Mutation Analysis of *Inhibitory Guanine Nucleotide Binding Protein Alpha* (*GNAI*) Loci in Young and Familial Pituitary Adenomas

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**Abstract**

Pituitary adenomas are neoplasms of the anterior pituitary lobe and account for 15–20% of all intracranial tumors. Although most pituitary tumors are benign they can cause severe symptoms related to tumor size as well as hypopituitarism and/or hypersecretion of one or more pituitary hormones. Most pituitary adenomas are sporadic, but it has been estimated that 5% of patients have a familial background. Germline mutations of the tumor suppressor gene *aryl hydrocarbon receptor-interacting protein (AIP)* predispose to hereditary pituitary neoplasia. Recently, it has been demonstrated that *AIP* mutations predispose to pituitary tumorigenesis through defective inhibitory GTP binding protein (*Gαi*) signaling. This finding prompted us to examine whether germline loss-of-function mutations in *inhibitory guanine nucleotide (GTP) binding protein (GNAI)* loci are involved in genetic predisposition of pituitary tumors. To our knowledge, this is the first time *GNAI* genes are sequenced in order to examine the occurrence of inactivating germline mutations. Thus far, only somatic gain-of-function hot-spot mutations have been studied in these loci. Here, we have analyzed the coding regions of *GNAI1, GNAI2*, and *GNAI3* in a set of young sporadic somatotropinoma patients (*n* = 32; mean age of diagnosis 32 years) and familial index cases (*n* = 14), thus in patients with a disease phenotype similar to that observed in *AIP* mutation carriers. In addition, expression of *Gαi* proteins was studied in human growth hormone (GH), prolactin (PRL), adrenocorticotropic hormone (ACTH)-secreting and non-functional pituitary tumors. No pathogenic germline mutations affecting the *Gαi* proteins were detected. The result suggests that loss-of-function mutations of *GNAI* loci are rare or nonexistent in familial pituitary adenomas.

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**Introduction**

Pituitary adenomas are neoplasms of the anterior pituitary lobe. They account for 15–20% of all the intracranial tumors [1] and approximately 16% of all the primary brain and central nervous system tumors [2]. The hallmarks of pituitary tumors are hormonal dysfunction, i.e hormonal hypersecretion or hypopituitarism and local symptoms related to the tumor mass. Compression of neighboring structures may cause headaches and visual impairment [3]. Pituitary adenomas are classified based on the pituitary cell of origin and the type of hormone secreted. The most common functional pituitary tumors hypersecrete prolactin (PRL) (40–45%). Patients with prolactinomas present with amenorrhea, infertility and galactorrhea in females, and infertility in males. Somatotropinomas hypersecrete growth hormone (GH) (20–25%), causing acromegaly with clinical features of enlarged extremities, coarse facial structure and comorbidities such as hypertension, cardiovascular disease and diabetes mellitus [4]. The rate of mortality associated to untreated acromegaly has been reported to be two to four times higher than that seen in the healthy population [5,6]. In many cases, slow progression of the symptoms delays the diagnosis [7]. Somatotropinoma during childhood or adolescence, before the growth of the long bones is complete, leads to gigantism. Tumors secreting adrenocorticotropic hormone (ACTH) (10–12%) cause Cushing's disease, which is characterized by hypercortisolism. The majority of the other adenomas are non-functioning (non-secreting) pituitary adenomas (NFPA) [4]. All in all, pituitary adenomas cause a heavy clinical burden due to increased morbidity and the treatment modalities involved, i.e neurosurgery, chronic medical therapies and radiotherapy.

