



Analysis of Somatic Variants in Growth Hormone Secreting Pituitary Adenomas by Whole Exome Sequencing

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Abstract

Background: Whole-genome and whole-exome sequencing (WES) studies of pituitary adenoma have reported on average three mutations per patient tumor and discovered calcium signaling pathway mediated role of tumorigenesis. The aim of this study is to analyze the somatic variants in growth hormone (GH) secreting pituitary adenoma using WES.

Material and Methods: Whole-exome sequencing was used to identify somatic mutations in GH-secreting pituitary adenomas from 14 patients (7-45 years), including a sample from a patient with germline *GPR101* duplication. A mean sequencing depth of 94x was achieved covering 98% of targeted exonic regions at a minimum depth of 10x.

Results: A total of 30 somatic variants were identified from 14 tumor samples. A recurrent hotspot mutation in *GNAS*, a well-established gene implicated in acromegaly, were identified in four patients. In addition, missense variants in *CACNA1H* and *WIPI1* were identified which have only been reported in single patients previously. We also observed variants in *GNAQ*, *RASGEF1B* and *PARP4* (this latter one in the *GPR101*-positive sample) genes that are involved in cAMP and calcium signaling pathways but have not been previously reported to be altered in acromegaly. Large deletions in chromosomes 1, 6, 10, 11, 12, 16, 17, 19 and 20 were also observed in at least eight patients.

Conclusion: By combining our findings with data from previous studies of GH-secreting adenomas, we conclude that pathways involving calcium and cAMP signaling are likely to be involved in the pathogenesis of somatotroph adenomas.

Keywords: Somatotropinoma; Acromegaly; Growth Hormone; Whole-Exome Sequencing

Introduction

Pituitary adenomas are usually benign, monoclonal neoplasms of anterior pituitary gland with an overall prevalence of 20% and clinically manifesting prevalence of 0.1% in the general population [1,2]. Invasive adenomas and carcinomas of the pituitary account for 35% and ~0.1-0.2% of all pituitary neoplasms, respectively, with the large majority being benign adenomas [2-4]. Although most pituitary adenomas are benign, the tumor size and hormonal status of the cell type that is affected ultimately determine the clinical phenotype. Prolactinomas are the most common (40-50%) hormone secreting pituitary tumors, followed by growth hormone (GH) secreting pituitary adenomas (15-20%). Other less common hormone secreting pituitary adenomas include adrenocorticotropin hormone (ACTH)-secreting adenomas, gonadotropin (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]) se-

creting adenomas, thyroid Stimulating Hormone (TSH)-secreting adenomas or mixed adenomas.

Clinically, pituitary tumors can present as sporadic or familial disease or syndromic or non-syndromic disease. The familial non-syndromic form (familial isolated pituitary adenomas (FIPA)) can be caused by *AIP* or *GPR101* mutations, while familial syndromic forms are due to *MEN1*, *PRKAC1A*, *SDHx*, or rarely *CDKN1B*, *PRKACB* and *DICER1* [5]. The majority (~93%) of pituitary tumors causing acromegaly are sporadic in nature with underlying genetic and epigenetic anomalies [6]. Two particular somatic gain-of-function mutations in *GNAS*, a gene encoding the α subunit of the stimulatory G protein, have been identified several decades ago, representing up to ~40% of somatotropinomas [7,8].



Identification of somatic genetic alternations in tumors is critical for understanding the molecular mechanism of tumorigenesis. The advancements in high-throughput sequencing and sophisticated analytical tools facilitate the unbiased genome-wide identification of somatic genetic mutations. Next-generation sequencing has been successful in elucidating the somatic mutational landscape of somatotropinomas [9-13], Cushing's disease [14-16], nonfunctioning pituitary adenomas [17] and other subtypes of pituitary tumors [9,12].

Whole-genome and whole-exome sequencing (WES) studies of GH-secreting pituitary adenoma have reported on average three mutations per patient tumor and discovered calcium signaling pathway mediated role of tumorigenesis. The low rate of somatic mutations in pituitary tumors also indicates the possible role of other mechanisms of tumorigenesis involving differential miRNA expression [18], epigenetic alterations [19,20] and copy number variations [9,21,22]. Recurrent copy number aberrations including the whole-chromosomal losses are also commonly observed in pituitary adenoma [9,13,23].

In this study, we report somatic variant profiles of 14 somatotropinomas by WES. We identified a recurrent hotspot mutation

in *GNAS* (4/14) and describe a deleterious somatic variant in *CACNA1H*, and likely benign variants in *WIP11*, *GNAS*, *GNAQ*, *RASGEF1B* and *PARP4*.

