RESEARCH

Screening for germline *KCNQ1* and *KCNE2* mutations in a set of somatotropinoma patients

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Abstract

Objective: Recently, mutations in *KCNQ1*, a potassium channel gene usually linked to long QT syndrome, were reported to cause maternally inherited gingival fibromatosis and growth hormone deficiency (GHD). Expression of the mutated *KCNQ1* with the auxiliary potassium channel subunit *KCNE2* was shown to reduce pituitary hormone secretion in functional experiments. Here, we investigated if germline mutations in *KCNQ1* and *KCNE2* were present in patients with somatotropinomas, which represent a model of growth hormone excess.

Design and methods: *KCNQ1* and *KCNE2* were screened for germline mutations in 53 patients with acromegaly by Sanger sequencing. Effects of the variants were predicted by *in silico* tools.

Results: Only deep intronic and synonymous polymorphisms were detected in *KCNQ1*. These findings were likely insignificant based on *in silico* predictions and the variants' frequencies in the general population. In *KCNE2*, a heterozygous c.22A>G, p.(Thr8Ala) mutation with unknown significance was found in three patients. It was present in the database controls with a frequency of 0.0038.

Conclusions: *KCNQ1* or *KCNE2* mutations do not appear to account for somatotropinoma formation, although larger patient series are needed to validate the findings.

Key Words

- ▶ *KCNQ1*
- ▶ *KCNE2*
- ▶ growth hormone
- ▶ pituitary adenoma
- ▶ acromegaly

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Introduction

Somatic growth and final height are the sum of multiple factors: nutritional status, general health, hormones, psychosocial well-being as well as inherited and epigenetic factors. Short or tall stature and body proportions also have huge social significance and they can predict adult life health and reproductive success (1, 2). Growth hormone (GH), which is secreted from the anterior pituitary somatotroph cells, regulates human growth under the influence of hypothalamic inputs such

as growth hormone-releasing hormone (GHRH) and somatostatin as well as several peripheral hormones.

GH-secreting pituitary adenomas, i.e. somatotropinomas, lead to acromegaly in adults and (acro-) gigantism in children and adolescents. Development of acromegaly is often insidious: most patients are diagnosed in their fifth decade of life with a median diagnostic delay of 4.5–5 years (3). Acromegaly patients have increased mortality compared to the general

population (4, 5). Somatotropinomas are the third most common type (9–16.5% of cases) of pituitary adenomas after prolactinomas and non-functioning adenomas (6). In approximately 5% of all pituitary adenoma cases, affected patients have a familial background, the vast majority of acromegaly-causing somatotropinomas being spontaneous (7). Somatotropinomas can present in familial tumor-causing syndromes or the predisposition to a pituitary adenoma can run in families without syndromic characteristics or even strong family history of the disease (7). However, in about half of the familial somatotropinoma cases, the possible underlying germline genetic defect cannot be identified, which implies that genes responsible for tumor formation remain undiscovered (8). Identification of new predisposing genes would enable earlier detection of pituitary adenomas and therefore improve clinical management of patients.

Recently, we showed that two missense mutations in *KCNQ1* (potassium voltage-gated channel subfamily Q member 1), underlie maternally inherited gingival fibromatosis and autosomal dominant growth hormone deficiency which, in some patients, expanded to multiple pituitary hormone deficiency (9). Intriguingly, *KCNQ1* was shown to be expressed in mouse hypothalamic GHRH neurons and somatotrophs (9), and in functional studies, co-expression of the mutant *KCNQ1* protein with *KCNE2* (potassium voltage-gated channel subfamily E regulatory subunit 2) lead to diminished hormone secretion from a mouse pituitary cell line (9). *KCNE2* is an auxiliary subunit that is required for the formation of functional potassium channels with *KCNQ1* in several secretory or excitable cells, for instance, in the stomach, thyroid and heart (10), and, as it seems, in the pituitary (9). Based on these findings, we hypothesized that germline mutations in these two genes could lead to an opposite phenotype, overproduction of GH, and thereby predispose to somatotropinoma formation in some somatotropinoma patients.