Most pituitary adenomas are sporadic but it has been estimated that 5% of affected patients have a familial background [8]. Pituitary adenomas occur as components of familial tumor...
syndromes such as multiple endocrine neoplasia type 1 (MEN1) [9,10], Carney’s complex (CNC) [11,12] and MEN4 [13]. Furthermore, in 2006, Vierimaa et al. found that germline mutations in the *aryl hydrocarbon receptor interacting protein (AIP)* gene cause pituitary adenoma predisposition (PAP) [14]. AIP mutations are mostly associated with somatotropinomas (70%), although cases with prolactinomas, NFPA and Cushing’s syndrome have also been reported [15,16]. The patients with AIP mutations are typically young (mean age at diagnosis 25 years) and do not necessary have a strong family history of the disease. AIP associated pituitary tumors are often large and invasive and resistant to the effects of available treatments, such as somatostatin analogues, which are used in acromegaly [17–19]. Familial occurrence of pituitary tumors is also the main feature in familial isolated pituitary adenoma (FIPA) [8,20]. Subsequently, it was found that AIP germline mutations explain 15–20% of FIPA patients and 50% of families with familial isolated somatotropinomas (IFS) [15]. Thus, the majority of FIPA families appear to be influenced by some other, as yet unidentified genes responsible for familial clustering of pituitary tumors. Identification of new predisposing genes would enable earlier detection of pituitary adenomas and contribute to clinical management of patients.

The *stimulatory guanine nucleotide (GTP) binding protein alpha* (GNAS; encoding Gz\(a_i\) subunit) has been found to be mutated in 30–40% of sporadic somatotropinomas. These somatic gain-of-function mutations lead to constitutive activation of cyclic adenosine monophosphate (cAMP) synthesis and increased proliferation through cAMP mediated mitogenic signaling [21–24]. Activating mutations on *GNAS* are also associated to McCune-Albright syndrome [25,26]. Along with well-established GNAS mutations, somatic mutations in other Gz family members, namely GNAQ and GNA11, have been linked to tumorigenesis in melanocytic neoplasms [27,28].

We have recently demonstrated that AIP loss-of-function mutations predispose to pituitary tumorigenesis through defective inhibitory GTP binding protein (Gz\(a_i\)) signaling and consequent elevated intracellular cAMP concentrations [29]. We found that Gz\(a_2\) and Gz\(a_4\) proteins are not capable of inhibiting cAMP synthesis during AIP deficiency and that Gz\(a_4\) protein levels are significantly reduced in AIP-mutated somatotropinomas. As the AIP protein seems to be an essential regulator of Gz\(a_i\) signaling, the possibility that inactivating germline mutations in GNA1 loci (encoding Gz\(a_i\) subunits) would predispose to pituitary adenomas prompted us to investigate the role of these genes in pituitary tumorigenesis. Here we sequenced all the coding exons of GNA1 loci in familial and sporadic pituitary adenomas patients and familial index cases, thus in patients with a disease phenotype similar to that observed in AIP mutation carriers.

### Materials and Methods

**Gz\(a_i\) immunohistochemistry**

To investigate the expression of Gz\(a_i\) proteins in human pituitary tumors, Gz\(a_{1,2,3}\) and Gz\(a_{4,5}\) immunostainings were performed in four prolactinomas, six somatotropinomas, three ACTH and four NFPA tumors. All tumors were AIP mutation negative. Antibodies used were mouse monoclonal antibody against Gz\(a_1\) (SPM397, sc-36536, Santa Cruz, 1: 40), rabbit polyclonal antibody against Gz\(a_2\) (T19, sc-7276, Santa Cruz, 1: 60) and mouse polyclonal antibody against Gz\(a_3\) (H00002773-B01P, Abnova Corp. Taïpei city, Taiwan, 1: 50). Anti-mouse/rabbit/ rat secondary antibody, Poly-HRP-GAM/R/R (DPVB55HRP, Immunologic, Duiven, Netherlands) and DAB chromogen (Lab Vision Corporation, Fremont, CA, USA, Thermo Fisher Scientific, Watham, MA, USA) were used for detection. Immunostaining protocol was applied as described [30]. The staining intensities of Gz\(a_i\) proteins were scaled as negative (0), weak (1), moderate (2), or strong (3). The images were taken and edited by Leica DM LB microscope (Meyer Instruments, Houston, TX, USA), Olympus DP50 camera (Olympus Corporation, Tokyo, Japan) and Studio Lite software (Lacor, Lincoln, NE, USA).