Materials and Methods

Patient subjects and tissue samples

Patients diagnosed with GH-secreting pituitary adenomas at the Neurosurgical Department of Sungkyunkwan University Samsung Changwon hospital were recruited to the study. We analyzed the formalin-fixed, paraffin-embedded (FFPE) samples at the pathological archives which were obtained for recent 5 years. And the blood samples were routinely obtained before treatment including surgery and any medication at the department. All 14 patients provided written consent for use of their biological samples for diagnosis and research purposes. Pituitary tumors surgically resected at the time of endonasal endoscopic transsphenoidal surgery from the patients were collected, washed and frozen immediately. Peripheral blood from the patients was also collected and stored. Details of the patients including sex, age at diagnosis, pathological reports and levels of hormones for all patients are provided in Table 1. The median age of the patients at the time of diagnosis was 32 years (range 7-56).

Table 1: Clinical details of patients with somatotroph macroadenomas.

Patient	Age at diagnosis	Sex	Immunohistochemical Staining						Hormone Level			Size of tumor (mm)	Treatment	FU
			GH	PRL	TSH	ACTH	FSH	LH	GH (µ/L)	IGF-1 (ULN)	PRL (ULN)			
1	26	M	+	-	-	-	-	-	3.7	0.96	0.58	11x16x20	TSS	Cured
2	28	F	+	+	-	-	-	-	227	7.8	0.94	46x31x26	TSS, EBRT, D2R agonist	Sudden death
3	40	F	+	+	-	-	-	-	2.3	2.05	0.4	18x10x11	TSS, GKRS	GH deficit
4	20	F	+	-	-	-	-	-	12.9	2.1	2.3	24x15x14	TSS	GH deficit
5	36	M	+	+	-	-	-	-	18.7	1.57	0.24	14x13x17	TSS	Cured
6	7	F	+	+	-	-	-	-	88	5.2	10.3	16x19x12	TSS, GKRS, D2R agonist	Stable disease
7	30	M	+	+	-	+	-	-	123	2.6	0.5	22x15x31	TSS, craniotomy, IMRT	Follow-up loss
8	35	M	+	-	-	-	-	-	9.5	4.75	2.6	11x10x15	TSS	Cured
9	44	F	+	-	-	-	-	-	12.2	2.66	2.5	14x17x14	TSS	Stable disease
10	45	M	+	-	-	-	-	-	49.1	0.94	0.62	18x13x15	TSS, EBRT	Cured
11	31	F	+	+	-	-	-	-	12.2	4.04	0.5	27x35x25	TSS	Cured
12	32	M	+	+	-	-	-	-	348	5.8	0.6	26x32x25	TSS	Active disease
13	56	M	+	-	-	-	-	-	11.4	5.1	42	20x12x18	TSS	Cured
14	32	M	+	-	-	-	-	-	11.8	0.6	0.33	12x10x11	TSS, GKRS	Cured

EBRT: External Beam Radiation Therapy; D2R: Dopamine D2 Receptor; GH: Growth Hormone; GKRS: Gamma Knife Radiosurgery; IMRT: Intensity-Modulated Radiation Therapy; TMZ: Temozolomide; TSS: Transsphenoidal Surgery

DNA isolation and whole exome sequencing

FFPE tumor specimens and the peripheral blood collected from patients were subjected to genomic DNA isolation using DNeasy Blood and Tissue kit (QIAGEN, USA). The quality of genomic DNA was checked using the Agilent 2100 BioAnalyzer and the quantity was measured using NanoDrop and Qubit fluorometer 3.0. About 1 µg of genomic DNA was fragmented using Covaris LE220 for library preparation. The DNA fragment library was further enriched for targeted exome using probes against 357,999 exons spanning 21,522 genes in the human genome using SureSelectXT Human All Exon V5 capture kit (Agilent Technologies, USA). The quality of library was assessed using the Agilent 2100 BioAnalyzer. Indexed exome libraries from multiple samples were then pooled and subjected to paired-end sequencing with read length of 100 bp on an Illumina HiSeq 2500 platform. Raw data from the sequencing was then demultiplexed to generate fast q files for individual samples.

Whole exome sequencing data analysis to identifying somatic mutations

Sequencing data analysis was carried out using an in-house computational pipeline established following the guidelines previously established for variant discovery [24]. In summary, the sequencing quality of data was assessed using FastQC (v0.10.1) and all low-quality bases with Phred score <20 from read ends were

soft masked from mapping against human genome primary assembly (GRCh37) using BWA (v0.7.10) [25]. The raw alignment was coordinate sorted using Samtools (0.1.19) [26], then processed to mask duplicate reads using MarkDuplicates (Picardtools v1.107) to filter for reads resulting from PCR duplicates during library preparation. Further steps of realignment around known indels and base quality score recalibration was carried out using Genome Analysis Toolkit (GATK v2.8-1) [27]. The somatic mutations were identified by comparing variants identified between pituitary tumor sample and corresponding blood samples for each patient using VarScan (v2.3.7) [28], and MuTect (v1.1.4) [29]. Sequence variants specifically observed in tumor DNA and completely absent in germline DNA were considered as somatic variants. Loss of heterozygosity in tumor genomes was detected using VarScan by comparing against the germline sequence. Annotation of identified somatic mutations was carried out using ANNOVAR (release: March 2015) [30] and SnpEff (v4.1) [31] against their curated reference databases for hg19. Variants with minor allele frequency (MAF) <0.01 were considered after comparing with variants from populations studies such as dbSNP, 1000 genomes project, EVS and ExAC [32]. Further, functional effects of the mutations were predicted using SIFT and Polyphen-2 scores. The shortlisted variants were validated by visualization of aligned data using integrated genomics viewer (IGV) [33].