Subjects and methods

We studied a set of 44 sporadic and one familial (patient 852, who had an affected sister) cases with GH-secreting pituitary adenomas. Moreover, whole-genome sequencing (WGS) data from eight patients with somatotropinomas were exploited. The tumors of these eight patients were *Gsp* mutation negative. The germline WGS data did not reveal mutations in known genes linked to inherited forms of pituitary adenoma (11). All patients had been

previously sequenced negative for *AIP* (aryl hydrocarbon receptor-interacting protein) and *CDKN1B* (cyclin-dependent kinase inhibitor 1B) (12) mutations as well as the *GPR101* (G protein-coupled receptor 101) variant p.Glu308Asp.

Patient age at diagnosis ranged from 14 to 56 years with the mean age of 39 years (Table 1). One of the studied cases was a giant (ST10) and the rest had typical acromegaly phenotypes. All patients were operated in Finland. Forty-nine cases represented the Finnish population and four had non-Finnish origins: these patients were from Estonia, Spain, Italy and Tunis (Table 1). Informed consent was obtained from all patients, and in the case of minor/children, a parent or guardian gave the consent. The study was approved by the Ethics Committee of the Hospital district of Helsinki and Uusimaa, and it was conducted in accordance with the Declaration of Helsinki.

Peripheral blood samples were collected from the patients for DNA extraction. DNA was extracted from EDTA blood samples by a non-enzymatic procedure (13). The coding exons and exon–intron boundaries of *KCNQ1* (ENSG00000053918, ENST00000155840.9, Ensembl release 90) and *KCNE2* (ENSG00000159197, ENST00000290310.3) were then PCR amplified. The PCR conditions are available upon request and the primers are provided in Supplementary Table 1 (see section on supplementary data given at the end of this article). The PCR products were purified with ExoProStar treatment (GE Healthcare Life Sciences) and sequenced from the forward direction using the ABI BigDyeTerminator Cycle Sequencing Kit (v3.1) and ABI Prism 3730xl DNA Analyzer automated sequencer (Applied Biosystems). The DNA sequences were aligned and read with Sequencher 4.9 software (Gene Codes Corporation, Ann Arbor, MI, USA). In addition to the Sanger sequenced samples, *KCNQ1* and *KCNE2* were manually verified from the WGS normal blood-derived DNA. Both gene regions, including all the exons and intronic regions equivalent to the regions gained from the Sanger-sequenced amplicons, were analyzed.

Allele frequencies of the identified variants were validated from the Genome Aggregation Database (gnomAD) (<http://gnomad.broadinstitute.org/>) (14). This database contains WGS and exome data from 138,632 individuals including 12,897 Finnish samples. Effects of the identified variants on transcripts were predicted with Human Splicing Finder (<http://www.umd.be/HSF3/>) (15) and MutationTaster (<http://www.mutationtaster.org/>) (16) online tools. Additionally, we utilized the Polyphen-2 (Polymorphism Phenotyping v2; (<http://genetics.bwh.harvard.edu/pph2/>)) (17) and SIFT (<http://sift.jcvi.org/>)

Table 1 Patient information and the variants detected in the coding regions of *KCNQ1* and *KCNE2*. ORIGIN LISÄTTÄVÄ.