**Patients**

This study included a set of 32 young sporadic GH-secreting pituitary adenoma cases in which three of the tumors were secreting both GH and PRL. Age at diagnosis for sporadic cases ranged from 14 to 56 years with a mean of 32 years (Table 1). A majority of the tumors were macroadenomas. The second set of samples included 14 index cases with a familial history of pituitary adenomas (Table 1). The hormones secreted by the tumors were GH (\(n = 11\)), PRL (\(n = 1\)), ACTH (\(n = 1\)) and NFPA (\(n = 1\)). All the patients had previously been sequenced negative for AIP. From familial cases 9/14 were earlier screened negative for large germline deletions of AIP [31]. The study and the consent procedures were approved by the Ethics Committee of the Hospital district of Helsinki and Uusimaa (HUS) (approval number: 408/13/03/03/2009) and the Institutional Review Board of the Department of Internal Medicine, General Hospital, Montebelluna (Treviso). Signed informed consent was obtained from all the study participants. In case of the minor/children, the consent was obtained from parent/guardian. Consents are stored and managed together with patient information in the central office/ambulatories where the access is restricted.

**Mutation Analysis on GNA1 loci**

The coding regions of GNA11 (ENST00000442586 and ENST00000351004; Ensemble release 75), GNA12 (ENST00000422163, ENST00000451956 and ENST00000266027), and GNA13 (ENST00000369851) were amplified and sequenced from blood-derived DNA. Also intronic regions flanking the exons were included in the analyses. PCR was carried out by mixing 0.25 µl 20 mM of each primers (Table 2), 5 ng/ul of DNA, 0.4 µl 40 mM of dNTP, 2.5 µl 10xPCR Buffer, and 0.1 µl AmpliTaq Gold DNA Polymerase (InVitrogen Life Science Technologies, Foster City, CA) in a final volume of 25 µl. PCR products were purified by using ExoSAP-IT PCR product cleanup reaction (Affymetrix, USB Products, CA, USA). DNA was sequenced by using BigDye v.3.1 sequencing chemistry and ABI3300x DNA sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were analyzed with Mutation Surveyor software V4.0.8 (SoftGenetics, State College, PA, USA).