Results and Discussion

Whole exome sequencing data analysis

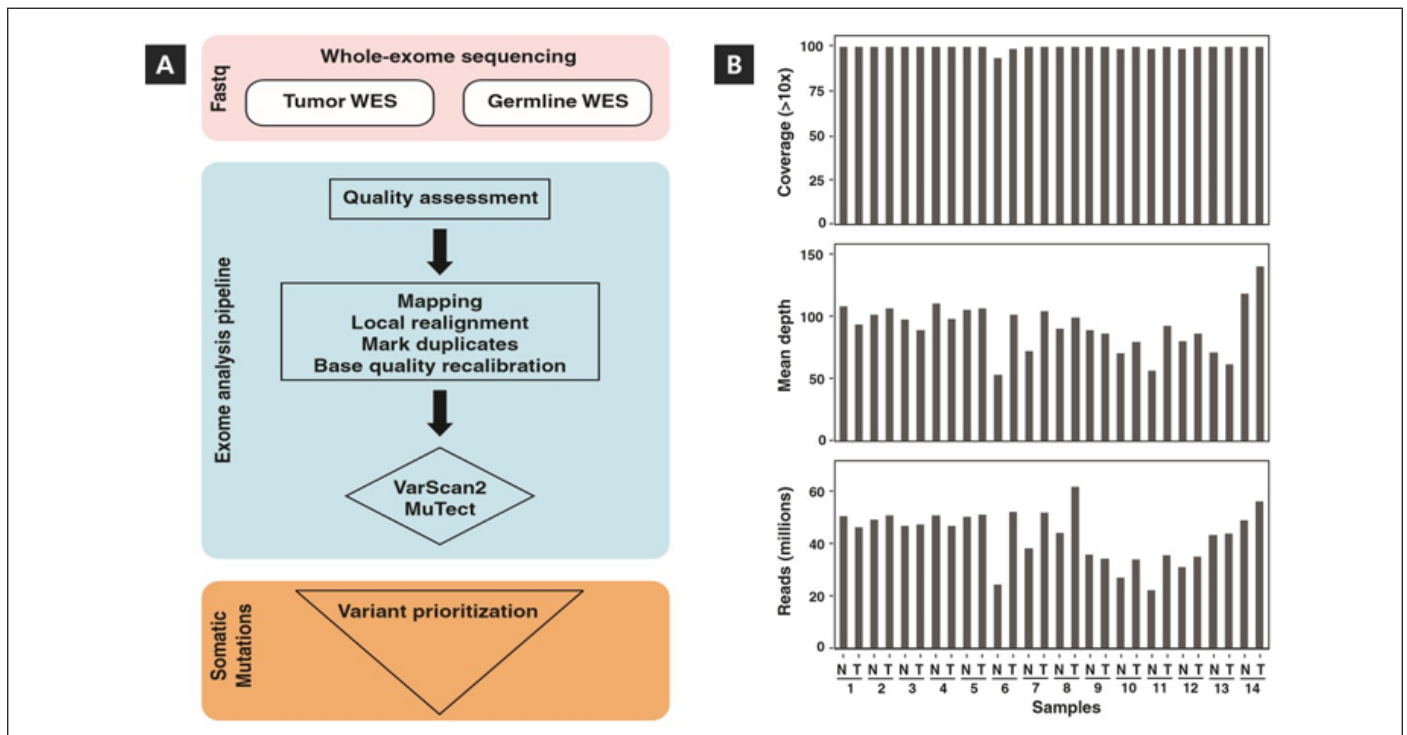


Figure 1: Schematic of data analysis workflow and alignment statistics. (A) Schematic workflow outlines the steps in whole-exome sequencing data analysis. The tumor and corresponding normal (germline) WES data were processed using an automated pipeline developed to implement the indicated workflow. Raw reads were aligned against human reference genome (hg19) and somatic variants were called to identify the mutations. (B) Plots show the number of paired-end reads generated along with alignment depth and coverage for each patient sample.

A total of 14 tumor-blood sample pairs from acromegaly patients were selected for WES (Table 1). WES analysis of tumor and matched blood samples from patients were carried out to identify somatic mutations in GH-secreting pituitary adenomas. Approximately 43 million paired-end reads of length 100 bp were generated per sample. Quality analysis of data revealed >95% of the sequenced bases were with Phred quality score of ≥ 20 which is good for reliable detection of variants. Raw data generated from sequencing of samples were analyzed by using an in-house exome analysis pipeline to map against the human genome reference (hg19). A schematic representation of data analysis steps and tools used for the processing of raw and aligned data are depicted in Figure 1A. This analysis resulted in achieving a mean depth of 90x and average coverage of 98% over the targeted regions at minimum depth of 10x. A summary of sample sequencing data along with number of reads, depth and coverage is shown in Figure 1B. Overall, the data quality was sufficient for discovery of somatic variants in the tumors by comparing against the germline DNA of individual

patients.

