Phenotypic and genotypic features of a large kindred with a germline AIP variant

Dal, Jakob; Nielsen, Eigil H; Klose, Marianne; Feldt-Rasmussen, Ulla; Andersen, Marianne; Vang, Søren; Korbonits, Márta; Jørgensen, Jens Otto L

Published in:
Clinical Endocrinology

DOI (link to publication from Publisher):
10.1111/cen.14207

Publication date:
2020

Document Version
Accepted author manuscript, peer reviewed version

DR JAKOB DAL (Orcid ID : 0000-0002-0610-7867)
DR J.O. JORGENSEN (Orcid ID : 0000-0001-7408-1526)

Article type : 1 Original Article - UK, Europe

Title page: **Phenotypic and genotypic features of a large kindred with a germline *AIP* variant**

Jakob Dal[1,2], Eigil H Nielsen[1], Marianne Klose[3], Ulla Feldt-Rasmussen[3], Marianne Andersen[4],, Søren Vang[5], Márta Korbonits[6] and Jens Otto L Jørgensen[7]

[1]Department of Endocrinology Aalborg University Hospital, 9000 Aalborg, Denmark
[2]Steno Diabetic Center Northjutland, 9000 Aalborg, Denmark
[3]Department of Endocrinology and Metabolism, Rigshospitalet, Copenhagen University Hospital, 2100 Copenhagen, Denmark
[4]Department of Endocrinology, Odense University Hospital, 5000 Odense, Denmark
[5]Department of Molecular Medicine, Aarhus University Hospital, 8200 Aarhus N, Denmark,
[6]Centre for Endocrinology, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, EC1M 6BQ, UK
[7]Department of Endocrinology and Internal Medicine and Medical Research Laboratories, Aarhus University Hospital, 8000 Aarhus C, Denmark

Corresponding author, JD: jakob.dal@dadlnet.dk
EIH: ehn@rn.dk
MK: marianne.christina.klose.01@regionh.dk
UFR: Ulla.Feldt-Rasmussen@regionh.dk
MA: Marianne.Andersen1@rsyd.dk

SV: vang@clin.au.dk
MK: m.korbonits@qmul.ac.uk
JOJ: joj@clin.au.dk

Word count: 2935
Short tile: Genotyping and phenotyping of familial acromegaly
Keywords: acromegaly, gigantism, IGF-I, GH, pituitary adenoma, AIP gene variant, familial acromegaly

Phenotypic and genotypic features of a large kindred with a germline *AIP* variant

Abstract

Context: Acromegaly is usually a sporadic disease, but familial cases occur. Mutations in the aryl hydrocarbon receptor-interacting protein (*AIP*) gene are associated with familial pituitary adenoma predisposition. However, the pathogenicity of some *AIP* variants remains unclear and additional unknown genes may be involved.

Objective: To explore the phenotype and genotype of a large kindred carrying the p.R304Q *AIP* variant.

Methods: The family comprised 52 family members at risk of carrying the p.R304Q *AIP* variant including a case with gigantism and one with acromegaly and several family members with acromegalic features. Nine family members (three trios) underwent exome sequencing to identify putative pathogenic variants.

Results: We identified 31 p.R304Q carriers and based on two cases with somatotropinomas the disease penetrance was 6%. We observed physical signs of acromegaly in several family members, which were independent of *AIP* status. Serum Insulin-like Growth Factor-I (IGF-I) levels in all family members were above the mean for age and sex [IGF-I SDS: +0.6 (CI95% +0.4-0.9), p<0.01]. Exome analysis identified two candidate genes: *PDE11A,* known to be associated with the development of adrenal tumors, and *ALG14* that co-segregated with the variant *AIP* gene. Ten asymptomatic p.R304Q family members (age>50 years) were screened for the *PDE11A* and *ALG14* variant; both variants were present in five of ten persons.

Conclusions: This large family adds new information on the p.R304Q *AIP* variant and data suggest two new candidate genes could be associated with growth hormone excess.

Introduction

Pituitary adenomas are some of the most frequent intracranial tumors with a prevalence of up to 800 per million people of which a small proportion is familial (hereditary) [1]. Mutations in the aryl hydrocarbon receptor-interacting protein gene (*AIP*) predispose to the development of pituitary adenomas [2] and account for ≈ 20% of the familial isolated pituitary adenoma patients and 4% of unselected sporadic pituitary adenoma cases, respectively [1,3]. The inheritance pattern is autosomal dominant with an age-dependent penetrance of 20-23 % [3]. The low disease penetrance could theoretically be due to additional disease-modifying genes [4]. FGFR4 has been suggested to influence acromegaly development, but it was not found to alter penetrance in *AIP* mutation positive families [5]. Members of the phosphodiesterase family regulate the cAMP pathway, which is known to play a key role in somatotroph tumorigenesis. PDE11A can modify adrenal adenoma risk [6,7] and their role in pituitary tumorigenesis has being studied [8,9]. The *AIP* gene is suggested to be a tumor suppressor gene and more than 100 different 'pathogenic' or 'likely pathogenic' germline *AIP* variants have been described [1,5] and additional variants are currently considered to be of unknown significance [3]. Patients with *AIP* mutations are predominantly young patients with large tumors that respond poorly to conventional treatment [1]. Identification of *AIP* mutation positive patients prompts the detection of carriers with otherwise unrecognized disease, potentially leading to earlier diagnosis and improved prognosis [5,10,11].

In the present study, we describe the genotype and phenotype of a large kindred with the p.R304Q *AIP* variant, including two index cases with gigantism and acromegaly, respectively.

