19 (2010) 2028–2038. [PubMed: 20190276]

[3]. Maret A, Ding C, Kornfield SL, Levine MA, Analysis of the GCM2 gene in isolated hypoparathyroidism: a molecular and biochemical study, J. Clin. Endocrinol. Metab 93 (2008) 1426–1432. [PubMed: 18182452]

[4]. D'Agruma L, Coco M, Guarnieri V, Battista C, Canaff L, Salcuni AS, Corbetta S, Cetani F, Minisola S, Chiodini I, Eller-Vainicher C, Spada A, Marcocci C, Guglielmi G, Zini M, Clemente R, Wong BY, de Martino D, Scillitani A, Hendy GN, Cole DE, Increased prevalence of the GCM2 polymorphism, Y282D, in primary hyperparathyroidism: analysis of three Italian cohorts, J. Clin. Endocrinol. Metab 99 (2014) E2794–2798. [PubMed: 25279501]

[5]. Mannstadt M, Holick E, Zhao W, Juppner H, Mutational analysis of GCMB, a parathyroid-specific transcription factor, in parathyroid adenoma of primary hyperparathyroidism, J. Endocrinol 210 (2011) 165–171. [PubMed: 21642377]

[6]. Kebebew E, Peng M, Wong MG, Ginzinger D, Duh QY, Clark OH, GCMB gene, a master regulator of parathyroid gland development, expression, and regulation in hyperparathyroidism, Surgery 136 (2004) 1261–1266. [PubMed: 15657585]

[7]. Wegner M, Riethmacher D, Chronicles of a switch hunt: gcm genes in development, Trends Genet. 17 (2001) 286–290. [PubMed: 11335039]

[8]. Okabe M, Graham A, The origin ofthe parathyroid gland, Proc. Natl. Acad. Sci. U. S. A 101 (2004) 17716–17719. [PubMed: 15591343]

[9]. Doyle D, Kirwin SM, Sol-Church K, Levine MA, A novel mutation in the GCM2 gene causing severe congenital isolated hypoparathyroidism, J. Pediatr. Endocrinol. Metab 25 (2012) 741–746. [PubMed: 23155703]

[10]. Mannstadt M, Bertrand G, Muresan M, Weryha G, Leheup B, Pulusani SR, Grandchamp B, Juppner H, Silve C, Dominant-negative GCMB mutations cause an autosomal dominant form of hypoparathyroidism, J. Clin. Endocrinol. Metab 93 (2008) 3568–3576. [PubMed: 18583467]

[11]. Evangelista L, Sorgato N, Torresan F, Boschin IM, Pennelli G, Saladini G, Piotto A, Rubello D, Pelizzo MR, FDG-PET/CT and parathyroid carcinoma: review of literature and illustrative case series, World J. Clin. Oncol 2 (2011) 348–354. [PubMed: 22022662]

[12]. Rubello D, Al-Nahhas A, Mariani G, Gross MD, Rampin L, Pelizzo MR, Feasibility and long-term results of focused radioguided parathyroidectomy using a low 37 MBq (1 mCi) 99mTc-sestamibi protocol, Int. Semin. Surg. Oncol 3 (2006) 30.

[13]. Mariani G, Gulec SA, Rubello D, Boni G, Puccini M, Pelizzo MR, Manca G, Casara D, Sotti G, Erba P, Volterrani D, Giuliano AE, Preoperative localization and radioguided parathyroid surgery, J. Nucl. Med 44 (2003) 1443–1458. [PubMed: 12960191]

[14]. Rosato L, Raffaelli M, Bellantone R, Pontecorvi A, Avenia N, Boniardi M, Brandi ML, Cetani F, Chiofalo MG, Conzo G, De Palma M, Gasparri G, Giordano A, Innaro N, Leopaldi E, Mariani G, Marcocci C, Marini P, Miccoli P, Nasi P, Pacini F, Paragliola R, Pelizzo MR, Testini M, De Toma G, Diagnostic, therapeutic and healthcare management protocols in parathyroid surgery: II consensus conference of the italian association of endocrine surgery units (U.E. C. CLUB), J. Endocrinol. Invest 37 (2014) 149–165. [PubMed: 24497214]

[15]. Schantz A, Castleman B, Parathyroid carcinoma.Astudyof70cases, Cancer 31 (1973) 600–605. [PubMed: 4693587]

[16]. Sandelin K, Auer G, Bondeson L, Grimelius L, Farnebo LO, Prognostic factors in parathyroid cancer: a review of 95 cases, World J. Surg 16 (1992) 724–731. [PubMed: 1413841]

[17]. Rodgers SE, Perrier ND, Parathyroid carcinoma, Curr. Opin. Oncol 18 (2006) 16–22. [PubMed: 16357559]

[18]. McClenaghan F, Qureshi YA, Parathyroid cancer, Gland Surg. 4 (2015) 329–338. [PubMed: 26312219]

[19]. Graham A, Richardson J, Developmental and evolutionary origins of the pharyngeal apparatus, Evodevo 3 (2012) 24. [PubMed: 23020903]

[20]. Grevellec A, Graham A, Tucker AS, Shh signalling restricts the expression of Gcm2 and controls the position of the developingparathyroids, Dev. Biol 353 (2011) 194–205. [PubMed: 21349263]

[21]. Zou D, Silvius D, Davenport J, Grifone R, Maire P, Xu PX, Patterning of the third pharyngeal pouch into thymus/parathyroid by Six and Eya1, Dev. Biol 293 (2006) 499–512. [PubMed: 16530750]