### Results

**Gz\(a_i\) immunohistochemistry**

To examine the Gz\(a_i\) protein expressions in human pituitary adenomas, Gz\(a_{1,2,3}\), Gz\(a_{4,5}\) expressions were immunohistochemically (IHC) analyzed in AIP mutation negative somatotropinomas, prolactinomas, NFPA and ACTH tumors. Weak and speckled cytoplasmic expression of Gz\(a_4\) was detected in GH- (mean±SD: 0.8±0.4) and PRL- (1±0.8) secreting tumors, whereas NFPA (1.8±0.5) and ACTH (1.7±0.6) tumors showed weak to moderate cytoplasmic expression (Figure 1). Consistent with the earlier observation in human GH-secreting tumors [29], Gz\(a_2\) was prominently expressed in the cytoplasm of the somatotropinomas (2.8±0.4). Prolactinomas displayed moderate to strong expression of Gz\(a_2\) (2.5±0.6), NFPA (1.8±0.5) and ACTH (1.7±0.6) adenomas showed moderate cytoplasmic and...
| Patient | Sex | Age at Dg | Age at Op | Origin | Clinical Dg | Tumor Size | GNAI1 | GNAI2 | GNAI3 | Affected family member(s) | Mutation |
|---------|-----|-----------|----------|--------|-------------|------------|-------|-------|-------|--------------------------|-----------|
| S1      | M   | 37        | –        | Spain  | GH          | Macro      | –     | –     | –     | –                        | c.468G>A  |
| S2      | M   | 40        | –        | Tunisia | GH          | Macro      | –     | –     | –     | –                        | c.138C>CT  |
| S3      | F   | 38        | –        | Finland | GH          | Macro      | –     | –     | –     | –                        | –         |
| S4      | M   | 14        | –        | Finland | GH          | Macro      | –     | –     | –     | –                        | –         |
| S5      | F   | 24        | –        | Italy   | GH/PRL      | NA         | –     | –     | –     | –                        | –         |
| S6      | F   | 24        | –        | Italy   | GH/PRL      | Macro      | –     | –     | –     | –                        | –         |
| S7      | F   | 23        | –        | Italy   | GH          | Macro      | –     | –     | –     | –                        | –         |
| S8      | M   | 22        | –        | Italy   | GH          | NA         | –     | –     | –     | –                        | –         |
| S9      | F   | 19        | –        | Italy   | GH          | Macro      | –     | –     | –     | –                        | –         |
| S10     | F   | 17        | –        | Italy   | GH          | Macro      | –     | –     | –     | –                        | –         |
| S11     | M   | 33        | –        | Italy   | GH          | Macro      | –     | –     | –     | –                        | c.468G>A  |
|         |     |           |          |         |             |            |       |       |       | c.105G>GA (rs2230350)    | c.987G>GA  |
|         |     |           |          |         |             |            |       |       |       | rs12721456 (rs61758987)  | rs762.707  |
| S12     | M   | 30        | –        | Italy   | GH          | Macro      | –     | –     | –     | c.468G>A  (rs12721456)   | c.138C>CT  |
| S13     | F   | 37        | –        | Italy   | GH          | Macro      | –     | –     | –     | –                        | –         |
| S14     | F   | 36        | –        | Italy   | GH          | Micro      | –     | –     | –     | –                        | –         |
| S15     | F   | 33        | –        | Italy   | GH          | Macro      | –     | –     | –     | –                        | –         |
| S16     | M   | 36        | –        | Italy   | GH          | Macro      | –     | –     | –     | –                        | –         |
| S17     | M   | 23        | –        | Italy   | GH          | Macro      | –     | –     | –     | –                        | –         |
| S18     | M   | 35        | –        | Italy   | GH          | Macro      | –     | –     | –     | c.468G>A  (rs12721456)   | c.138C>CT  |
|         |     |           |          |         |             |            |       |       |       | rs10241877 (rs762.707)   | –         |
| S19     | F   | 32        | –        | Italy   | GH          | Macro      | –     | –     | –     | –                        | –         |
| S20     | F   | 36        | –        | Italy   | GH          | Macro      | –     | –     | –     | c.468G>A  (rs12721456)   | –         |
| S21     | M   | 39        | –        | Italy   | GH          | Macro      | –     | –     | –     | –                        | (c.105G>GA) |
|         |     |           |          |         |             |            |       |       |       | rs2230350                | rs12721456 |
| S22     | M   | 38        | –        | Italy   | GH          | Micro      | –     | –     | –     | c.468G>A  (rs12721456)   | –         |
| S23     | M   | 26        | –        | Finland | GH          | NA         | –     | –     | –     | c.846T>C (rs762.707)     | –         |
| S24     | F   | 40        | –        | Italy   | GH          | NA         | –     | –     | –     | –                        | –         |
| S25     | M   | 23        | –        | Finland | GH/PRL      | Macro      | –     | –     | –     | c.846T>C (rs10241877)    | –         |
| S26     | F   | 43        | –        | Finland | GH          | NA         | –     | –     | –     | c.846T>C (rs10241877)    | –         |
Table 1. Cont.