Subjects and methods

Family tree, genetic screening and exome analysis

We examined the entire family tree of the two index patients (**cases 1** and **2,** described below) for five generations (fig. 1). Both index patients carried a p.R304Q *AIP* variant without detectable mutations in the *MEN1* gene. All family members at risk of carrying the *AIP* variant were offered genetic screening as well as serum measurements of IGF-I, GH and

prolactin. Carriers of the p.R304Q *AIP* variant were subsequently examined by a pituitary MRI in addition to annual measurements of GH, IGF-I and prolactin. Children in the family were followed by paediatricians from the age of 5 years with annual GH, IGF-I and prolactin measurements, growth charts, and a pituitary MRI after the age of 12 years.

Three trios, each consisting of two parents and one child (fig. 2) from the family, were selected for exome sequencing to identify putative mutations ('gene-x') that could be involved in the formation of pituitary adenomas. The first trio included index **case 1** with gigantism carrying the p.R304Q variant and his parents with an asymptomatic father carrying the p.R304Q variant and the mother without the p.R304Q variant (fig. 2A). The second trio included index **case 2** with acromegaly and her healthy husband and their son (**case 3,** described later), who exhibited acromegalic features without carrying the p.R304Q variant (fig. 2B). The third trio included a male carrier of the p.R304Q variant, who presented acromegalic features without biochemical or radiological evidence of acromegaly (**case 4,** described later) and his two parents where the mother was an asymptomatic carrier of the p.R304Q variant (fig. 2C).

Serum GH and IGF-I levels and height measurements

Analysis of GH and IGF-I was centralised and measured, as previously described [12]. IGF-I standard deviation scores (IGF-SDS) were calculated using age- and sex-specific reference values for each IGF-I measurement [13].

Target height estimates were calculated based on data from the Department of Growth and Reproduction at the Danish National Hospital. Target height was calculated using the mid-parental height +6.5 cm/-6.5 cm for males/females respectively, with a confidence interval of ±8.5 cm [14]. Standard deviations scores (SDS) for height were calculated based on a Danish reference cohort [15].

Exome analysis

Genomic DNA libraries were derived from blood samples. Libraries were made with Kapa Hyper Library Prep Kit (KapaBiosystems, Wilmington, MA, USA) followed by exome capture using Nimblegen SeqCap EZ Exome v3.0 Capture Kit (Roche). Indexed libraries were paired-end sequenced (2x 151bp) on an Illumina Nextseq500. On average, 147 (range 84-275)

million read pairs were sequenced per sample yielding a median exome coverage of 141 (range 83-268). Exome analysis: Fastq files were demultiplexed using bcl2fastq (v2.15) and were quality checked using fastQC and fastqScreen [16]. Adapter sequences were trimmed using TrimGalore (v0.4.1) and Cutadapt (v1.9). The paired end sequences were mapped using bwa mem (0.7.5a-r405) to the hg19 reference genome. PCR and optical duplicates were removed from each library independently (Picard package (v2.0.1, [17])and the final bam file was realigned (GATK v3.6 IndelRealigner [18]) and its base quality scores were adjusted in regions with technical artifacts (GATK BaseRecalibrator) using data from dbSNP, 1000G, Mills and 1000G gold standard indels. Basic alignment statistics were calculated using different Picard tools. SNPs and short indels were called using GATK HaplotypeCaller [18]. Individual samples were called in combination with a large combined database of other in-house sequenced samples (1658 gVCF files). This strategy increases the sensitivity at low coverage regions and powers the statistical filtering model that will evaluate the validity of the individual calls. Genomic variants were analyzed using Ingenuity Variant Analysis version 3.1.20150407 [19].

Statistics

Histogram and qq-plot were used to examine continuous variables for normal distribution. Normally distributed data were expressed as mean±95% confidence interval (CI) and non-normal distribution data as median±IQR (interquartal range). If data were not normally distributed, log transformation was applied to archive normal distribution for further statistical use. Student's paired or unpaired t-tests were used to compare variables within or between groups, respectively. The study was approved by the Danish Ethical Committee (1-10-72-117-14) and by the Danish Data Protection Agency (1-16-02-358-14). Consent has been obtained from each patient or subject involved in exome analyses after full explanation of the purpose and nature of all procedures used, including the use of pictures (case 3). The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Index cases and family members with acromegalic traits

Case 1: 37-year-old male presented in 2010 with joint pain, muscle weakness, and signs of gigantism including frontal bossing, prognathism, and enlarged hands and feet. The patient's height was 207 cm (+4 SDS-height) with a target height of 185±8.5 cm. The mean serum GH level during a day curve was 1.3 ug/l and the nadir GH during a glucose suppression test was 0.9 ug/l (< 0.4 ug/l). The serum IGF-I was 340 ug/l (3.3 IGF-SDS) and the prolactin level was normal. A contrast enhanced pituitary MRI (1.5 tesla) gave the suspicion of a small pituitary lesion but without a distinct pituitary adenoma (fig. 3). A whole body PET-CT using a DOTATOC tracer did not disclose pathologic uptake outside the sellar region. His medical history suggested the occurrence of a pituitary apoplexy approximately 10 years prior to the gigantism diagnosis with a self-limited episode of severe headache, nausea, and visual symptoms. The patient was treated with Lanreotide Autogel at a final dose of 120 mg/4 weeks resulting in normalization of serum IGF-I (183 ug/l, 0.0 IGF-SDS), fasting GH of 0.7 ug/L and a GH nadir of 0.57 ug/l. A subsequent MRI performed in 2012 indicated growth of the pituitary lesion and the presence of a pituitary microadenoma (fig. 3). Genetic testing revealed a variant of unknown significance in the *AIP* gene: c.911G>A, p.R304Q.

