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LETTER TO THE EDITOR

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The genome-wide mutational landscape of pituitary adenomas

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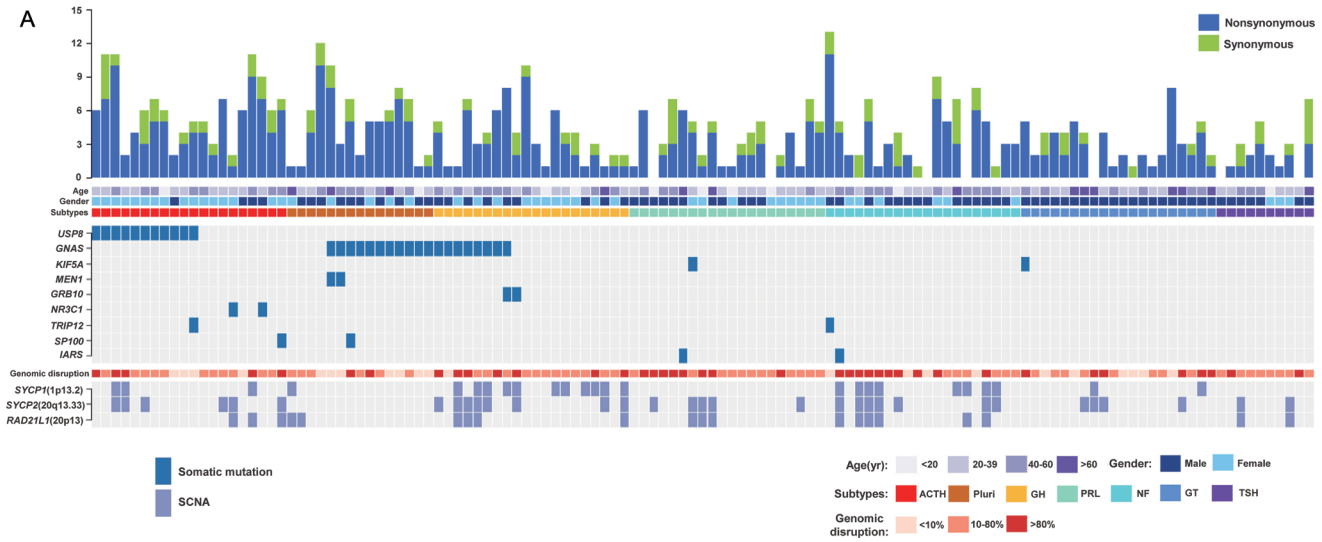
Dear Editor,