| Patient | Sex | Age at Dg | Age at Op | Origin | Clinical Dg | Tumor Size | Affected family member(s) | GNAI1 | GNAI2 | GNAI3 |
|---------|-----|-----------|-----------|--------|-------------|------------|--------------------------|-------|-------|-------|
| S27     | F   | 24        | –         | Finland| GH          | Macro      | –                        | –     | –     | –     |
| S28     | F   | 39        | –         | Estonia| GH          | Macro      | –                        | –     | –     | –     |
| S29     | M   | 40        | –         | Finland| GH          | NA         | –                        | –     | –     | –     |
| S30     | F   | 25        | –         | Italy  | GH          | NA         | –                        | –     | –     | –     |
| S31     | M   | 56        | –         | Finland| GH          | NA         | –                        | –     | c.846T>TC (rs10241877) | –     | –     |
| S32     | F   | 55        | –         | Finland| GH          | NA         | –                        | –     | –     | –     |
| F1      | F   | 40        | –         | Italy  | GH          | Micro      | NFPP (father)            | –     | –     | –     |
| *F2     | F   | 56        | NA        | Italy  | GH          | NA         | GH (aunt)                | –     | –     | –     |
| *F3     | M   | 56        | NA        | Italy  | NFPA        | NA         | GH (mother)              | –     | –     | –     |
| *F4     | F   | NA        | 67        | Italy  | ACTH        | NA         | GH (son)                 | –     | –     | –     |
| *F5     | F   | NA        | 36        | Italy  | PRL         | NA         | GH (aunt)                | –     | c.138C>CT (rs762707)   | –     | –     |
| *F6     | F   | NA        | 49        | Italy  | GH          | NA         | PRL (daughter)           | –     | –     | –     |
| *F7     | M   | 42        | NA        | Italy  | GH          | NA         | GH (cousin)              | –     | –     | –     |
| *F8     | M   | 36        | –         | Finland| GH          | NA         | GH (uncle)               | c.846T>TC (rs10241877) | –     | –     |
| F9      | F   | NA        | 59        | Finland| GH          | NA         | PRL (niece)              | –     | –     | (c.105G>GA) rs2230350 | –     |
| *F10    | M   | NA        | 44        | Italy  | GH          | NA         | NFPA (niece)             | c.468G>GA (rs12721456) | –     | –     |
| F11     | M   | 24        | NA        | Italy  | GH          | NA         | GH/PRL (sister)          | –     | –     | –     |
| F12     | F   | 36        | NA        | Italy  | GH          | NA         | GH (brother)             | –     | –     | –     |
| F13     | F   | 63        | NA        | Finland| GH          | NA         | ACTH (cousin)            | c.846T>TC (rs10241877) | –     | –     |
| *F14    | M   | 40        | NA        | Finland| GH          | Macro      | PRL (cousin)             | –     | –     | –     |