Case 2*:* A paternal aunt of the index patient was diagnosed with acromegaly in 1988 at the age of 37 years. At that point, she presented elevated fasting GH levels (30 ug/l), borderline elevated prolactin (22 ug/l (< 18 ug/l)), and a pituitary adenoma with a maximal diameter of 10 mm. Her height was 180 cm, whereas her target height was 165 cm corresponding to 1.7 SDS-height. In consistency with a presumed early disease onset, physical signs of acromegaly were noticeable on family-photos before the age of 22. One month prior to transsphenoidal surgery, the patient experienced an episode of severe headache, nausea and vomiting, which lasted for three days. At surgery signs of a recent pituitary apoplexy within a cystic pituitary adenoma was noted. Postoperatively, due to hypopituitarism, the patient was replaced with hydrocortisone, levothyroxine and GH.

Case 3: A 29-year-old healthy son of index case 2 exhibited acromegalic features with frontal bossing, a large jaw and large hands, but did not carry the p.R304Q variant and had normal IGF-I values 183 ug/l (0.72 IGF-SDS). The patient had remarkable physical similarities to his cousin with gigantism (case 1). His actual height was 188 cm, and his target height was 172 ± 6.5cm corresponding to 1.1 SDS-height (based on his father's and

on his grandparents' heights) as his mother's (case 2) height could not be used as she had early onset acromegaly.

Case 4: A 44-year-old male carrier of the p.R304Q variant who exhibited an acromegalic appearance with frontal bossing, prognathism, and enlarged hands and feet (shoe size 49, fig. 4). His height was 193 cm as compared to a target height of 187 cm± 6.5cm corresponding to 1.8 SDS-height, but without additional acromegalic features. He used no prescription medicine, but was diagnosed with migraine during adolescence. The episodes of migraine culminated when he reached the age of 35 years where he experienced periods with severe headaches, nausea and transient loss of sight. During the subsequent years, the migraine episodes became less severe and frequent. He displayed slightly elevated IGF-I levels estimated by two different assays: iSYS: 239 ug/l (2.37 IGF-SDS) and Liaison: 35.6 nmol/l (11.5-27.3 nmol/L), but a normal GH suppression during glucose tolerance testing with a nadir GH level of 0.1 ug/l. Two normal pituitary MRIs were performed at two-years interval.

p.R304Q *AIP* variant screening

A total of 159 individuals were identified covering five generations from a common ancestor couple who had 14 children. In the second generation, all individuals were deceased except for an 80-year-old male who carried the p.R304Q variant without evidence of acromegaly. None of his 13 siblings were diagnosed with acromegaly.

Genetic screening of 72 surviving family members identified six of the 14 siblings as carriers of the *AIP* p.R304Q variant putting at risk 52 family members of inheriting the mutation, including the two index cases (fig. 1). The sex distribution among the individuals at risk was equal (25 males/27 females). Among the 50 healthy predisposed family members, including both variant carriers (304Q+, n=29) and variant non-carrier (304Q-, n=21) individuals, IGF-I-SDS levels were significantly elevated (0.6 SDS (CI95% 0.4-0.9), $p<0.01$, fig. 5) and several members exhibited acromegalic traits independent of *AIP* variant status (304Q +: IGF-I 0.6 SDS (CI95% 0.2-0.9); 304Q -: IGF-I 0.7 SDS (CI95% 0.4-1.1), $p=0.5$). Among the 304Q + members, the average age was 39 years (CI95% 31.0-46.1). Based on the two patients with somatotropinomas, the disease penetrance was 6%. If we include

the case with NFPA, the prevalence was 10% and 13% among patients older than 30 years[11]. Additional hormone measurements showed normal serum levels of prolactin and a mean random GH concentration of 0.7 ug/l (CI95% 0.1-1.3).

One p.R304Q variant carrier (case 5) without symptoms or signs of hormonal hypersecretion presented with an intrasellar cystic lesion on the initial MRI and an intrasellar microadenoma (7 x 4 mm) on a second MRI 3 years later.

Exome analysis: **PDE11A and ALG14**

We performed a combined analysis of the nine samples (three trios, fig 2) in Ingenuity Variant Analysis (v5.2). From a total of 288,193 variants in 20,712 genes, a *PDE11A* variant showed up consistently in all nine subjects. *In silico* predictions of the pathogenicity of the *PDE11A* variant (chr2:178936272, c.893A>G, p.N298S, rs78328794) are differing: SIFT and AlignGVGD predict the variant: Benign ("C0" and "Tolerated", respectively) whereas Mutation Taster and PolyPhen2 predict the variant: Disease causing (prob:1) and Probably damaging (0.998) respectively (using Alamut Visual 2.14). The variant has a frequency of 0.11% in non-Finnish Europeans (gnomAD r2.1.1.). There are no functional studies available for this variant and it is not significantly associated with to testicular [20] or prostate tumours [21].

Another variant in the *ALG14* gene also co-segregated with the *AIP* p.R304Q variant. *In silico* predictions of the *ALG14* variant (chr1:95538342, c.113G>T, p.S38I, rs139521179) are: AlignGVGD: Benign (C0), Mutation Taster: Disease causing (prob. 0.974), SIFT: Tolerated (0.15) and PolyPhen2: Possibly damaging (0.17). The frequency of the variant in gnomAD (r2.1.1) is 0.37% in non-Finnish Europeans. Individuals carrying the *PDE11A* or *ALG14* variants are shown in figure 2, including case 1-4.

Asymptomatic p.R304Q family members (n=10, M=7/F=3) were screened for the *PDE11A* and *ALG14* variant; 3 subjects had the *PDE11A* variant, three the *ALG14* variant and two subjects harbored both variants (fig 1).

Discussion

In this study, we describe a very large family carrying the *AIP* p.R304Q variant. The family

includes one index case with gigantism (case 1) and a second case with early-onset acromegaly (case 2), both of whom had a history suggestive of a pituitary apoplexy. During the family screening, we identified one case of a clinically non-functioning pituitary adenoma (case 5) that we suspected to be a phenocopy, and we identified two healthy family members (case 3 and 4) with acromegalic traits independent of *AIP* status. Based on the two patients with somatotropinomas, the disease penetrance was 6%.