Patient	Sex	Age at Dg	Age at Op	Clinical Dg	<i>KCNQ1</i>		MAF ^c	<i>KCNE2</i>		MAF ^c
331	M	39		GH	–			–		
332	F		39	GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
335	M	42	42	GH	–			–		
336	F	25		GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
337	F	43	53	GH	c.1638G>A ^b , p.(Ser546=)	rs1057128	0.2013	–		
338	M	38		GH	–			–		
340	M	38	60	GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
341	F	39		GH	–			–		
343	M	42	42	GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
344	M	36		GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
345	M	38		GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
346	M	29		GH	–			c.22A>G, p.(Thr8Ala)	rs2234916	0.0038
347	M	30	34	GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
348	F	42	42	GH	–			–		
349	M	38	40	GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
350	F	36	36	GH	–			–		
351	M	40	40	GH	–			–		
352	M	33	33	GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
353	F	38	39	GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
415	F	39	45	GH	c.1638G>A, p.(Ser546=)	rs1057128	0.2013	–		
416	F	40	40	GH	c.1638G>A ^b , p.(Ser546=)	rs1057128	0.2013	–		
417	F	42	43	GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
418	F	44	44	GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
419	F	22	38	GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
420	F	33	33	GH	–			–		
421	M	35		GH	–			–		
422	F	32	47	GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
					c.1638G>A ^b , p.(Ser546=)	rs1057128	0.2013	–		
423	M	34	46	GH	c.1638G>A, p.(Ser546=)	rs1057128	0.2013	–		
445	F	39	39	GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
720	F	36		GH	–			–		
721	M	43		GH	–			–		
725	M	40		GH	c.1638G>A ^b , p.(Ser546=)	rs1057128	0.2013	–		
813	F		43	GH	c.1638G>A, p.(Ser546=)	rs1057128	0.2013	–		
860	F		31	GH	–			–		

(Continued)

Table 1 Continued.

Patient	Sex	Age at Dg	Age at Op	Clinical Dg	<i>KCNQ1</i>		MAF ^c	<i>KCNE2</i>		MAF ^c
861	M	47	47	GH	–			c.22A>G, p.(Thr8Ala)	rs2234916	0.0038
862	F	53		GH/PRL	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
833	M		44	GH	c.1638G>A ^b , p.(Ser546=)	rs1057128	0.2013	–		
842	M		60	GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
847	F	26		GH	c.1638G>A, p.(Ser546=)	rs1057128	0.2013	–		
848	F	53		GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
849	M	44	44	GH	–			–		
852 ^{d(I)}	M	37	37	GH	–			–		
856	M	25		GH	–			–		
857	M	41		GH/PRL	c.1638G>A, p.(Ser546=)	rs1057128	0.2013	–		
858	F	33		GH	c.1638G>A, p.(Ser546=)	rs1057128	0.2013	–		
ST5 ^a	M	56		GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
ST6 ^{a(T)}	M	40		GH	–			c.22A>G, p.(Thr8Ala)	rs2234916	0.0038
ST7 ^{a(E)}	F	40		GH	c.1638G>A, p.(Ser546=)	rs1057128	0.2013	–		
ST8 ^a	F	55		GH	–			–		
ST9 ^a	F	38		GH	c.1638G>A, p.(Ser546=)	rs1057128	0.2013	–		
ST10 ^{a,e}	M	14		GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
ST11 ^a	F	24		GH	c.1986C>T, p.(Tyr662=)	rs11601907	0.1748	–		
ST12 ^{a(S)}	M	37		GH	c.1638G>A, p.(Ser546=)	rs1057128	0.2013	–		

^aWhole genome sequencing data from Välimäki et al. 2015 (11); ^bHomozygous change; ^cMinor allele frequency (MAF); control allele frequencies: <http://gnomad.broadinstitute.org/> (14); ^dFamilial background; ^eGiant.

Dg, diagnosis; E, Estonian; GH, growth hormone; I, Italian; Op, operation; PRL, prolactin; S, Spanish; T, Tunisian.

(18) tools and the Clinvar database (<https://www.ncbi.nlm.nih.gov/clinvar/>) in the functional predictions of the c.22A>G, p.(Thr8Ala) missense mutation found in *KCNE2*. The significance of the allele frequency differences between the cases and database controls were calculated with Fisher's exact test by using R software. $P < 0.05$ was accepted to indicate statistical significance.