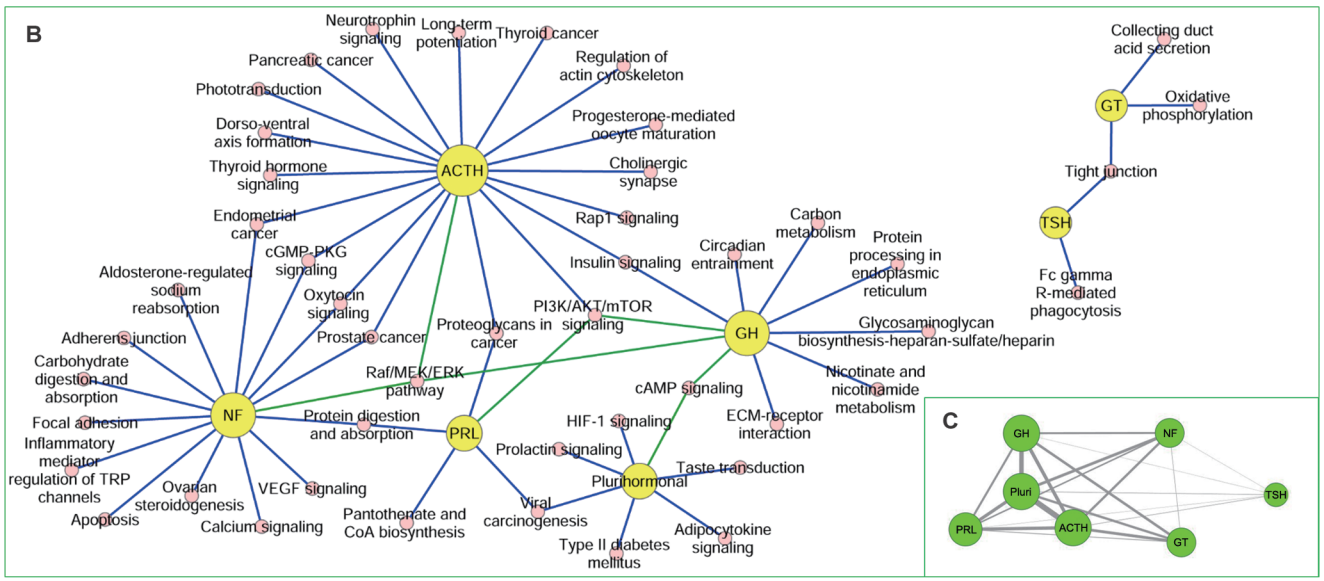
Pituitary adenomas (PAs) are one of the most common intracranial tumors, which can result in significant morbidity and can cause mortality either by exerting central pressure effects from the pituitary mass or by secreting excessive pituitary hormones [1]. Depending on their capability to produce hormones, PAs are classified as clinically functioning and nonfunctioning (NF). Functioning PAs include 6 subtypes, characterized by hypersecretion of prolactin (PRL), growth hormone (GH), adrenocorticotropin (ACTH), gonadotropins including follicle stimulating hormone and luteinizing hormone (GT), thyrotropin (TSH) or multiple hormones (plurihormonal), respectively. We and other group recently reported the recurrent genetic mutations in ACTH-PAs [2, 3]. Previous studies also identified genetic alterations in GH-PAs by whole-genome and -exome sequencing [4, 5] and in 7 NF-PAs by exome analysis [6]. Due to challenges in collection and preparation of PA samples, exome-wide sequencing of other subtypes, including the PRL-, GT-, TSH-, and plurihormonal PA subtypes, has not been reported yet.

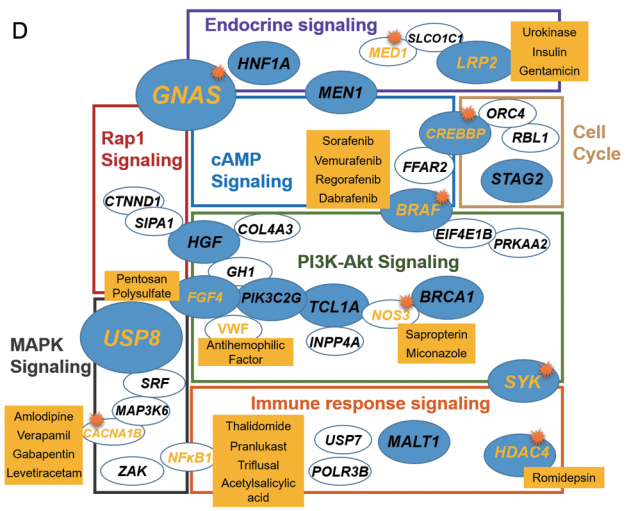
To provide a comprehensive genetic landscape of all 7 subtypes of PAs, we examined the somatic mutational landscape of 125 PAs, including 20 NF-PAs, 20 PRL-PAs, 20 GH-PAs, 20 ACTH-PAs, 20 GT-PAs, 10 TSH-PAs, and 15 plurihormonal PAs. Plurihormonal PAs in this series secreted GH+PRL (n = 11), GH+ACTH (n = 2), or GH+TSH (n = 2). The analysis of 20 ACTH-PAs included 12 tumor samples analyzed in our previous study [2]. The clinical characteristics of these patients are summarized in Supplementary information, Table S1A. The detailed methods are described in Supplementary information, Data S1 and Figure S1A. We identified 412 somatic mutations in 125 PAs (Supplementary information, Table S1B). This analysis confirmed a relatively low number of somatic mutations per tumor across all 7 PA subtypes (mean = 3.3 mutations per exome; Figure 1A) [3-6]. The predominant substitution was C>T/G>A transitions which accounted for 41% of all substitutions across all PA subtypes, followed by T>C/A>G transitions (23%), similar to other intracranial tumors (Supplementary information, Figure S1B).