Finally, based on a trio analysis, we identified two potential disease-modifying genes *PDE11A* and *ALG14* by exome analysis.

Genetic screening for *AIP* variants in acromegaly has so far identified 32 patients as carriers of the p.R304Q variant [5,22,31,32,23–30], but the pathogenic role remains uncertain (18). Patient characteristics among the p.R304Q carriers are in some cases similar to overtly pathogenic *AIP* variants with respect to younger age at diagnosis, large tumors and a family history, but there are cases with Cushing disease, which is not typical of the spectrum of tumor types associated with *AIP* mutations [25]. Furthermore, two healthy persons with homozygous p.R304Q variant have been described in the gnomAD database [33] and lack of loss of heterozygosity in somatotropinoma cells has been reported [34]. In line with this observation, most of the *in vitro* studies (using various techniques, such as protein half-life and cell proliferation) do not support a pathogenicity of the p.R304Q variant [27,30,35–40]. However the β-galactosidase quantitative two-hybrid assay found a borderline loss of interaction between p.R304Q variant and PDE4A5 indicating pathogenicity [8,36]. Taken together, the impact of the 304Q variant is still unclear, but the majority of data points toward a less harmful variant compared to other *AIP* mutations. The formal American College of Medical Genetics classification of this variant is 'Variant of uncertain significance"[41].

We observed acromegalic features (case 3) and elevated IGF-I levels in family members in the absence of the *AIP* variant (fig 5). This could indicate the presence of hitherto unidentified regulatory or genetic factors predisposing to increased production and activity of IGF-I. The low disease penetrance in *AIP* mutations and the variance in clinical phenotypes in a large kindred has previously been ascribed to unidentified disease-modifying genes [5]. Based on three trios, including the two cases with somatotropinomas, two

healthy persons with acromegalic features and five healthy relatives, we identified two potential disease-modifying candidate genes through exome sequencing: *PDE11A* and *ALG14*.

The *PDE11A* is a member of the PDE family, with higher expression in pituitary tumours than in normal tissue, but no significant difference in *PDE11A* variants are reported between pituitary adenoma patient compared to controls [9]. The *PDE11A* gene contains 23 exomes and four splice variants have been identified with a dual-specificity for both cAMP and cGMP [42]. *In vitro* studies of *PDE11A* sequence variants that were predicted *in silico* to affect function showed a decreased enzymatic activity, leading to higher levels of cAMP and cGMP in HEK293 cells [43]. Similarly, an increased level of cAMP and a decreased *PDE11A* immunostaining were present in adrenocortical tumours tissues harboring the missense variants, compared with tumours with wild-type *PDE11A* sequence [44] . Data on adrenal, testicular and prostate tumours, and even in familial breast cancer[45], suggest that defects in *PDE11A* predispose to these tumours, but no data are available for pituitary [6,7,9]. A study on *PDE11A* variants in acromegaly patients without known *AIP* mutations did not find an increase in *PDE11A* variants, but patients with the variants tended to exhibit a more aggressive phenotype [9]. Moreover, *in silico* assessment of the *PDE11A* variant was classified as "damaging". In the present family, all cases with acromegaly or acromegalic features carried the *PDE11A* variant but it also occurred in 50% of the tested healthy p.R304Q carriers (fig 1), which questions its pathogenic role. Currently it is not known whether genetic, epigenetic or environmental factors regulate disease penetrance, this is active field of research in several 'monogenic' diseases.

The human ALG14 protein consists of 216 amino acids and forms a hetero-dimeric complex together with ALG13. It is located in the endoplasmic reticulum membrane where it is involved in N-linked glycosylation that is essential for glycoprotein folding and stability [46]. *ALG14* mutations have been associated with severe intellectual disability and other neurological disorders, but so far not with pituitary disease or growth disturbances [47,48]. The *ALG14* variant was in general predicted to be "tolerant" by the *in silico* analyses. In the present family, the *ALG14* variant occurred in 50% of the tested healthy p.R304Q carriers (fig 1)

The fact that only two family members presented with somatotropinomas and

no tumour tissue was available limited the possibilities of genetic analyses. It would be interesting to examine changes in the *PDE11A* and *ALG14* gene in other cases with the p.R304Q *AIP* variant and in other familial isolated pituitary adenoma cases. In our large family, we are now following 14 asymptomatic individuals carrying the p.R304Q variant with annual blood sampling. Therefore, the p.R304Q *AIP* variant remains a variant of unknown significance, but new cases with pituitary diseases could help to shed light on the role of this variant in somatotroph cell function or pituitary adenoma predisposition.

Funding

This project was supported by an unrestricted grant from Novartis.

Disclosure

JD and MK: unrestricted research grant and lecture fees from Pfizer. UFR: research salary from the Novo Nordisk Foundation. JOLJ has received unrestricted grants and lecture fees from IPSEN, Novartis and Pfizer and served on advisory boards for Novartis and Pfizer.

1. Beckers A, Aaltonen LA, Daly AF, Karhu A. Familial isolated pituitary adenomas (FIPA) and the Pituitary adenoma predisposition due to mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene. *Endocr Rev*. 2013;34(2):239-277. doi:10.1210/er.2012-1013
2. Vierimaa O, Georgitsi M, Lehtonen R, et al. Pituitary adenoma predispostion caused by germline mutations in the AIP gene. *Science (80-)*. 2006;312(5777):1228-1230. doi:10.1086/275028
3. Gadelha MR, Kasuki L, Korbonits M. The genetic background of acromegaly. *Pituitary*. 2017;20(1):10-21. doi:10.1007/s11102-017-0789-7
4. Tateno T, Asa SL, Zheng L, Mayr T, Ullrich A, Ezzat S. The FGFR4-G388R polymorphism promotes mitochondrial STAT3 serine phosphorylation to facilitate pituitary growth hormone cell tumorigenesis. *PLoS Genet*. 2011;7(12):1-13. doi:10.1371/journal.pgen.1002400