Dg: diagnosis, Op: operation, S: sporadic, F: familial, M: male, F: female, NA: not available, Micro: <10 mm, Macro: >10 mm. *Screened negative for AIP germline deletions by MLPA. doi:10.1371/journal.pone.0109897.t001
| Primer     | Sequence (5'–3')              | T\textsubscript{m} (°C) | Transcript         |
|------------|-------------------------------|-------------------------|-------------------|
| Ga\textsubscript{i1}_{ex1} _F | GGATTCCCCGTGCTTTGGA          | 60                      | ENST00000442586   |
| Ga\textsubscript{i1}_{ex1} _R | GTTTCAACCGGGCCGAGGG           |                         |                   |
| Ga\textsubscript{i1}_{ex2\&3} _F | CACACAGAGAGAGCTGGGTG         | 60                      | ENST00000351004   |
| Ga\textsubscript{i1}_{ex2\&3} _R | GGTCCTGAAGTTGAACAGCC         |                         |                   |
| Ga\textsubscript{i1}_{ex4} _F | AAGGAATTCGCTATTGCC           | 60                      | ENST00000351004   |
| Ga\textsubscript{i1}_{ex4} _R | AATGTGTCAGCCAATTCGCG         |                         |                   |
| Ga\textsubscript{i1}_{ex5} _F | GTTTGGATGACTTTATTGGCG        | 60                      | ENST00000351004   |
| Ga\textsubscript{i1}_{ex5} _R | TCTCCCAACCTTTTTGGTCC         |                         |                   |
| Ga\textsubscript{i1}_{ex6} _F | CCCATAAGTCCTCTCCTCCCTC       | 62 x1, 61 x1, 60 x2, 59 x2, 58 x2 | ENST00000351004   |
| Ga\textsubscript{i1}_{ex6} _R | CTTGGCAACCTTCCTTTGGTCC       |                         |                   |
| Ga\textsubscript{i2}_{ex1c} _F | TCACCCACATCACCGTCTAA         | 59                      | ENST00000422163   |
| Ga\textsubscript{i2}_{ex1c} _R | ACGCGTCCTCTTGCAACTA          |                         |                   |
| Ga\textsubscript{i2}_{ex1d} _F | CGCTGTCCATTGCTCTCAT          | 60                      | ENST00000451956   |
| Ga\textsubscript{i2}_{ex1d} _R | GCACATGTGAGCATTCGAG          |                         |                   |
| Ga\textsubscript{i2}_{ex2} _F | AGCTGAAGTGTGACGCTGTG         | 58                      | ENST00000266027   |
| Ga\textsubscript{i2}_{ex2} _R | CTTGGCAACCTTTTCAGGTGAG       |                         |                   |
| Ga\textsubscript{i2}_{ex3\&4} _F | ATGTGAGATTGGTGCTGTG          | 58                      | ENST00000266027   |
| Ga\textsubscript{i2}_{ex3\&4} _R | GATCTGCACTTTCCGAGGCG         |                         |                   |
| Ga\textsubscript{i2}_{ex5} _F | CCAAGAATACCCTAGGCTTG         | 60                      | ENST00000266027   |
| Ga\textsubscript{i2}_{ex5} _R | GCAAAAGCAGCTAGTGCC           |                         |                   |
| Ga\textsubscript{i2}_{ex6} _F | CTACCTGAAGCCTTGGAGCGTA       | 58                      | ENST00000266027   |
| Ga\textsubscript{i2}_{ex6} _R | CTTGGCAACCTTTTCAGGTGAG       |                         |                   |
| Ga\textsubscript{i2}_{ex7\&8} _F | AAATGGGATGAAAGAGCTCCC       | 58                      | ENST00000266027   |
| Ga\textsubscript{i2}_{ex7\&8} _R | TGTTCTGACATTGCTAATTCC       |                         |                   |
| Ga\textsubscript{i2}_{ex9} _F | CTTGCTGCACACGTAGAGATG        | 58                      | ENST00000266027   |
| Ga\textsubscript{i2}_{ex9} _R | CGCTTGTGTCTTCCCCCCAGCG       |                         |                   |
| Ga\textsubscript{i3}_{ex1} _F | GCAGTTTCCGTTGGTGAG           | 58                      | ENST00000369851   |
| Ga\textsubscript{i3}_{ex1} _R | GTCACACCTACAGCATCAG          |                         |                   |
| Ga\textsubscript{i3}_{ex2\&3} _F | TAGGACCCGTTGGCTACCTC        | 60                      | ENST00000369851   |
| Ga\textsubscript{i3}_{ex2\&3} _R | TTGTTGTCTAAATCTTCCCCC       |                         |                   |
| Ga\textsubscript{i3}_{ex4} _F | CTGGCCTGAAGAAGCTTGCG         | 60                      | ENST00000369851   |
| Ga\textsubscript{i3}_{ex4} _R | TCAACCTCTTTCAAGGTGCCG        |                         |                   |
| Ga\textsubscript{i3}_{ex5} _F | GCAAGTCTTCTTTCCAGGC          | 58                      | ENST00000369851   |
| Ga\textsubscript{i3}_{ex5} _R | GTTGGCAAAACCTGTGAGGCG        |                         |                   |
| Ga\textsubscript{i3}_{ex6} _F | TGGTATGTCCTCTCCTCCCA         | 60                      | ENST00000369851   |
| Ga\textsubscript{i3}_{ex6} _R | CAAGAGCACATCACGTAGCCTACA     |                         |                   |

T\textsubscript{m}: annealing temperature.

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occasional nuclear Gαq staining. All tumor types displayed moderate cytoplasmic expression of Gαq (GH: 2.3±0.5, PRL: 2.2±0.8, ACTH: 1.6±0.6, NFPA: 1.8±0.5). Weak to moderate nuclear Gαq staining was also observed in all tumor types (GH: 1.3±0.8, PRL: 0.8±0.5, ACTH: 1.3±0.6, NFPA: 1.5±0.6).

**GNAI loci mutation analysis**

All the GNAI coding exons (23 amplicons per sample) were successfully sequenced and analyzed in 32 young sporadic somatotropinoma and 14 index familial cases (Table 1). In **GNAI1**, earlier reported synonymous heterozygous variations were detected in exon 6 (rs12721456/5 samples) and in exon 7 (rs10241877/8 samples). In **GNAI2**, one reported heterozygous and synonymous variation was found in exon 4 (rs762707/3 samples). Also in **GNAI3**, only previously observed heterozygous synonymous variations were detected in exon 1 (rs2230350/3 samples) and exon 8 (rs61758987/1 sample). None of these variants modified amino acid sequence, indicating the polymorphic nature of these changes. Additionally, several reported and unreported variations were observed in intronic regions (Table S1). All the intronic variants located outside of the splice site consensus sequences and are thus not assumed to affect splicing events.