Recurrently-mutated genes are shown in Figure 1A. We confirmed mutations in *G protein subunit α* (*GNAS*), *ubiquitin-specific protease 8* (*USP8*), *Nuclear Receptor Subfamily 3 Group C Member 1* (*NR3C1*), and *Menin 1* (*MEN1*) in PAs and identified new somatic variants in these genes. Activating mutations of *GNAS* have long been known to play a role in pathogenesis of GH-PAs [7]. In this study, *GNAS* mutations were identified in 54% of all GH-secreting tumors, including 8 of 20 GH-monosecreting tumors and 11 of 15 plurihormonal tumors that secreted GH and another hormone. The high frequency of *GNAS* mutations in GH-PAs is consistent with previous findings [4]. *GNAS* alterations included p.R201C (n = 14), p.R201H (n = 1), p.Q227L (n = 3) and a novel p.G49R (n = 1) mutation. USP8 is a deubiquitinating enzyme that protects growth factor receptors including EGFR from degradation. Highly frequent *USP8* mutations in ACTH-PAs have been shown by our group and others [2, 3]. In this study, *USP8* mutations were identified in 11 out of 20 ACTH-PAs, among which all mutation types have been described previously [2, 3]. We also identified novel truncation mutations in the glucocorticoid receptor gene *NR3C1* in two ACTH-PAs that did not contain *USP8* mutations: p.Q632X-p.S512fs and p.R714P-p.630_630del. This is consistent with previous findings implicating mutations targeting *NR3C1* as an uncommon contributor to ACTH-PA pathogenesis [2]. We additionally identified two novel somatic loss-of-function mutations in *MEN1*, including c.208_214del:p.D70fs and c.G608A:p.W203X, both in plurihormonal PAs producing GH and PRL from patients without a history of familial MEN syndrome and without germline *MEN1* mutations. While germline *MEN1* mutations have long been linked to PAs, *MEN1* mutations have also been reported as rare somatic events in sporadic PAs [8].

We also identified recurrently-mutated *kinesin heavy chain isoform 5A* (*KIF5A*) and *growth factor receptor-bound protein 10* (*GRB10*) as novel targets in two PA patients, respectively. KIF5A is a member of the kinesin


A
Nonsynonymous
Synonymous
Age
Gender
Subtypes
USP8
GNAS
KIF5A
MEN1
GRB10
NR3C1
TRIP12
SP100
IARS
Genomic disruption
SYCP1(1p13.2)
SYCP2(20q13.33)
RAD21L1(20p13)
Somatic mutation
SCNA
Age(yr): <20 20-39 40-60 >60 Gender: Male Female
Subtypes: ACTH Pluri GH PRL NF GT TSH
Genomic disruption: <10% 10-80% >80%