5. Hernández-Ramírez LC, Gabrovska P, Dénes J, et al. Landscape of familial isolated and young-onset pituitary adenomas: Prospective diagnosis in AIP mutation carriers. *J Clin Endocrinol Metab*. 2015;100(9):E1242-E1254. doi:10.1210/jc.2015-1869
6. Boikos S, Horvath A, Heyerdahl S, et al. Phosphodiesterase 11A Expression in the Adrenal Cortex, Primary Pigmented Nodular Adrenocortical Disease, and other Corticotropin-independent Lesions. *Horm Metab Res*. 2008;40(5):347-353. doi:10.1055/s-2008-1076694
7. Libé R, Horvath A, Vezzosi D, et al. Frequent phosphodiesterase 11A gene (PDE11A) defects in patients with Carney Complex (CNC) caused by PRKAR1A mutations: PDE11A may contribute to adrenal and testicular tumors in CNC as a modifier of the phenotype. *J Clin Endocrinol Metab*. 2011;96(1):208-214. doi:10.1210/jc.2010-1704
8. Bizzi MF, Bolger GB, Korbonits M, Ribeiro-Oliveira Jr. A. Phosphodiesterases and cAMP Pathway in Pituitary Diseases. *Front Endocrinol (Lausanne)*. 2019;10. doi:10.3389/fendo.2019.00141
9. Peverelli E, Ermetici F, Filopanti M, et al. Analysis of genetic variants of phosphodiesterase 11A in acromegalic patients. *Eur J Endocrinol*. 2009;161(5):687-694. doi:10.1530/EJE-09-0677
10. Williams F, Hunter S, Bradley L, et al. Clinical experience in the screening and management of a large kindred with familial isolated pituitary adenoma due to an aryl hydrocarbon receptor interacting protein (AIP) mutation. *J Clin Endocrinol Metab*. 2014;99(4):1122-1131. doi:10.1210/jc.2013-2868
11. Marques P, Caimari F, Hernández-Ramírez LC, et al. Significant benefits of AIP testing and clinical screening in familial isolated and young-onset pituitary tumors. 2020;1:1-40. doi:10.1210/clinem/dgaa040.
12. Dal J, Klose M, Heck A, et al. Targeting either GH or IGF-I during somatostatin analogue treatment in patients with acromegaly: A randomized multicentre study. *Eur J Endocrinol*. 2018;178(1). doi:10.1530/EJE-17-0546
13. Bidlingmaier M, Friedrich N, Emeny RT, et al. Reference intervals for insulin-like growth factor-1 (IGF-I) from birth to senescence: Results from a multicenter study using a new automated chemiluminescence IGF-I immunoassay conforming to recent international recommendations. *J Clin Endocrinol Metab*. 2014;99(5):1712-1721.

doi:10.1210/jc.2013-3059
14. Predicted adult height. http://vækstkurver.dk/estimeret_sluthoejde.html. Accessed April 7, 2019.
15. Dansih Growth curves. http://vækstkurver.dk/sds_tabeller.html. Accessed April 7, 2019.
16. Babraham Bioinformatics. 2015;2:161202-161204. http://www.bioinformatics.babraham.ac.uk/projects/. Accessed April 7, 2019.
17. picard SourceForge.net. https://sourceforge.net/projects/picard/. Accessed April 7, 2019.
18. Poplin R, Ruano-Rubio V, DePristo MA, et al. Scaling accurate genetic variant discovery to tens of thousands of samples. *bioRxiv*. 2017:201178. doi:10.1101/201178
19. QIAGEN Bioinformatics. https://www.qiagenbioinformatics.com/. Accessed April 7, 2019.
20. Pathak A, Stewart DR, Faucz FR, et al. Rare inactivating PDE11A variants associated with testicular germ cell tumors. *Endocr Relat Cancer*. 2015;22(6):909-917. doi:10.1530/ERC-15-0034.Rare
21. Faucz FR, Horvath A, Rothenbuhler A, et al. Phosphodiesterase 11A (PDE11A) genetic variants may increase susceptibility to prostatic cancer. *J Clin Endocrinol Metab*. 2011;96(1):135-140. doi:10.1210/jc.2010-1655
22. Cazabat L, Libè R, Perlemoine K, et al. Germline inactivating mutations of the aryl hydrocarbon receptor-interacting protein gene in a large cohort of sporadic acromegaly: mutations are found in a subset of young patients with macroadenomas. *Eur J Endocrinol*. 2007;157(1):1-8. doi:10.1530/eje-07-0181
23. Cazabat L, Bouligand J, Salenave S, et al. Germline AIP mutations in apparently sporadic pituitary adenomas: Prevalence in a prospective single-center cohort of 443 patients. *J Clin Endocrinol Metab*. 2012;97(4):663-670. doi:10.1210/jc.2011-2291
24. Cuny T, Pertuit M, Sahnoun-Fathallah M, et al. Genetic analysis in young patients with sporadic pituitary macroadenomas: Besides AIP don't forget MEN1 genetic analysis. *Eur J Endocrinol*. 2013;168(4):533-541. doi:10.1530/EJE-12-0763
25. Tuncer FN, Çiftçi Doğanşen S, Serbest E, et al. Screening of AIP Gene Variations in a Cohort of Turkish Patients with Young-Onset Sporadic Hormone-Secreting Pituitary

Adenomas . *Genet Test Mol Biomarkers*. 2018;22(12):702-708. doi:10.1089/gtmb.2018.0133