Somatic mutation profile of acromegaly

Next-generation sequencing of tumor genomes enables detection of driver genes that alter the physiology of cells to turn them cancerous. To identify somatic mutations in acromegaly, variants identified from tumor and normal patient genomes were compared. From the variant analysis of the 14 patient samples, we identified a total of 30 genes with somatic variants that alter their protein sequences and has a $MAF < 0.01$. We observed up to 4 somatic changes per tumor across the 14 patient samples (Table 2). The most recurrent mutations were observed in the *GNAS* gene (5 out of 14 patients), which is a well-established gene known to be mutated in acromegaly. Four out of five patients carried the well-known hot spot mutation p.R201C in *GNAS* gene, while the fifth patient had a novel and predicted benign *GNAS* variant p.G553D. In addition, we identified somatic variants in *CACNA1H*, *WIP11*, *RASGEF1B*, *PARP4* and *GNAQ* genes (Table 2).

Table 2: Details of identified variants in patients with somatotroph macroadenomas.

Patient	Family History	Germline change	Somatic mutation in pituitary tumor	Somatic mutation database	In silico score *PON-P2	ϕPolyphen2 HVAR prediction	∫REVEL (score)
1	No	None	None	N/A	N/A	N/A	N/A
2	No	None	<i>GNAS</i> c.C601T, p.R201C <i>WIP11</i> c.A1244G, p.E415G	COSM27887 Absent	0.781 0.899	Deleterious Possibly damaging	0.943 0.224
3	No	None	<i>CACNA1H</i> c.A4532T, p.Y1511F	COSM16297, 4 p.Y1511C	0.625	Deleterious	0.862
4	Yes	None	<i>GNAQ</i> c.A286T, p.T96S	COSM404628	0.552	Benign	0.3
5	Yes	None	<i>GNAS</i> c.C601T, p.R201C	COSM27887	0.781	Deleterious	0.943
6	No	<i>GPR101</i> duplication	<i>PARP4</i> c.C3241T, p.L1080F	COSM231303	0.282	Benign	0.085
7	No	<i>RET</i> c.C2556G, p.I852M	None	COSM4573611	0.803	Deleterious	0.65
8	No	None	None	N/A	N/A	N/A	N/A
9	No	None	None	N/A	N/A	N/A	N/A
10	No	None	<i>GNAS</i> c.G1658A, p.G553D	Absent	0.624	Benign	0.28
11	No	None	None	N/A	N/A	N/A	N/A
12	No	None	<i>GNAS</i> c.C601T, p.R201C	COSM27887	0.781	Deleterious	0.943
13	No	None	<i>RASGEF1B</i> c.A328C, p.K109Q	Absent	0.675	Deleterious	0.306
14	No	None	<i>GNAS</i> c.C601T, p.R201C	COSM27887	0.781	Deleterious	0.943

*PON-P2 score is a probability score for pathogenicity of variant.

ϕPolyphen2 predictions represent the possible impact of an amino acid substitution on the structure and function of a human protein.

∫REVEL score is based on an ensemble method for predicting the pathogenicity of missense variants on the basis of multiple individual prediction tools, the closer score to 1, the higher the chance the variant being damaging. Threshold usually taken at 0.75.

Interestingly, the *CACNA1H* p.Y1511F change in the calcium voltage-gated channel subunit alpha1 H has not been previously described and is rendered deleterious by several prediction programs. A different change affecting the same amino acid was seen in the COSMIC database (p.Y1511C). We found a novel missense change in *WIP11*, also a calcium pathway protein playing a role in calcium-mediated autophagy. The *RASGEF1B* p.K109Q variant is rare and predicted to be deleterious by Polyphen, but not by REVEL. The *RASGEF1B* gene is a guanine-nucleotide exchange factor and known

interactor of Ras family of proteins which are essential regulators of cell proliferation, survival, differentiation, actin organization, vesicular trafficking, and gene expression. The *PARP4* p.L1080F somatic change was previously seen in the COSMIC database and predicted to be benign. Genetic changes seen in this study are listed in Table 2 and depicted in Figure 2A.

Although WES has its limitations to detect large chromosomal variations, we compared tumor normal DNA sequencing data to identify large deletions or amplifications of chromosomal segments

which were previously implicated in somatotroph tumorigenesis. Analysis of genome-wide loss of heterozygosity events in our WES data revealed somatic deletions in chromosomes of at least eight patients. Complete hemizygous deletion of chromosome 6 and 19 were observed in patient #3 and #12, respectively. Other small focal deletions in chromosome 1, 6, 10, 11, 12, 16, 17, 19 and 20 were observed in patients #2, #5, #9, #10, #13 and #14 as showed

in Figure 2B. Previous studies of GH-secreting pituitary adenomas using next generation sequencing approach have also reported chromosome losses (chromosomes 1, 6, 13, 14, 15, 16, 18 and 22) and complex rearrangements [9-13]. In a recent unpublished study, increased somatic copy-number variation was associated with DNA damage resulting from enhanced cAMP activity in sporadic somatotroph adenomas [34].