B
ACTH
NF
PRL
GH
GT
TSH
Pluri
Neurotrophin signaling
Long-term potentiation
Thyroid cancer
Pancreatic cancer
Regulation of actin cytoskeleton
Phototransduction
Progesterone-mediated oocyte maturation
Dorso-ventral axis formation
Thyroid hormone signaling
Cholinergic synapse
Endometrial cancer
Rap1 signaling
cGMP-PKG signaling
Insulin signaling
Aldosterone-regulated sodium reabsorption
Oxytocin signaling
PI3K/AKT/mTOR signaling
Adherens junction
Prostate cancer
Proteoglycans in cancer
Carbohydrate digestion and absorption
Raf/MEK/ERK pathway
Focal adhesion
Protein digestion and absorption
Inflammatory mediator regulation of TRP channels
Ovarian steroidogenesis
VEGF signaling
Apoptosis
Calcium signaling
Pantothenate and CoA biosynthesis
Viral carcinogenesis
Type II diabetes mellitus
Adipocytokine signaling
Prolactin signaling
HIF-1 signaling
cAMP signaling
ECM-receptor interaction
Taste transduction
Plurihormonal
Nicotinate and nicotinamide metabolism
Glycosaminoglycan biosynthesis-heparan-sulfate/heparin
Circadian entrainment
Carbon metabolism
Protein processing in endoplasmic reticulum
Collecting duct acid secretion
Oxidative phosphorylation
Tight junction
Fc gamma R-mediated phagocytosis
C
GH
NF
Pluri
TSH
PRL
ACTH
GT


D
Endocrine signaling
HNF1A
SLCO1C1
MED1
LRP2
Urokinase
Insulin
Gentamicin
GNAS
MEN1
Rap1 Signaling
cAMP Signaling
Sorafenib
Vemurafenib
Regorafenib
Dabrafenib
CREBBP
ORC4
RBL1
Cell Cycle
FFAR2
STAG2
CTNND1
SIPA1
BRAF
HGF
COL4A3
EIF4E1B
PRKAA2
PI3K-Akt Signaling
GH1
Pentosan Polysulfate
FGF4
PIK3C2G
TCL1A
NOS3
BRCA1
VWF
Antihemophilic Factor
INPP4A
Sapropterin
Miconazole
MAPK Signaling
USP8
SYK
SRF
MAP3K6
Immune response signaling
Amlodipine
Verapamil
Gabapentin
Levetiracetam
CACNA1B
Thalidomide
Pranlukast
Triflusal
Acetylsalicylic acid
NFκB1
USP7
MALT1
HDAC4
ZAK
POLR3B
Romidepsin

family of proteins, which participates in a multisubunit complex to promote intracellular organelle transport. Somatic *KIF5A* mutations are found in prostate cancer [9], but had not been linked to PAs. We identified somatic mutations targeting the stalk (p.Y749C) and tail (p.T938I) domains of KIF5A in two PAs. *GRB10* encodes an intracellular adapter protein which interacts with several receptor tyrosine kinases and downstream signaling molecules to regulate secretion of insulin and other peptides [10]. We identified novel mutations in *GRB10* including p.L357F and p.T155S, both in GH-PAs (2 out of 20 GH-PAs, 10%), one of which also contained a *GNAS* mutation, while the other one did not. *GRB10* mutations in GH-PAs implicate *GRB10* in the pathogenesis of GH-PAs. In addition, mutations of other genes, such as *IARS*, *SP100* and *TRIP12*, occurred in two cases (Figure 1A).

We next focused on somatic copy number alternations (SCNA) in our panel of 125 PAs (Supplementary information, Figure S1C). We found that 18% (22/125) of samples had SCNA involving < 10% of the genome; and 32% (40/125) had considerably greater levels of genomic disruption, with > 80% of the genome involved. We observed frequent loss of chromosomes 11q13.2 (q-value = 1.01E-18) and 11p15.5 (q-value = 1.14E-16), consistent with the previous study [11]. We also observed loss of 1p36.31 (q-value = 1.0098E-18), 9q34.11 (q-value = 2.39E-18), 16p13.3 (q-value = 5.82E-18), and 3p21.31 (q-value = 1.34E-17), and frequent gain of 20q13.33 (q-value = 1.47E-29), 3p22.3 (q-value = 3.10E-22), 1q31.3 (q-value = 5.06E-18), 7q21.11 (q-value = 1.54E-17), and 16q12.2 (q-value = 2.59E-21). Our copy number data revealed frequent gains in regions encoding cohesin complex genes including synaptonemal complex genes *SYCP1* on 1p13.2 and *SYCP2* on 20q13.33 and RAD21 cohesin complex like 1 (*RAD21L1*) gene on 20p13 (Figure 1A and Supplementary information, Figure S1C). The identification of frequent amplifications in cohesin complex genes implicates cohesin deregulation in PA pathogenesis [12]. We found no significant association between SCNA as well as gains of cohesin complex regions and clinical features and PA subtypes.