[22]. Grigorieva IV, Thakker RV, Transcription factors in parathyroid development: lessons from hypoparathyroid disorders, Ann. N. Y. Acad. Sci 1237 (2011) 24–38. [PubMed: 22082362]

[23]. Fernandez-Ranvier GG, Khanafshar E, Jensen K, Zarnegar R, Lee J, Kebebew E, Duh QY, Clark OH, Parathyroid carcinoma, atypical parathyroid adenoma, or parathyromatosis? Cancer 110 (2007) 255–264. [PubMed: 17559137]

Function	mRNA				Protein		
	SNP for mRNA	Accession	Position	Allele Change	Accession	Position	Residue Change
missense	Rev	NM 004752.3	916	**T**AT→ **G**AT	NP 004743.1	282	**Y [Tyr]→ D[Asp]**

Fig. 1.

Gene model and wild type sequence of the Glial Cell Missing homolog 2 gene (*GCM2*) at the locus of the SNP (position 916) responsible for the Y282D variant. Rev = reverse, Tyr = tyrosine, Asp = aspartic acid. https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=61734277.

Table 1

Surgical excision area: n = number of patients (%).

		Thyroid lobectomy		Parathyroidectomy					Lymph node
				superior		inferior		ectopic	
	n	right	left	right	left	right	left		
Sporadic parathyroid carcinoma	23	10 (43)	8 (35)	7 (30)	6 (26)	5 (22)	6 (26)	9 (39)	2 (9)
Atypical adenoma	39	10 (26)	14 (36)	8 (21)	12 (31)	4 (10)	18 (46)	16 (41)	5 (13)

Table 2

Histological criteria of malignancy for parathyroid tumors.

Histological criteria	Sporadic parathyroid carcinoma	Atypical adenoma
Local invasion of adjoining structures	X	
Distant or lymph node metastasis	X	
Vascular invasion	X	
Capsular rupture	X	
Thick fibrous bands	X	X
Pleomorphic cells and trabecular patterns	X	X
Mitosis	X	X

Table 3

Distribution of the clinical data within the patient groups: serum calcium levels pre- and post-surgical treatment, PTH levels pre-surgical treatment, vitamin D levels pre-surgical treatment, and intra-surgical decrease in PTH levels after removal of the pathological tissue (IOPTH).

	Sporadic parathyroid carcinoma		Atypical adenoma		
Parameter	**median**	**range [min-max]**	**median**	**range [min-max]**	**p-value**
pre-surgical calcium level [mmol/1]	3.1	2.6–3.9	3.1	2.3–4.5	0.971
pre-surgical PTH level [ng/1]	527	91–1387	368	43–4000	0.488
pre-surgical vitamin D level [nmol/1]	41	30–65	44	17–117	0.910
IOPTH [%]	95	58–98	91	−17–97	0.090
post-surgical calcium level [mmol/1]	2.5	2.0–3.1	2.2	1.8–3.9	0.227

Table 4

Identified SNP of the *GCM2* gene (region c.717–c.1023 of Gcm2).

Identified SNP	n
NM_004752.3c.844 T>G **Y282D** hetero	6
NM_004752.3c.900 T>C p.Asn300 hetero	2
NM_004752.3c.905 C>T p.Thr302Ile hetero	1

Table 5

Distribution of the gene variants within the groups.

	Number of patients	Sporadic parathyroid carcinoma (SPC)	Atypical adenoma (AA)	p-value SPC vs. AA
Total number of patients	62	23	39	
Without gene variant	53 (85.5%)	19 (82.6%)	34 (87.2%)	
With gene variant	9 (14.5%)	4 (17.4%)	5 (12.8%)	0.715
With gene variant Y282D	6 (9.7%)	4 (17.4%)	2 (5.1%)	0.153
With gene variant other than Y282D	3 (4.8%)	0	3 (7.7%)	

Table 6

Influence of the gene variant on clinical parameters. Values are presented as means ± SD.

Group distribution	n	pre-surgical calcium level [mmol/1]	pre-surgical PTH level [ng/1]	pre-surgical vitamin D level [nmol/1]	IOPTH [%]	post-surgical calcium level [mmol/1]
Group 1: without gene variant	53	3.11 ± 0.518	631 ± 761.6	48 ± 23.6	84.7 ± 22.1	2.39 ± 0.348
Group 2: with any gene variant	9	3.40 ± 0.390	436 ± 220.3	39 ± 21.5	89.7 ± 7.71	2.69 ± 0.290
Group 3: with gene variant Y282D	6	3.57 ± 0.312	476 ± 284	65 ± SD	86.6 ± 10.6	2.73 ± 0.289
Group 4: with gene variant other than Y282D	3	3.07 ± 0.332	382 ± 131	31 ± 15.8	92.9 ± 2.55	2.60 ± 0.329
p-value group 1 vs. group 2 without vs. any gene variant		0.025	0.956	0.646	0.992	0.007
p-value group 1 vs. group 3 without vs. gene variant Y282D		0.005	0.956	0.437	0.768	0.010
p-value group 1 vs. group 4 without vs. gene variant other than Y282D		0.871	0.873	0.266	0.796	0.233
p-value group 3 vs. group 4 gene variant Y282D vs. gene variant other than Y282D		0.167	0.743	0.500	0.700	0.667