Results

To evaluate if germline mutations in *KCNQ1* and *KCNE2* could increase susceptibility to somatotropinoma, we amplified and Sanger-sequenced all coding exons and exon–intron boundaries of these genes from the patient genomic DNA. *KCNQ1* and *KCNE2* were also screened for somatic mutations in the eight previously

whole-genome sequenced samples. No somatic hits were detected. Effects of the found variants on gene function were predicted with several *in silico* tools, and the gnomAD database (14) was used as a variant frequency control set.

From the blood-derived DNA, we detected two synonymous coding variants in *KCNQ1*, c.1638G>A, p. (Ser546=) (rs1057128) and c.1986C>T, p.(Tyr662=) (rs11601907). According to Human Splicing Finder and MutationTaster, these synonymous variants do not likely affect splicing. The c.1638G>A, p.(Ser546=) variant had a frequency of 0.2013 and the c.1986C>T, p.(Tyr662=) variant 0.1748 in the gnomAD database control population (Table 1). Taken together, the *in silico* predictions and frequencies in the general population suggest that these variants are benign. Moreover, both of the variants are silent that do not alter the amino acid sequence of

the protein. All other variants detected in *KCNQ1* were located deep in introns (13 >bp from exon borders) and common in the gnomAD control population and/or were not predicted to affect splicing (Supplementary Table 2).

In three patients, one heterozygous missense variant in *KCNE2*, c.22A>G, p.(Thr8Ala) (rs2234916), was detected with an allele frequency of 0.028 among the patients. The frequency of this variant was 0.0038 in the gnomAD database, in which it was detected in a homozygous state in eight individuals. Among the Finnish controls (12,897 individuals), the variant frequency was 0.0038 as well. A significant difference in the allele frequencies between the patients and controls was found (OR, 7.73; 95% CI, 1.56–23.32; $P=0.008$). This variant was disease causing according to MutationTaster, deleterious according to SIFT

(score 0) and probably damaging according to PolyPhen-2 (score, 0.991). To evaluate allelic imbalance at the chromosomal region of c.22A>G, p.(Thr8Ala), we explored the WGS data of patient ST6. The sequences were obtained from the normal blood-derived DNA and the respective tumor tissue (11). Somatic copy-number profiling did not reveal allele gain or loss at the locus (Fig. 1).

Discussion

Endocrine pituitary cells express numerous voltage-gated sodium, calcium, potassium and chloride ion channels (19). Ionic mechanisms play an essential role in the regulation of hormone secretion in pituitary cells,

Figure 1

Whole-genome sequence reads of patient ST6 showing a heterozygous c.22A>G *KCNE2* variant in DNA extracted from peripheral blood leukocytes and respective tumor tissue. Both blood and tumor tissues show close to 50–50% allele fraction. Only representative portion of the reads is seen in the figure. Coverage: number of total reads (61 reads cover the position), G: number of reads containing the G allele (51% and 52%). Red read: + direction, green read: – direction. An in-house tool BasePlayer (<https://doi.org/10.1101/126482>) was used for the variant analysis.

such as GH-secreting somatotrophs. The status of electrical signaling in the pituitary cells is dependent on the release of stimulating and inhibiting neurohormones from the hypothalamus (19). These neurohormones act by stimulating and inhibiting G protein-coupled receptors (GPCRs) and $G\alpha_S$ and $G\alpha_i/o$ protein-coupled receptors (19, 20, 21). Both $G\alpha_S$ and $G\alpha_i$ signaling are known to be essential factors in the formation of somatotropinomas via cyclic adenosine monophosphate (cAMP) synthesis (21). cAMP is a mitogenic factor in somatotrophs, and increase in the intracellular cAMP concentration leads to the activation of protein kinase A (PKA) as well as further mitogenic signaling in GH-secreting somatotrophs (19, 20, 21).