To determine whether any molecular pathways are preferentially targeted by mutations in different PA subtypes, we performed gene set enrichment analysis using annotation based on the KEGG database ($P < 0.05$). NF-, GH-, PRL-, plurihormonal and ACTH-PAs were enriched for somatic mutations in overlapping molecular pathways such as Raf/MEK/ERK, PI3K/AKT/mTOR, cGMP-PKG, oxytocin, insulin and cAMP signalings (Figure 1B). Interconnection based on pathway analysis between each subtype also suggested that GH-, PRL-, plurihormonal and ACTH-PAs are closely related with each other (Figure 1C). On the other hand, TSH- and GT-PAs were enriched for somatic mutations in a distinct set of molecular pathways. In contrast to the other pituitary-secreted hormones which are polypeptides, TSH and GT are glycoproteins which share an identical α subunit. Moreover, TSH- and GT-secreting pituitary cells are both dependent on the transcription factor GATA binding protein 2 (GATA-2) during differentiation, while ACTH-, GH- and PRL-secreting cells are GATA-2 independent [13]. Our pathway analysis raises the possibility that TSH- and GT-PAs may share features of their molecular pathogenesis.

Medical treatment for PAs is limited and patients frequently require lifelong treatment. Genomic alterations identified in targetable genes may be useful to identify PA patients who could potentially benefit from targeted treatment in future clinical trials. For example, *USP8* mutation, by activating EGFR-MAPK signaling, accounts for about 50% of ACTH-PAs [2, 3]. This provides

Figure 1 (A) Somatic mutational and SCNA landscape of PAs. Tumor subtypes based on hormone secretion profile, patient age and gender, mutations in recurrently-mutated genes, number of mutations in each tumor subtype and relative number of different single-nucleotide substitutions in each tumor subtype are shown for 125 PAs. Copy number gain of *SYCP1*, *SYCP2*, and *RAD21L1*, as well as overall percentage of the genome disrupted by copy number variations, are all shown based on GISTIC analysis. **(B)** Significantly enriched pathways in each subtype based on pathway enrichment analysis. Bipartite network of the association between subtypes and pathways, where a yellow node represents a subtype, a pink node represents a pathway, and the line between these two types of nodes indicates that the pathway was significantly ($P < 0.05$) associated with the subtype. Blue lines illustrate associations between a PA subtype and a molecular pathway based on our data in combination with previous whole-exome sequence data. The green lines show the associations between PA subtypes and well-known pathways based on prior publications. **(C)** Interconnections between each subtype based on pathway analysis. Each pair of subtypes is linked by a line with width corresponding to the number of pathways (containing at least two mutated genes) shared by both subtypes. Node size reflects the number of pathways shared by any other subtypes. **(D)** The landscape of potential drug targets in 125 PAs. Genes encoding potential drug targets in seven altered pathways are shown. Genes mutated in PAs in this study are shown in ovals. Genes in blue ovals indicate cancer-related genes. Gene names in brown indicate potential drug targets, with their FDA-approved drugs shown in adjacent brown boxes. Orange stars indicate that drugs tested in phases I-III clinical trials are available to target the gene.

