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IMPACT OF AIP AND GA_{I-2} PROTEINS ON CLINICAL FEATURES OF SPORADIC GH-SECRETING PITUITARY ADENOMAS

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Introduction

In sporadic acromegaly, downregulation of AIP protein of the adenomas associates with invasive tumour features and reduced response to somatostatin analogue treatment. AIP is a regulator of Gαi signaling, but it is not known how the biological function of the Gαi pathway is controlled.

Aim

To study somatic GNAS and AIP mutation status, AIP and Gαi-2 protein expressions, Ki67 proliferation indices, and clinical parameters in patients having primary surgery because of acromegaly at a single centre between years 2000-2010.

Results

Sixty patients (F/M 31/29, mean age 49 (median 50), mean follow-up 7.7 (range 0.6-14.0) yrs) underwent primary surgery. Of the 60 adenoma specimens, four (6.8%) harboured an *AIP* and 21 (35%) an activating *GNAS* (*Gsp*+) mutation. All adenomas stained positive for Gαi-2, and 55/56 *AIP* mutation negative adenomas stained positive for AIP protein. Altogether 13/56 (23%) adenomas had low AIP protein levels, and 14/56 (25%) low Gαi-2 staining. A regression model including Gαi-2, Ki 67 proliferation indices and GH (measured three months after surgery), best explained the variance in the AIP protein level (p=6.03x10^{-9}). The majority (43%) was explained by $G\alpha_{i-2}$ level only. *Gsp*+ status was not related to AIP or Gαi-2 protein levels, but associated with lower KNOSP grade (p= 0.0018, r= 0.332), tumours restricted to the sella (p= 0.026, r= 0.320), and higher preoperative prolactin concentrations (p= 0.017, r= 0.032). However, the associations were not significant after correction for multiple testing.

Table 1. Comparison between patients treated by primary surgery only and patients with multimodal treatment

	Total, n	Primary surgery only (n=37)	Primary surgery + any other treatment modality (n=20)	ES	p value	Sg	q
Gender, F/M, n	53	18/18	9/8	0.027	1	NS	1
Mean age at diagnosis, years	53	51.4	47.8	0.110	0.433	NS	0.874
Mean preoperative GH, ug/l	53	42.7	129.8	0.538	**3.76x10^{-5}**	**	0.001
Preoperative IGF-1, %ULN/100	50	2.1	2.4	0.122	0.395	NS	0.874
Size (micro vs. macro)	53	5/31	2/15	0.029	1	NS	1
KNOSP grade > 2, n (%)	53	4 (11.1)	8 (47.1)	0.397	**0.006**	**	0.043
Somatic GNAS mutation, n (%)	53	16 (44.4%)	4 (23.5)	0.199	0.225	NS	0.783
Gαi-2 protein level 3 or < 3, n (%)	53	10 (27.8)	5 (29.4)	0.017	1	NS	1
AIP protein level 3 or < 3, n (%)	53	7 (19.4)	5 (29.4)	0.110	0.490	NS	0.877
Mean Ki-67 proliferation index, < 1-6	51	1.6	1.6	0.029	0.839	NS	1
Mean 3-month postoperative GH, ug/l	52	3.4	22.3	0.632	**7.53x10^{-7}**	**	3.19x10^{-5}
Mean 3-month postoperative IGF-1, %ULN/100	50	0.54	1.44	0.593	**7.28x10^{-6}**	**	1.32x10^{-4}
In remission at last follow-up visit, %	52	97.1	64.7	0.441	**0.003**	**	0.029
Mean follow-up time, yrs	52	7.1	8.8	0.280	**0.044**	*	0.231

Table 2: Clinical characteristics in 60 sporadic somatotroph adenomas according to tumour type

	AIP mutation positive (n=4, 6.7%)	GNAS mutation positive (n=21, 35.0%)	Wildtype (n=35, 58.3%)
Demographics			
Gender, M/F (n)	3/1	8/13	18/17
Mean age at diagnosis, years (SD)	31 (10)	52 (15)	49 (11)
Mean follow-up time, years (SD)*	11 (4)	7 (3)	8 (3), 2 missing
Tumor characteristics			
Tumor size, micro/macro (%)	0/100	10/90	14/86
Suprasellar extension, n (%)	3 (75)	6 (29)	21 (60), 1 missing
KNOSP grade 2 or above, n (%)	3 (75)	5 (24)	18 (52), 2 missing
Ki-67 < 3%, n (%)	3 (75), 1 missing	15 (71), 1 missing	29 (83), 1 missing
Biochemical measurements at diagnosis			
Mean GH at diagnosis, µg/l (SD)	44 (36)	56 (62), 14 missing	75 (85)
Mean IGF-1 at diagnosis, x ULN (SD)	1,9 (0,9)	2,5 (1,3)	2,1 (1,1), 3 missing
Mean PRL at diagnosis, mU/l (SD)	12000 (24000)	2900 (9500)	320 (290)
Hypopituitarism at diagnosis, n (%)	2 (50)	9 (43)	15 (43)
Treatment			
Reoperation, n (%)	1 (25)	1 (5)	4 (11)
Radiotherapy, n (%)	1 (25)	1 (5)	4 (11)
Suppressive medical therapy, n (%)	3 (75)	4 (19), 1 missing	13 (37), 3 missing
Somatostatin analogue, n (%)	1 (25)	4 (19), 1 missing	12 (34), 3 missing
Cabergoline, n (%)	3 (75)	2 (10), 1 missing	5 (14), 3 missing
Pegvisomant, n (%)	0 (0)	1 (5), 1 missing	2 (6), 3 missing
Preoperative medical treatment			
SSA, n (%)	0 (0)	1 (5)	4 (11)
Cabergoline, n %)	0 (0)	3 (14)	0 (0)

