

**Supplemental table 1: Clinical and genetic characteristics of the 10 patients harboring a variant of undetermined significance\* in this study.**

M, man; W, woman; macro, macroadenoma, defined by a diameter >10 mm; micro, microadenoma, defined by a diameter <10 mm; NA, not available; NR, not previously reported \*Classification of sequence variants using ACMG guidelines(1)

<b>MEN1 (NM_130799)</b>								
<b>N°</b>	<b>Sex</b>	<b>Size of prolactinoma</b>	<b>Age at diagnosis of tumor</b>	<b>Familial context</b>	<b>Exon</b>	<b>Nucleotide variant</b>	<b>Protein mutation</b>	<b>Ref</b>
1	W	macro	25	0	2	c.-16C>G	p.(?)	NR
2	W	micro	21	0	3	c.654+48C>T	p.(?)	NR
3	M	macro	29	0	4	c.691G>C	p.(Asp231His)	(2)
4	M	macro	4	0	7	c.948A>T	p.(Glu316Asp)	(2)
5	M	macro	19	Grand-father: macroprolactinoma	9	c.1186-96G>T	p.(?)	NR
<b>AIP (NM_003977)</b>								
6	M	macro	20	0	2	c.174G>C	p.(Lys58Asn)	(3)Same patient
7	M	micro	15	0	3	c.325G>A	p.(Ala109Thr)	NR
8	M	micro	50	Father: macroprolactinoma	6	c.788-12C>A	p.(?)	NR
<b>CDKN1B (NM_004064)</b>								
9	W	macro	26	2 first cousins: somatotroph adenomas	1	c.-213C>T	p.(?)	NR
10	M	NA	15	0	2	c.586C>T	p.(Arg196Cys)	NR

**Supplemental table 2: Clinical and genetic characteristics of the 12 patients harboring a likely benign variant\* in this study.**

M, Man; W, Woman; macro, macroadenoma, defined by a diameter >10 mm; micro, microadenoma, defined by a diameter <10 mm; NA, not available \*Classification of sequence variants using ACMG guidelines

<b>MEN1 (NM_130799)</b>								
<b>N°</b>	<b>Sex</b>	<b>Size of adenoma</b>	<b>Age at diagnosis</b>	<b>Familial cases</b>	<b>Exon</b>	<b>Nucleotide variant</b>	<b>Protein mutation</b>	<b>Ref</b>
1	W	macro	20	0	2	c.-10G>A	p.( ?)	NR
2	W	micro	21	0	2	c.1-22C>A	p.( ?)	(4)
3	M	macro	29	0	4	c.655-6C>T	p.( ?)	(5)
4	W	micro	27	0	8	c.1170G>C	p.(Pro390Pro)	NR
<b>AIP (NM_003977)</b>								
5	W	NA	19	0	1	c.26G>A	p.Arg9Gln	(6)
6	W	micro	30	Cousin : prolactinoma	5	c.753G>A	p.(Leu251Leu)	NR
7	M	macro	27	0	6	c.911G>A	p.(Arg304Gln)	(7)
8	W	micro	33	0	6	c.911G>A	p.(Arg304Gln)	(7)
9	W	NA	42	0	6	c.911G>A	p.(Arg304Gln)	(7)
<b>CDKN1B (NM_004064)</b>								
10	W	NA	17	0	1	c.-386C>T	p.( ?)	NR
11	W	macro	38	0	1	c.-266C>G	p.( ?)	NR
12	W	macro	16	0	1	c.-61T>C	p.( ?)	NR

**Supplemental table 3: Prevalence of *AIP* and *MEN1* mutations in patients with prolactinomas in literature (Publications with a number of patients with prolactinoma > 9).** Series for which variants were not available or not reliable to a phenotype or series for which prevalence could not be calculated were excluded. Only patients with genetic sequencing are reported.