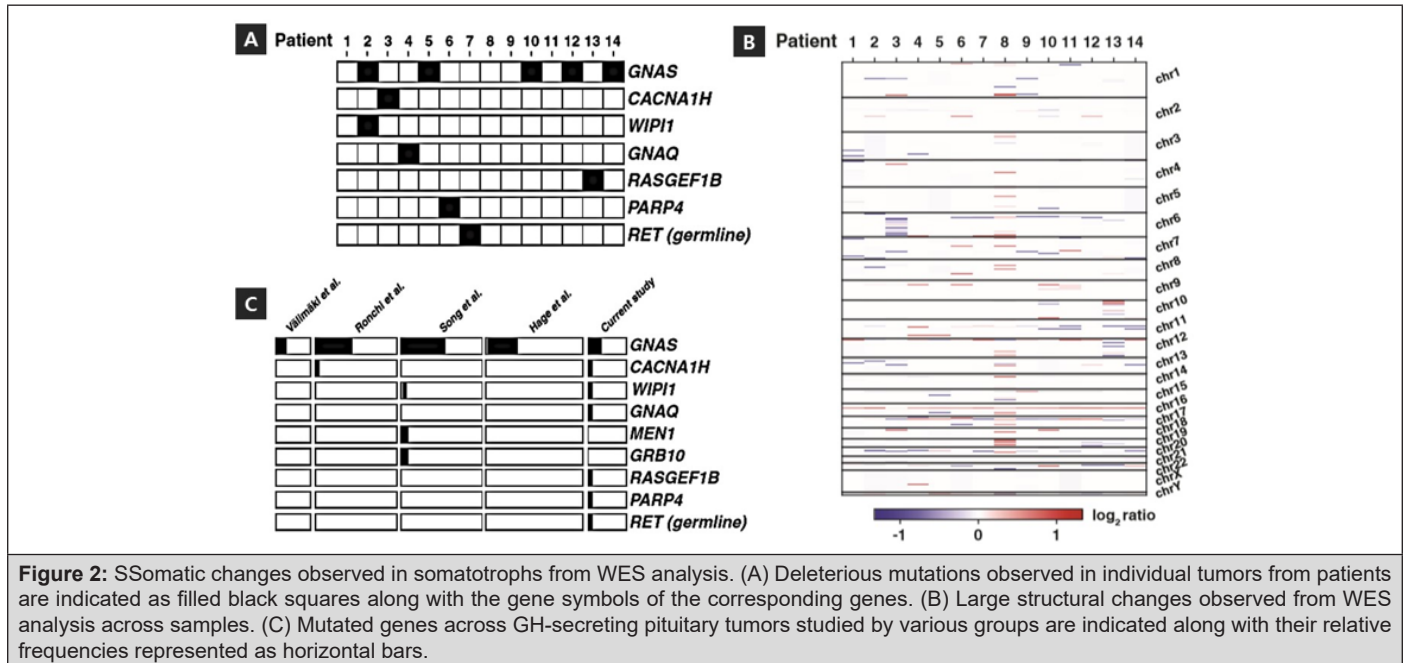


Figure 2: Somatic changes observed in somatotrophs from WES analysis. (A) Deleterious mutations observed in individual tumors from patients are indicated as filled black squares along with the gene symbols of the corresponding genes. (B) Large structural changes observed from WES analysis across samples. (C) Mutated genes across GH-secreting pituitary tumors studied by various groups are indicated along with their relative frequencies represented as horizontal bars.

Patient #4 with a benign somatic *GNAQ* variant (not seen in germline but seen in COSMIC previously) is a FIPA case as her mother was also affected by acromegaly. No tissue sample is available from the mother. Patient #6 was positive for *GPR101* duplication and the benign *PARP4* variant was observed in the tumor. Patient #7 with a recurrent tumor was identified with a germline variant p.I852M in the *RET* gene. He has normal calcitonin, calcium and parathormone levels, and normal urinary metanephrens. This *RET* variant has been described in medullary thyroid carcinoma [35] and Multiple Endocrine Neoplasia 2A patients [36] and most likely represents a benign variant, despite the prediction scores [37]. Acromegaly has been described in a few patients with *RET* mutations, but these probably represent coincidences, rather than linked pathogenetically to the *RET* mutations [38,39]. Physical reexamination of a subset of patients with *GNAS* mutations in tumor did not reveal any skin lesions and their bone scans were normal, therefore ruling out McCune-Albright syndrome. The presence of variants was not correlating to the sparsely or densely granulated nature of the tumours, or to size and invasiveness.