**Discussion**

Many G proteins have been linked to tumor development, starting with the discovery that somatic gain-of-function mutations of codons 201 and 227 in the GNAS gene are responsible in one third of the sporadic somatotropinomas with elevated cAMP levels [23,32]. Activating GNAS hot-spot mutations have been detected in many other tumor types. For instance, biliary tract, thyroid, pancreatic, colon, and testis tumors are common targets of somatic GNAS mutations. Additionally, activating somatic hotspot mutations have been reported in GNAQ (Gαq) and GNA11 (Gα11) genes in melanomas and meningal tumors [33]. Somatic mutations in other Gα subunit genes have been detected, albeit in a low frequency.

Proteins of the inhibitory Gα subfamily, Gαi/Gαo, mediate several cellular and metabolic functions [34–37]. Unlike the Gαs, Gα11, Gαq, and Gαo subunits are involved in the hormonal inhibition of adenylate cyclase (AC) activity with subsequent decrease of intracellular cAMP levels [38,39]. Previous studies have been focusing on screening GNAI2 somatic hot-spot mutations (termed gip2 oncogene) in codons 179 and 205. Somatic gip2 mutations have been found in ovarian, adrenal, ACTH and NFPA tumors [32,40,41]. However, other studies have failed to confirm these initial findings [42–48]. Although isolated somatic mutations of GNAI genes have also been observed in next-generation sequencing efforts, further experiments are needed to validate the existence and relevance of these findings [49,50].

In our original study, we found that AIP deficiency is associated in pituitary tumorigenesis via reduced Gαi signaling followed by elevated cAMP concentrations [29]. In the current study, we searched for germline mutations in GNAI loci in pituitary adenoma patients compatible with the AIP phenotype; young patients with somatotropinoma and familial index cases (Table 1). Also protein expressions of Gαq, Gαi, and Gαo were examined in human GH-, PRL-, ACTH- and non-secreting (NFPA) pituitary adenomas. We have earlier shown that Gαq and Gαi proteins are expressed in human somatotropinomas [29]. Here we observed that also the Gαq protein, although at low levels, is present in GH-secreting pituitary adenomas. Moreover, immunoreactions against all three Gαi proteins were detected in human prolactinomas, ACTH and NFPA tumors (Figure 1), suggesting a biological role of these proteins in these tumor types as well.

We screened for germline mutations in the GNAI loci in sporadic somatotropinoma patients (n = 32) and familial index cases (n = 14) characterized by the AIP phenotype (Table 1). No pathogenic mutations were observed in any of the patients studied. All the detected variants were either known polymorphisms or located in intronic regions. Although certain intronic variants may...
cause impaired splicing, the observed variants were not proximal to known splice sites. We acknowledge that the sample size in the present study is insufficient to draw a definite conclusion of the involvement of GNAI germline mutations in genetic predisposition of pituitary tumors. Moreover, due to the small sample size there is no adequate power to detect possible associations between the observed variant alleles and a pituitary tumor phenotype.

To our knowledge, this is the first time that all the coding exons of GNA11, GNA12 and GNA13 have been sequenced to detect germline loss-of-function mutations in a set of selected pituitary adenoma patients. All in all, our sequencing results suggest that germline mutations of the GNAI loci seem not to be associated to, or are rare in familial pituitary tumorigenesis. However, a larger set of samples, somatic mutation screenings, copy number profiling and additional cellular works would provide a more comprehensive result of the role of GNAI genes in pituitary tumorigenesis.

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Supporting Information

Table S1 Intronic variations in GNAI loci. (DOCX)

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Author Contributions

Conceived and designed the experiments: HD ID AK. Performed the experiments: HD ID. Analyzed the data: HD ID AK. Contributed reagents/materials/analysis tools: LK OK CSJ EDM AK. Wrote the paper: HD ID LK OK CSJ EDM AK. Supported the writing of the manuscript: HD ID LK OK CSJ EDM AK.

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