Potassium channel genes can also be implicated in tumorigenesis, including angiogenesis, tumor invasion and growth (22). For example, recent findings suggest that *KCNQ1* is a key regulator and a target gene in the Wnt/ β -catenin signaling pathway (23), which is implicated in pituitary development and tumorigenesis of craniopharyngiomas (24). Rapetti-Mauss *et al.* (23) showed that *KCNQ1* suppresses the Wnt/ β -catenin signaling pathway and forms complexes with β -catenin and E-cadherin in the cell membrane. High expression of *KCNQ1* was associated with high expression of E-cadherin. When *KCNQ1* was suppressed, E-cadherin and β -catenin moved to the cytosol from the membrane, which was associated with proliferation of colorectal cancer cells and epithelial-to-mesenchymal transition (EMT) (23).

KCNQ1 is present in the somatotrope cell membrane (9), and E-cadherin is expressed in somatotropinomas (25). In a study by Fougner and colleagues, downregulation and redistribution of E-cadherin were associated with large tumors and partly to tumor invasiveness: Specifically, translocation of E-cadherin to the nucleus and subsequent loss of its membranous expression was demonstrated in a subset of exceptionally large, somatostatin analog (SMS)-resistant somatotropinomas (25). Wnt pathway inhibitors have been found to be downregulated in GH-secreting pituitary tumors (26, 27), suggesting that Wnt signaling, possibly including *KCNQ1* and E-cadherin, may participate in somatotropinoma formation.

In our patient set, we did not find any potentially pathogenic *KCNQ1* changes. Two identified variants were synonymous substitutions and common in controls. In addition to *KCNQ1*, we screened the coding region of *KCNE2* in our acromegaly patients. *KCNE2* forms complexes with *KCNQ1* in gastric parietal cells (28), it participates in the proliferation and activity of

gastric secretory epithelial cells and its downregulated expression is implicated in gastric cancer (29, 30). In the present study, three patients carried the heterozygous c.22A>G, p.(Thr8Ala) (rs2234916) missense variant, which was the only *KCNE2* variant found in our patient series. The allele frequency of this variant in the patient group was significantly higher compared to the general population ($P=0.008$). Intriguingly, this finding suggests an association of the c.22A>G, p.(Thr8Ala) variant with acromegaly. In past publications, the c.22A>G, p.(Thr8Ala) variant has been described as potentially increasing the risk of congenital or acquired long QT syndrome and drug-induced cardiac arrhythmia, and it has been found in both healthy individuals and affected patients (31, 32, 33, 34, 35, 36, 37, 38).

Anatomical pathology diagnosis revealed that all three tumors with the p.(Thr8Ala) variant were typical acidophilic GH-secreting adenomas without significant atypia. Tumors of 861 and ST6 showed low proliferation rates (Ki-67 1–2%) and were p53 negative. No Ki-67 and p53 immunostaining were performed for the third tumor. Available electrocardiograms (ECG) showed that patient 861 had a normal QT interval. Patient ST6 had a prolonged QT interval during transient diabetic ketoacidosis (DKA). Prolonged QT interval occurs frequently during DKA (39, 40, 41). In the case of our patient, the QT interval returned to normal after recovery and was normal in all subsequent ECGs.

We have recently identified *KCNQ1*, a gene previously implicated in cardiac arrhythmia syndromes, as a new factor associated with human growth and the anterior pituitary function (9). In the current study, we identified two coding *KCNQ1* and one *KCNE2* germline variants in somatotropinoma patients. While the definite exclusion of the pathogenic role of genetic variants is often challenging, our observations suggest that these variants are not associated with somatotropinoma tumorigenesis. However, our patient cohort was limited in size. Therefore, additional germline and/or somatic mutation screenings in larger patient cohorts with early onset and familial cases are needed, and the expression of *KCNQ1* and *KCNE2* in tumor tissues might be worth investigating. Especially the possible association of the *KCNE2* c.22A>G, p.(Thr8Ala) variant with acromegaly requires further studies.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/EC-18-0123>.

Declaration of interest

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

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