Mutation data in five independent cohorts provided an opportunity to compare our results to the genetic profiles of other somatotropinomas (Figure 2C). A summary and a partial list of genes with variants reported by these recent publications are provided in Table 2 & 3. Mutations in *GNAS* are the only mutations recurrent (Figure 3A). The identification of variants in *CACNA1H* and *WIPI1* in

this study and a previous study, together with other calcium pathway protein changes emphasize the key role of calcium signaling in tumorigenesis of GH-secreting pituitary adenomas (Figure 3B & 3C). Sequencing of additional pituitary tumors in the future may lead to discovery of new driver genes contributing to understanding of tumorigenesis in acromegaly.

Global comparison of variants across various pituitary adenomas

Clinically non-functioning pituitary adenomas were analyzed in three studies [9,12,17] which did not identify any recurrent mutations. While recurrent *GNAS* mutations at two sites (codon 201 and 227) are seen in somatotroph adenomas, and recurrent *USP8* mutations at the binding site of 14-3-3 protein in corticotroph adenomas, truncating mutations of *NR3C1* was observed in adrenocorticotroph adenomas and *MEN1* somatic loss-of-function changes in plurihormonal adenomas producing growth hormone and prolactin [12]. The majority of the identified mutations in these genes were observed in known hot spot regions and only a few were novel. To enable general observations in somatotroph tumors, we compared the mutation data from current study to all five recent studies. Mutations in *CACNA1H* and *WIPI1* were seen in only two patients with GH-hypersecretion including cases from this study. Non-overlapping genetic mutation profiles between various subtypes of pituitary adenomas suggests a possible role of distinct

mechanisms of tumorigenesis and secretion of hormones. This comparison suggests a low mutation burden across the pituitary tumors and mutations in classical cancer genes such as *TP53* and *RAS* are almost never seen [5].

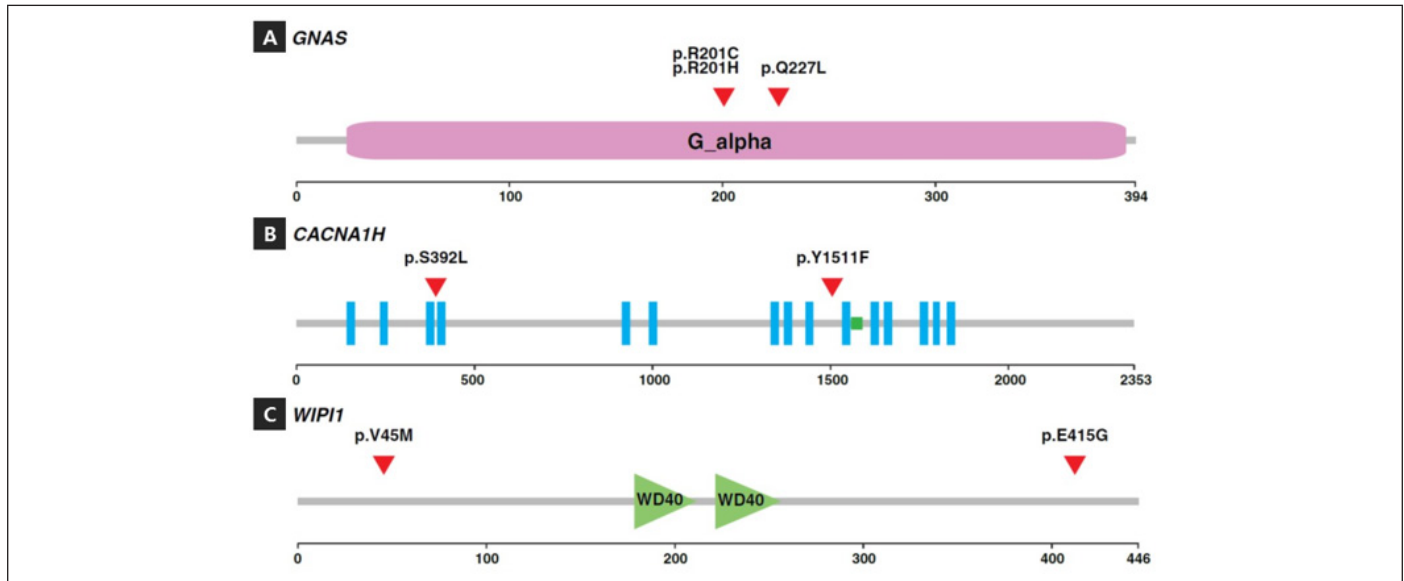


Figure 3: Mutations identified in genes with multiple mutations. The domain structures along with the locations of the substitutions (marked by red triangles) are shown for *GNAS* (A), *CACNA1H* (B) and *WIPI1* (C) The scale at the bottom marks amino acid positions in the protein sequences.

Biological relevance of genes with variants

Table 3: Comparison of outcomes from various studies of genome sequencing the GH-secreting pituitary adenomas.