Ref	N	Age (years)	Family history	Size of tumors	Sequenced genes	Prevalence of reported variants (n)	Prevalence of reported (L) after reclassification (n)	Characteristics of patients with mutations
(6)	132	34.6 ± 15.9	sporadic	40 micro 92 macro	<i>AIP</i>	Total : 4.5% (6/132) micro: 5% (2/40) macro: 4.3% (4/92)	Total : 1.5% (2/132) micro: 0% (0/40) macro 2.2% (2/93)	M, 16 yo, macroPRL W, 30 yo, giant PRL (primary amenorrhea)
(8)	74	<30	sporadic	macro	<i>AIP</i> and <i>MEN1</i>	Total: 10.8% (8/74) <18: 16.7% (5/30) 18-30: 10% (3/30) <i>AIP</i> : 5.4% (4/74)  <i>MEN1</i> : 5.4% (4/74)	Total: 6.8% (5/74): <18: 16.7% (5/30) 18-30: 0% (0/30)  <i>AIP</i> : 2.7% (2/74) <i>MEN1</i> : 4% (3/74)	Same patients than in the presence study <i>AIP</i> : W, 15, macro M, 13, macro <i>MEN1</i> : M, 16 yo, macro F, 13 yo, macro F, 17yo, macro
(3)	61	<30	sporadic	macro	<i>AIP</i>	Total: 11.5% (7/61)	Total: 3.3% (2/61)	M, 16 yo, NA M, 28 yo, macro
(9)	43	<40	sporadic	12 micro 31 macro	<i>AIP</i>	0	0%	-
(10)	38	<40	sporadic	macro	<i>AIP</i>	2.6% (1/38)	0%	-
(11)	38	NA	sporadic	NA	<i>AIP</i>	0		-
(12)	35	NA	18 +17 FIPA families	NA	<i>AIP</i>	0 mutation among 36 members of 18 homogenous FIPA families with prolactinoma  3 variants among 26 heterogenous FIPA families with at least one member with prolactinoma (11.5%)	0 mutation among 36 members of 18 homogenous FIPA families with prolactinoma  3 variants among 26 heterogenous FIPA families with at least one member with prolactinoma (11.5%)	1 W, micro, age NA, 2 M, macro, aged 10 and 40 yo
(13)	25	<40	sporadic	NA	<i>AIP</i>	4% (1/25)		1 W, macro, 22 yo

(14)	21	<50	sporadic	NA	<i>AIP</i>	0		-
(7)	19	<30	sporadic	5 micro 14 macro	<i>AIP</i>	0		-
(15)	17	15 to 61 years	2 familial and 15 sporadic	7 micro 10 macro	<i>AIP</i>	0		-
(16)	11	NA	sporadic	NA	<i>AIP</i>	0		-
(17)	10	<40	2 familial and 8 sporadic	Macro	<i>AIP</i> and <i>MEN1</i>	0		-

**Supplemental Table 4:** Reclassification of some variants previously reported in patients with isolated prolactinoma according to ACMG-AMP guidelines.

NA: not available

Variant	Phenotype in literature (sex, age of adenoma diagnosis, size at diagnosis)	Ref	Previous classification	New classification	Arguments
<i>AIP: c.26G&gt;A, p.(Arg9Gln)</i>	Woman, 14 yo, macroPRL	(6)	Pathogenic	Likely benign	BS1 Prevalence in general population (allelic frequency 0.02% in gnomADv2.1 last access 24/08/2023, 69 entries) BP4 Multiple lines of computational evidence suggest no impact on gene or gene product. Not in a UNIPROT defined domain
<i>AIP: c.100-18C&gt;T, p.(?)</i>	Woman, 29 yo, macroPRL	(18)	Likely pathogenic	Benign	BA1 Prevalence in general population (allelic frequency 0.31% in gnomADv2.1 last access 04/08/2023 889 entries and 6 homozygous) BP4 Multiple lines of computational evidence suggest no impact on gene or gene product.
<i>AIP: c.166C&gt;T, p.(Arg56Cys)</i>	Man, 26 yo, macroPRL	(3)	Pathogenic	Undetermined significance	PM2 Not absent from control but relatively rare (allelic frequency 0.002% in gnomAD v2.1 last access 04/08/2023, 5 entries) Conflicting <i>in silico</i> predictions PM1 In PPIase FKBP-type domain (31 - 121) PP4 Phenotype of the patient (Giant invasive prolactinoma)
<i>AIP: c.174G&gt;C, p.(Lys58Asn)</i>	Man, 20 yo, macroPRL	(8)	Likely pathogenic	Undetermined significance	PM2 Not absent from control but relatively rare (allelic frequency 0.0008% in gnomAD v2.1 last access 04/08/2023, 2 entries) Conflicting <i>in silico</i> predictions PM1 In PPIase FKBP-type domain(31 - 121) PP4 Phenotype of the patient (Giant invasive prolactinoma)