26. Daly AF, Tichomirowa MA, Petrossians P, et al. Clinical characteristics and therapeutic responses in patients with germ-line AIP mutations and pituitary adenomas: An international collaborative study. *J Clin Endocrinol Metab*. 2010;95(11):373-383. doi:10.1210/jc.2009-2556
27. Igreja S, Chahal HS, King P, et al. Characterization of aryl hydrocarbon receptor interacting protein (AIP) mutations in familial isolated pituitary adenoma families. *Hum Mutat*. 2010;31(8):950-960. doi:10.1002/humu.21292
28. Leontiou CA, Gueorguiev M, Van Der Spuy J, et al. The role of the aryl hydrocarbon receptor-interacting protein gene in familial and sporadic pituitary adenomas. *J Clin Endocrinol Metab*. 2008;93(6):2390-2401. doi:10.1210/jc.2007-2611
29. Preda V, Korbonits M, Cudlip S, Karavitaki N, Grossman AB. Low rate of germline AIP mutations in patients with apparently sporadic pituitary adenomas before the age of 40: A single-centre adult cohort. *Eur J Endocrinol*. 2014;171(5):659-666. doi:10.1530/EJE-14-0426
30. Schöfl C, Honegger J, Droste M, et al. Frequency of AIP gene mutations in young patients with acromegaly: A registry-based study. *J Clin Endocrinol Metab*. 2014;99(12):E2789-E2793. doi:10.1210/jc.2014-2094
31. Tichomirowa MA, Barlier A, Daly AF, et al. High prevalence of AIP gene mutations following focused screening in young patients with sporadic pituitary macroadenomas. *Eur J Endocrinol*. 2011;165(4):509-515. doi:10.1530/EJE-11-0304
32. Occhi G, Grimaldi F, Castello R, et al. Prevalence of AIP mutations in a large series of sporadic Italian acromegalic patients and evaluation of CDKN1B status in acromegalic patients with multiple endocrine neoplasia. *Eur J Endocrinol*. 2010;163(3):369-376. doi:10.1530/EJE-10-0327
33. https://gnomad.broadinstitute.org/variant/11-67258382-G-A. gnomAD browser I AIP p.Arg304Gln. https://gnomad.broadinstitute.org/variant/11-67258382-G-A. Accessed October 31, 2019.
34. Mothojakan N, Ferraù F, Dang M, et al. Polymorphism or mutation? - The role of the R304Q missense AIP mutation in the predisposition to pituitary adenoma. In:

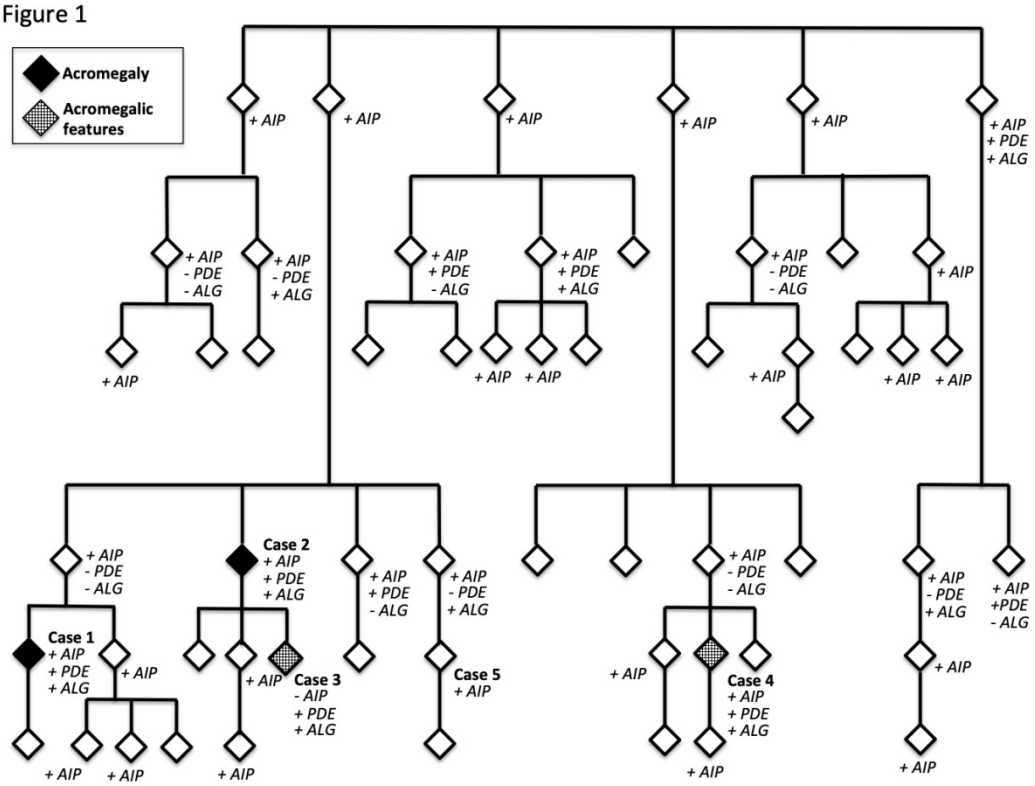
Endocrine Abstracts. ; 2016:p167. doi:https://www.endocrine-abstracts.org/ea/0044/ea0044p167