	Välämäki et al. [13]	Ronchi et al [11]	Song et al. [12]	Bi et al. [9]	Hage et al. [10]	This study 2019
Journal (year)	J Clin Endocrin Metab	Eur J Endocrinol	Cell Research	Clin Cancer Res	J Clin Endocrin Metab	
Patients Number	12	67	35	5	43	14
Technology	WGS (40x)	WES (>100x)	WES	WES (108x)	Array-CGH Targeted Sanger	WES
Phenotype studied	GH-secreting pituitary tumor	GH-secreting pituitary tumor	Multiple subtypes of pituitary tumor	Multiple subtypes of pituitary tumor	GH-secreting pituitary tumor	GH-secreting pituitary tumor
Mean number of mutations	2.3	3	3.3	-	-	
Copy number changes studied	Yes	No	No	Yes	Yes	No
Number of patients w/o any mutations	2	5	-	-	-	
Mutations	GNAS (25%)	GNAS (31.4%)	GNAS (54%)	GNAS (0%)	GNAS (30%)	GNAS (36%)
cAMP/ATP signaling pathway	SUPV3L1 / ATPAF2 ATAD2B / DICER1 AOX1	14/36 -39%				
Calcium signaling pathway	C2CD3 / RYR1 SSR3 / ACTN4 UBR4	8/36 -22%				

CGH: Comparative Genomic Hybridization; GH: Growth Hormone; WES: Whole Exome Sequencing; WGS: Whole Genome Sequencing

Table 4: List of mutated genes by various studies on GH-secreting pituitary adenomas.

Gene	Signaling pathway	Välämäki et al. [13]	Ronchi et al. [11]	Song et al. [12]	Bi et al. [9]	Hage et al. [10]	This study 2019
<i>GNAS</i>	cAMP	3	16	19		13	5
<i>CACNA1H</i>	calcium		1				1
<i>WIPI1</i>	calcium			1			1
<i>GNAQ</i>	cAMP						1
<i>RASGEF1B</i>							1
<i>PARP4</i>							1
<i>MEN1</i>				2			

<i>GRB10</i>				2		
<i>SUPV3L1</i>	cAMP	1				
<i>C2CD3</i>	calcium	1				
<i>ATPAF2</i>	cAMP	1				
<i>ATAD2B</i>	cAMP	1				
<i>AOX1</i>	cAMP	1				
<i>SSR3</i>	calcium	1				
<i>ACTN4</i>	calcium	1				
<i>RYR1</i>	calcium	1				
<i>DICER1</i>	cAMP	1				
<i>UBR4</i>	calcium	1				
<i>PRKAA2</i>	cAMP		1			
<i>GRK3</i>	cAMP		1			
<i>ATP6V0A1</i>	cAMP		1			
<i>CCR10</i>	cAMP		1			
<i>CHRM3</i>	cAMP		1			
<i>OR51B4</i>	cAMP		1			
<i>CAPN1</i>	calcium		1			
<i>DMD</i>	calcium		1			
<i>GRIN2B</i>	calcium		1			
<i>JHP2</i>	calcium		1			
<i>MAN1A1</i>	calcium		1			
<i>PCDH11X</i>	calcium		1			
<i>PROCA1</i>	calcium		1			
<i>SLIT2</i>	calcium		1			
<i>SPTA1</i>	calcium		1			
<i>TESC</i>	calcium		1			
<i>GAGE2B</i>					1	
<i>KRTAP4-11</i>					1	
<i>NBPF1</i>					1	
<i>NBPF10</i>					1	
<i>POTEC</i>					1	
<i>SBNO1</i>					1	
<i>CACNA1B</i>	calcium			1		
<i>SP100</i>				1		
Others				103		80

Table 5: List of mutated genes and associated pathways.

Pathway	Gene Symbol	Gene Name
GTPase signaling	RABGAP1L	RAB GTPase activating protein 1 like
	RIN2	RPM1 interacting protein 2
	ARHGAP35	Rho GTPase activating protein 35
	AMPD1	Adenosine monophosphate deaminase 1
	TBCD	Tubulin folding cofactor D
	RABGAP1L	RAB GTPase activating protein 1 like
Calcium signaling	EFCAB2	EF-hand calcium binding domain 2
	LRP1B	LDL receptor related protein 1B
	FLG	Filaggrin
	WIP1	WD repeat domain, phosphoinositide interacting 1

	CACNA1H	Calcium channel, voltage-dependent, T type, alpha 1H subunit
	ANXA9	Annexin A9
	ITGAV	Integrin alpha V
	RPTN	Repetin
	ZAN	Zonadhesin
	NVL	AAA ATPase domain-containing protein
Others	RASGEF1B	RasGEF domain family member 1B
	ARHGEF15	Rho guanine nucleotide exchange factor (GEF) 15
	NCAM1	Neural cell adhesion molecule 1
	SMURF2	E3 ubiquitin-protein ligase SMURF2
	WDR36	WD repeat domain 36