a rationale for an effective treatment for patients with *USP8*-mutated ACTH-PAs by inhibiting USP8 catalytic activity or by anti-EGFR therapy, although anti-EGFR therapy has been shown to be promising *in vitro* and in animal models of ACTH-PAs in general [14]. We further sought to determine whether any potentially actionable cellular pathways are disrupted by mutations in PAs. We found 7 pathways enriched for cancer-related genes that were mutated in our series of 125 PAs (Figure 1D): cAMP signaling, cell cycle, PI3K-Akt signaling, immune response signaling, MAPK signaling, endocrine signaling and Rap1 signaling pathways. We next analyzed 48 existing drugs which target a critical molecule in one of these 7 pathways. Among those 48 drugs, 21 drugs are FDA-approved and 27 drugs are in phases I-III clinical trials. Genes encoding drug targets inthese pathways included *FGF4*, *GNAS*, *HDAC4*, *NFkB1*, *NOS3* and *SYK* (Supplementary information, Table S1C). Altogether, 28% of PA patients in this series had tumors with a mutation in a potentially actionable gene in one of these 7 pathways. This analysis suggests that using drugs to directly target the disrupted pathways may not be a straight forward approach in PAs, as potentially actionable pathways disrupted by somatic mutations were heterogeneous among PAs and were only found in a minority of tumors.

We then attempted to identify links between our mutational data and the clinical characteristics of PA patients. We retrospectively collected clinical information of these patients, including age, tumor size, clinical presentation, and invasion for our entire set of 125 PAs (Supplementary information, Table S1A). Invasiveness is one of the most important clinical phenotypes of PAs. Invasive PAs are always life threatening due to severe symptoms, high mortality and morbidity after surgery, and high incidence of post-operative recurrence. In our cohort, 157 mutations were detected in the invasive tumor group and 222 mutations were in the non-invasive group. As a whole, no significant difference was observed in the number of mutations or the number of targetable mutations or pathways between the invasive and non-invasive tumors. In GH-PAs, *GNAS* mutation has been linked to tumor invasion and drug resistance to somatostatin analogs such as octreotide in several studies but not others [15]. In our cohort, *GNAS* mutation was inversely correlated with tumor invasiveness as determined by pathologic analysis, in which 40% of the *GNAS*-mutated tumors showed invasion compared to 60% of the *GNAS*-WT tumors with invasion ($P = 0.036$). Also, *GNAS* mutation was associated with drug resistance, with 15.4% of the *GNAS*-mutated tumors showing resistance versus 84.6% of the *GNAS*-WT tumors showing resistance ($P = 0.001$). We also confirmed that ACTH-PAs with mutated *USP8* were significantly smaller in size than those with wild-type *USP8* (mean = 1.0 vs 1.9 cm in maximum dimension, $P < 0.001$) [2]. No other significant associations were observed between mutations and basic clinical data, either among the group of all PAs or among any PA subtypes.

In summary, we for the first time present a currently largest genome-wide mutational and SCNA landscape of PAs and a comprehensive genetic landscape for all 7 PA subtypes. We confirmed *GNAS* mutations in GH-PAs, *USP8* mutations in ACTH-PAs, and revealed new somatic variants of *GNAS*, *MEN1* and *NR3C1*. Further, we identified *KIF5A* and *GRB10* as novel recurrently-mutated genes in PAs. Molecular pathways related to specific pituitary hormones were differentially targeted by genetic alterations in the various PA subtypes, and a subset of tumors contained genetic alterations in pathways that may be amenable to therapeutic interventions. This study provides insights into PA pathogenesis and may allow for the design of functional studies that further delineate the biologic basis of the various PA subtypes. We estimated that exome sequencing of 20 samples would identify genes mutated at 30% frequency among a single subtype. Further studies with much larger sample sizes will be needed to identify driver mutations occurring at a lower frequency in these subtypes. Future studies will also be needed to identify whether miRNAs, noncoding regions, or methylation-related events may contribute to the pathogenesis of PAs.

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(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)