35. Aflorei ED, Klapholz B, Chen C, et al. In vivo bioassay to test the pathogenicity of missense human AIP variants. *J Med Genet*. 2018;55(8):522-529. doi:10.1136/jmedgenet-2017-105191
36. Bolger GB, Bizzi MF, Pinheiro S V, et al. cAMP-specific PDE4 phosphodiesterases and AIP in the pathogenesis of pituitary tumors. *Endocr Relat Cancer*. 2016;23(5):419-431. doi:10.1530/erc-15-0205
37. Hernández-Ramírez LC, Martucci F, Morgan RML, et al. Rapid proteasomal degradation of mutant proteins is the primary mechanism leading to tumorigenesis in patients with missense AIP mutations. *J Clin Endocrinol Metab*. 2016;101(8):3144-3154. doi:10.1210/jc.2016-1307
38. Hernández-Ramírez LC, Morgan RML, Barry S, D'Acquisto F, Prodromou C, Korbonits M. Multi-chaperone function modulation and association with cytoskeletal proteins are key features of the function of AIP in the pituitary gland. *Oncotarget*. 2018;9(10):9177-9198. doi:10.18632/oncotarget.24183
39. Fainstein Day P, Loto MG, Glerean M, Picasso MFR, Lovazzano S, Giunta DH. Incidence and prevalence of clinically relevant pituitary adenomas: Retrospective cohort study in a health management organization in Buenos Aires, Argentina. *Arch Endocrinol Metab*. 2016;60(6):554-561. doi:10.1590/2359-3997000000195
40. Morgan RML, Hernández-Ramírez LC, Trivellin G, et al. Structure of the TPR Domain of AIP: Lack of Client Protein Interaction with the C-Terminal α-7 Helix of the TPR Domain of AIP Is Sufficient for Pituitary Adenoma Predisposition. *PLoS One*. 2012;7(12):20-25. doi:10.1371/journal.pone.0053339
41. LSue Richards, PhD1 , Nazneen Aziz, PhD2, 16 , Sherri Bale, PhD3 , David Bick M, , Soma Das P, Julie Gastier-Foster, PhD6, 7, 8, Wayne W. Grody, MD, PhD9, 10, 11, Madhuri Hegde P, et al. Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology Sue. *Genet Med*. 2015;17(5):405-424. doi:10.1038/gim.2015.30.Standards
42. Azevedo MF, Faucz FR, Bimpaki E, et al. Clinical and molecular genetics of the

phosphodiesterases (pdes). *Endocr Rev*. 2014;35(2):195-233. doi:10.1210/er.2013-1053

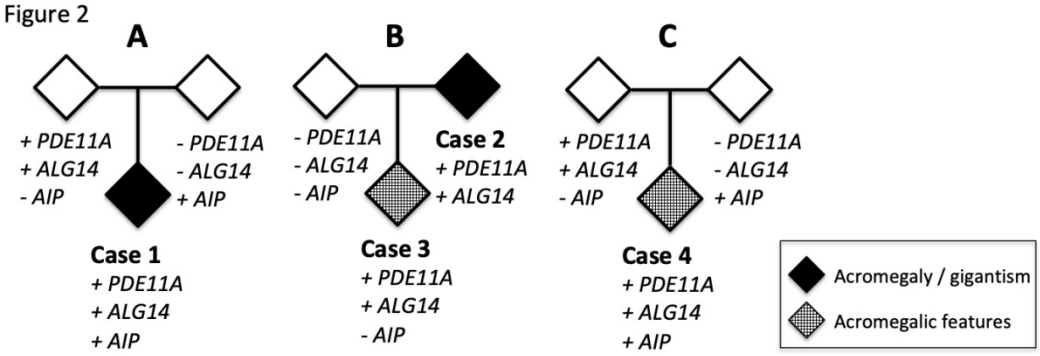
43. Horvath A, Giatzakis C, Robinson-White A, et al. Adrenal hyperplasia and adenomas are associated with inhibition of phosphodiesterase 11A in carriers of PDE11A sequence variants that are frequent in the population. *Cancer Res*. 2006;66(24):11571-11575. doi:10.1158/0008-5472.CAN-06-2914
44. Libé R, Fratticci A, Coste J, et al. Phosphodiesterase 11A (PDE11A) and genetic predisposition to adrenocortical tumors. *Clin Cancer Res*. 2008;14(12):4016-4024. doi:10.1158/1078-0432.CCR-08-0106
45. Shahi RB, De Brakeleer S, Caljon B, et al. Identification of candidate cancer predisposing variants by performing whole-exome sequencing on index patients from BRCA1 and BRCA2-negative breast cancer families. *BMC Cancer*. 2019;19(1):1-11. doi:10.1186/s12885-019-5494-7
46. Gao XD, Tachikawa H, Sato T, Jigami Y, Dean N. Alg14 recruits Alg13 to the cytoplasmic face of the endoplasmic reticulum to form a novel bipartite UDP-N-acetylglucosamine transferase required for the second step of N-linked glycosylation. *J Biol Chem*. 2005;280(43):36254-36262. doi:10.1074/jbc.M507569200
47. Kvarnung M, Taylan F, Nilsson D, et al. Genomic screening in rare disorders: New mutations and phenotypes, highlighting ALG14 as a novel cause of severe intellectual disability. *Clin Genet*. 2018;94(6):528-537. doi:10.1111/cge.13448
48. Engel A. Congenital Myasthenic Syndromes in 2018. *Curr Neurol Neurosci Rep*. 2018;36(2):367-378. doi:10.1016/j.ncl.2018.01.007