AIP: c.469-2A>G, p.(?)	Man, 16 yo, macroPRL	(6)	Pathogenic	Likely pathogenic	PVS1 Null variant in a gene where LOF is a known mechanism of disease. PM2 Absent from controls (gnomADv2.1 last access 04/08/2023)
AIP: c.563G>A, p.(Arg188Gln)	Woman, 24 yo, microPRL	(6)	Pathogenic	Undetermined significance	PM2 Absent in population databases (gnomADv2.1 last access 25/08/2022) BP7 Multiple lines of computational evidence suggest no impact on gene or gene product PM1 In the Repeat domain :TPR 1 (179 - 212)
AIP: c.584T>C, p.(Val195Ala)	Man, 12 yo, macroPRL	(3)	Pathogenic	Undetermined significance	PM2 Not absent from control but relatively rare (allelic frequency 0.002% in gnomAD v2.1 last access 04/08/2023, five entries) BP7 Multiple lines of computational evidence suggest no impact on gene or gene product PM1 In the Repeat domain: TPR 1 (179 - 212) PP4 Phenotype of the patient (Giant invasive prolactinoma)
AIP: c.591G>A, p.(Glu197Glu)	Woman, 26 yo, microPRL	(18)	Pathogenic	Likely benign	BS1 Prevalence in general population (allelic frequency 0.007% in gnomADv2.1 last access 04/08/2023, 20 entries) BP7 Multiple lines of computational evidence suggest no impact on gene or gene product
AIP: c.803A>G, p.(Tyr268Cys)	Man, 28 yo, macroPRL	(3)	Pathogenic	Likely pathogenic	PM2 Not absent from control but very rare (allelic frequency 0.0005% in gnomAD v2.1 last access 04/08/2023, one entry) PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product PM1 In Repeat domain: TPR 3( 265 - 298) PP4 Phenotype of the patient (Giant

					invasive prolactinoma)
<i>AIP: c.807C&gt;T, p.(Phe269Phe)</i>	Man, 10 yo, Size NA	(3)	Pathogenic	Undetermined significance	BS1 Prevalence in general population (allelic frequency 0.06% in gnomADv2.1 last access 04/08/2023, 154 entries) BP7 Multiple lines of computational evidence suggest no impact on gene or gene product PS3 <i>In vitro</i> and <i>in vivo</i> studies showing an effect on AIP mRNA expression (19)
<i>AIP: c.911G&gt;A, p.(Arg304Gln)</i>	Man, 36 yo, microPRL	(18)	Pathogenic	Benign	BA1 Prevalence in general population (allelic frequency 0.16% in gnomADv2.1 last access 04/08/2023, 437 entries, 2 homozygous) BP7 Multiple lines of computational evidence suggest no impact on gene or gene product
	Woman, 25 yo, microPRL	(6)	Pathogenic	Benign	BS3 <i>In vitro</i> studies showing a lack of impact on the stability of the AIP protein (19)
	Woman, 16 yo, macroPRL	(13)	Pathogenic	Benign	Not in a UNIPROT defined domain
<i>AIP: c.974G&gt;A, p.(Arg325Gln)</i>	Woman, 18 yo, macroPRL	(6)	Pathogenic	Likely benign	Not absent from control but relatively rare (allelic frequency 0.006% in gnomAD v2.1 last access 04/08/2023, 16 entries) BP7 Multiple lines of computational evidence suggest no impact on gene or gene product BS3 <i>In vitro</i> studies showing a lack of impact on the stability of the AIP protein (20, 21) Not in a UNIPROT defined domain
<i>MEN1: c.691G&gt;C, p.(Asp231His)</i>	Man, 29 yo, macroPRL	(8)	Likely pathogenic	Undetermined significance	PM2 Absent in population databases (gnomADv2.1 last access 25/08/2022) PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product
<i>CDKN1B: c.272C&gt;T, p.(Pro91Leu)</i>	Woman, 19 yo, macroPRL	(22)	Likely pathogenic	Undetermined significance	PM2 Not absent from control but very rare (allelic frequency 0.0004% in gnomAD v2.1 last access 04/08/2023, one entry) BP7 Multiple lines of computational evidence suggest no impact on gene or gene product





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