Activated calcium and cAMP signaling pathways lead to an increase in cytosolic free calcium which triggers secretion of GH. In addition, ATP released along with pituitary hormones induces intracellular free calcium accumulation which in turn leads to secretion of GH [40]. Recurrent mutations of *GNAS*, *CACNA1H* and *WIP1* genes suggest a vital role of cAMP and calcium signaling in acromegaly. Activating mutations identified in *GNAS* gene were associated with production of cAMP from ATP by activation of adenylate cyclase. *CACNA1H* is a member of alpha-1 subunit family of the voltage-dependent calcium channel complex and directly mediates calcium flux. *WIP1* plays an important role in calcium-mediated autophagy during increased levels of cytosolic calcium. The

Ras protein family interactor *RASGEF1B* is a novel gene found with changes in this tumor type and mutations of *PARP4* were previously reported in thyroid and breast cancers. Overall, a total of 15 and 12 genes involved in cAMP and calcium signaling, respectively were found changed in somatotropinomas as observed in current or previous studies (Table 3 & 4). A pathway diagram of calcium signaling adopted from Wiki-Pathways (<https://www.wikipathways.org/instance/WP536>) is presented in Figure 4 with the mutated genes highlighted in red. Identification of mutations across several of these genes strongly suggest the dysregulation of the calcium and cAMP signaling which are potentially involved in pituitary tumorigenesis (Table 5).

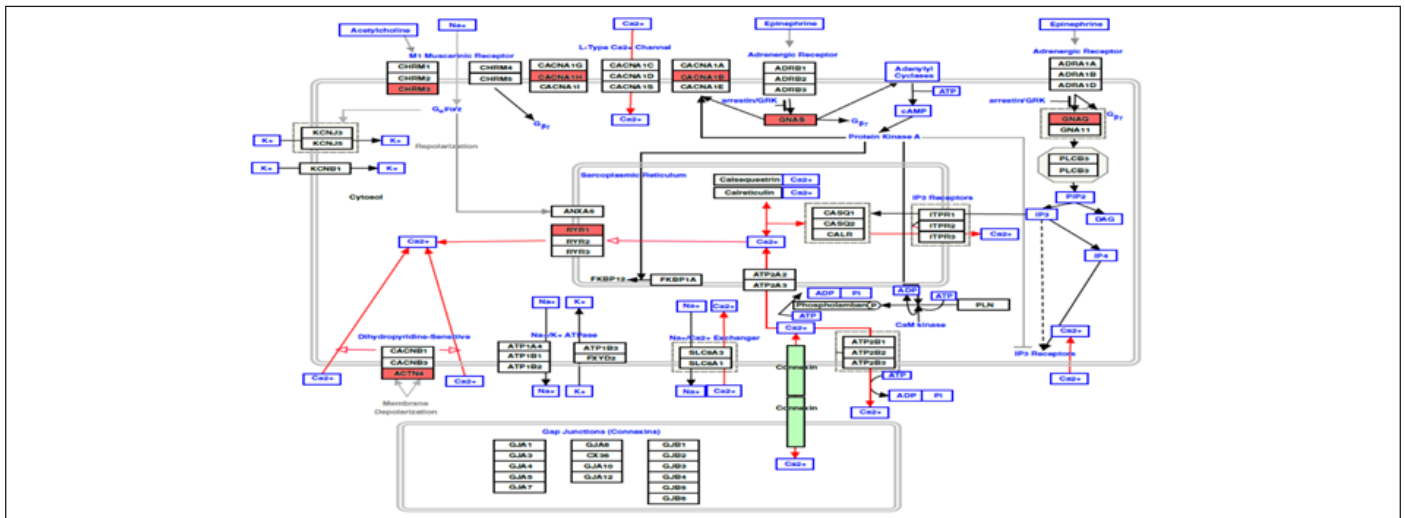


Figure 4: Calcium signaling pathway (<https://www.wikipathways.org/instance/WP536>) with mutated genes (marked in red) associated with acromegaly from multiple studies is shown. Only a subset of molecules involved in calcium signaling is provided for brevity.

Conclusions

Somatotroph adenomas are mostly driven by somatic mutations. Next-generation sequencing-based platforms enable large-scale analysis of patient samples to decipher mutational landscapes of tumors. We undertook WES of GH-secreting pituitary tumors, including one with germline *GPR101* duplication, to detect somatic changes. We identified recurrent mutations in *GNAS*, a well-established gene implicated in causing acromegaly, in four out of 14 patients. Combined analysis of somatic variants from this study with

recent studies on somatotropinomas identified two additional candidate genes of importance, *CACNA1H* and *WIP1* in two independent cohorts. Variants in other associated genes *GNAQ*, *RASGEF1B* and *PARP4* were also identified in individual samples. No somatic mutations of current relevance were identified in five tumors, although large deletions were present in two of them. Our data suggest that whole-exome sequencing have limitations identifying definite cause of pituitary adenomas and points to the direction of studying epigenetic changes, structural changes or whole genome sequencing should to fully understand somatotroph tumorigenesis.

Disclosure/Conflict of Interest

The authors report no competing financial interests or conflicts of interest.

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