*time between diagnostic MRI and last clinical follow-up visit

Table 3: Linear regression model of AIP protein expression level. For each predictor (Gαi-2 level, Ki-67 and three-month postoperative GH concentration), coefficient (b), standard error (SE), t statistic, p-value and 95% confidence intervals are reported. The constant term b0 of the regression model y=b0+∑bixi+ε denoted by (Constant). Statistically significant with *p<0.05 and ***p <0.001.

Variable	b	SE	t	p	CI lower	CI upper
(Constant)	0.39	0.10	3.92	**2.81x10^{-4}*** **	0.19	0.59
Gai-2	0.68	0.09	7.33	**2.33x10^{-9}*** **	0.49	0.86
KI-67	-0.06	0.03	-2.12	**0.04***	-0.13	0.00
Three-month GH	-0.003	0.002	-1.07	0.29	-0.01	0.00

n=52, degrees of freedom df=48, adjusted R2=0.55, F=21.4, BIC=26.1, p=6.03x10-9***

Figure 1

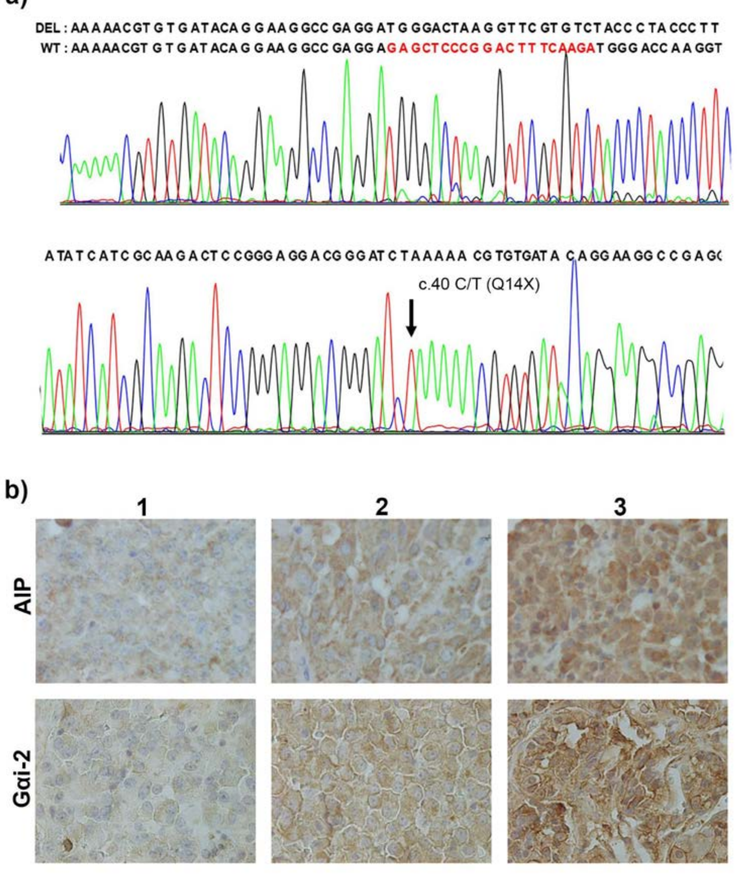

Figure 1. a) *AIP* exon 1 mutations found in sequenced somatotropinomas; a short out-of-the-frame c.70>89delGAGCTCCCGGACTTTCAAGA deletion (upper panel) and a Finnish Q14X nonsense mutation (lower panel) with LOH. In upper panel is shown the wild-type (WT) and the mutant allele sequences. The deleted region is colored red in the WT sequence. The exact deletion breaking points were confirmed by sequencing the amplicon in both directions. **b)** Immunostainings for AIP and $G\alpha_{i-2}$ proteins in sporadic somatotropinomas. The upper panel shows a diffuse cytoplasmic AIP immunoreactions and the lower panel cytoplasmic/membranous $G\alpha_{i-2}$ stainings in corresponding pituitary tumors. 1= weak, 2=moderate and 3= strong immunoreaction intensity.

Conclusions

We demonstrate, for the first time, that AIP protein expression associates with $G\alpha_{i-2}$ protein intensities in sporadic somatotropinomas. This may indicate a synergetic effect on somatostatin signaling. Low AIP protein levels associate with higher proliferation activity and higher postoperative serum GH, indicating more aggressive adenomas. The *AIP* mutation rate of 6.8% is fairly high and probably reflects the genetic composition of the Finnish population.