Figure 1



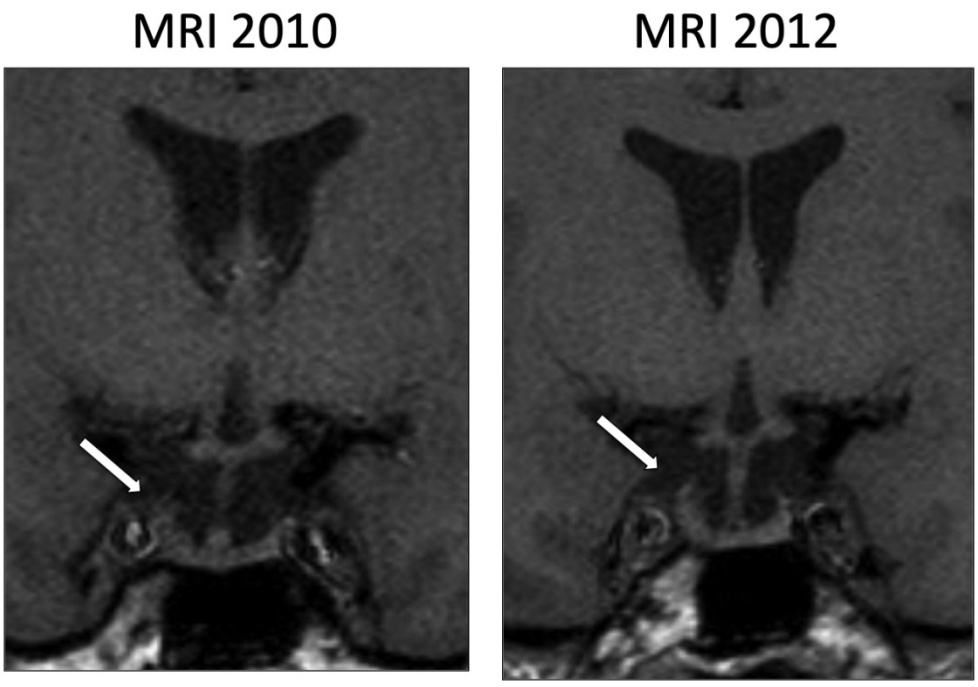
cen_14207_f1.jpg

Figure 2

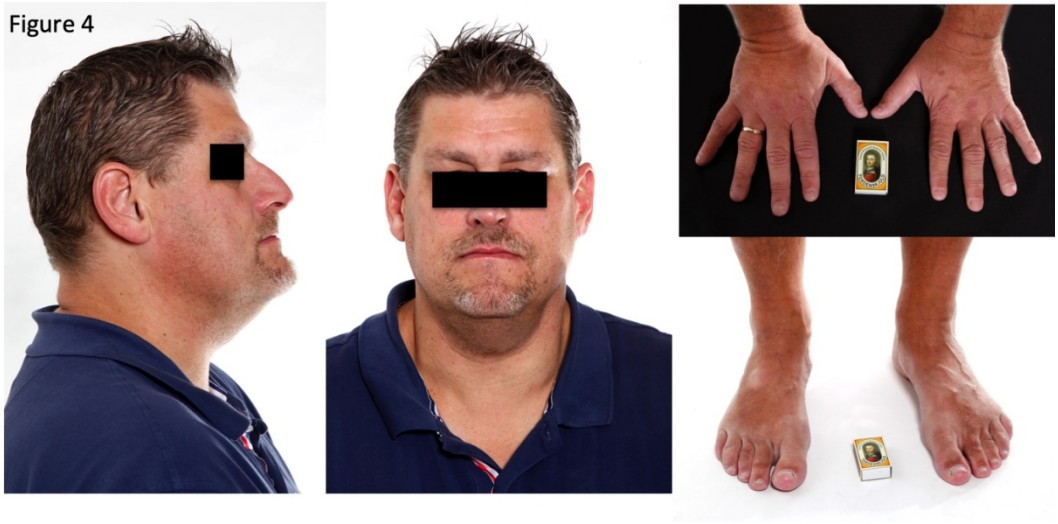


cen_14207_f2.jpg

Figure 3

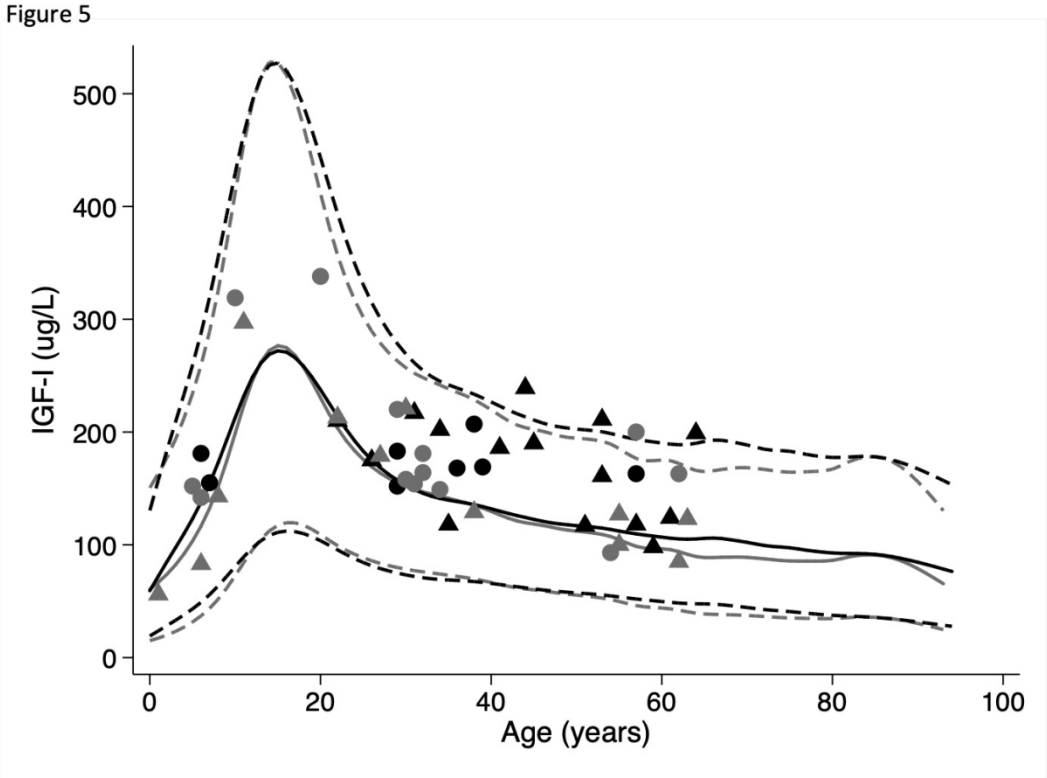


cen_14207_f3.jpg



cen_14207_f4.jpg

Figure 5



cen_14207_f5.jpg