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Genetic testing in prolactinomas: a cohort study

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58

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61

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63

64 **Abstract:** 251 words

65 Background: Prolactinomas represent 46 to 66% of pituitary adenomas, but the prevalence
66 of germline mutations is largely unknown. We present here the first study focusing on
67 hereditary predisposition to prolactinoma.

68 Objective: We studied the prevalence of germline mutations in a large cohort of patients
69 with isolated prolactinomas.

70 Materials and methods: A retrospective study was performed combining genetic and clinical
71 data from patients referred for genetic testing of *MEN1*, *AIP*, and *CDKN1B* between 2003
72 and 2020. *SF3B1* was Sanger sequenced in genetically-negative patients.

73 Results: 506 patients with a prolactinoma were included: 80 with microprolactinoma
74 (15.9%), 378 with macroprolactinoma (74.7%), 48 unknown; 49/506 in a familial context
75 (9.7%). Among these, 14 (2.8%) had a (likely) pathogenic variant in *MEN1* or *AIP*, and none in
76 *CDKN1B*. All positive patients had developed a macroprolactinoma before age 30. The
77 prevalence of germline mutations in patients with isolated macroprolactinoma under 30 was
78 4% (11/258) in a sporadic context, and 15% (3/20) in a familial context. Prevalence in
79 sporadic cases younger than 18, was 15% in men (5/33) and 7% in women (4/57). No R625H
80 *SF3B1* germline mutation was identified in 264 patients with macroprolactinomas.

81 Conclusion: we did not identify any (likely) pathogenic variants in patients over 30 years of
82 age, either in a familial or sporadic context, and in in a sporadic context in our series or the
83 literature. Special attention should be paid to young patients and to familial context.

84

85 **Significance Statement:**

86 Approximately 5% of pituitary adenomas are thought to be hereditary, leading to a genetic
87 testing for patients with a macroadenoma identified at a young age. The problem is that
88 these recommendations are mainly based on data from hereditary acromegaly since large
89 studies on the prevalence of germline predisposition in prolactinoma are lacking, and this
90 poses a cost-effectiveness issue. We present here the largest series of prolactinoma patients
91 ever to have undergone genetic analysis. Moreover, we performed a literature review on
92 similar studies and reclassified variants according to the state-of-art, to provide evidence
93 supporting recommendations for germline genetic testing in prolactinomas.

94 INTRODUCTION

95 It is generally accepted that genetic testing can be proposed to patients in whom a
96 prolactinoma was diagnosed at an early age or in a familial context (1, 2, 3, 4, 5).
97 Nevertheless, the prevalence of germline mutation in patients with prolactinoma is not well
98 known, because most previous studies have focused on somatotropinomas. Pituitary
99 adenomas, also referred to as pituitary neuroendocrine tumors (PitNETs), are generally
100 benign tumors of the anterior pituitary, either hormonally active or not, with the potential to
101 be invasive of surrounding structures. The overall prevalence of PitNETS is estimated to be 1
102 case per 1,064 in the population, among which approximatively 5 to 7% are inherited (6). In
103 that setting, PitNETs may be either the sole manifestation of the disease, as seen in Familial
104 Isolated Pituitary Adenomas (FIPA) syndrome, which is secondary to a mutation in the *AIP*
105 gene (OMIM 605555) in 20% of cases (7, 8); or may be one of the lesions that are
106 predisposed to in a more complex disease, as is seen in Multiple Endocrine Neoplasia
107 syndrome type I (MEN1; OMIM 131100), which arises due to an inactivating mutation in the
108 *MEN1* gene (9, 10). In addition to PitNETs, MEN1 predisposes patients to primary
109 hyperparathyroidism and duodeno-pancreatic endocrine tumors, while, more rarely,
110 mutation in the *CDKN1B* gene causes PitNETs in Multiple Endocrine Neoplasia type 4
111 syndrome (MEN4; OMIM 610755) (11).

112 Based on large retrospective analyses, prolactinomas represent 46 to 66% of clinically
113 relevant PitNETs based on large retrospective analysis (12, 13, 14, 15, 16). Prolactinomas
114 develop from lactotroph cells and lead to hyperprolactinemia which, in turn, usually results
115 in central hypogonadism. While, the average prevalence of prolactinomas is estimated to be
116 around 10 cases per 100,000 individuals in men and 25-60 cases per 100,000 individuals in
117 women, the specific prevalence of hereditary forms of prolactinoma is not well known (17).
118 A recurrent hotspot mutation (R625H) in *SF3B1* has recently been identified as a somatic
119 driver mutation in 20% of surgically-treated prolactinomas (18) and is therefore a potential
120 candidate gene for genetic predisposition.

121 Our study aimed to assess the prevalence of germline mutations in *AIP*, *MEN1*, and *CDKN1B*
122 in a large cohort of patients with prolactinomas (Figure 1). In a second step, we focused on
123 the cohort who were screened based on the French recommendations proposed in 2011 and
124 last revised in 2016, namely, sporadic macroprolactinomas diagnosed before 30 years of age
125 (with tolerance for microadenomas in a pediatric context) and prolactinomas occurring in a

126 familial context (recommendations SFE-TENGEN-ANPGM: <https://anpgm.fr> and
127 <https://www.reseau-gte.org/tengen/>). Additionally, for those patients who were negative
128 for this first genetic panel, we performed germline genetic sequencing of the *SF3B1* hotspot
129 mutation. Finally, we conducted a literature review examining the prevalence of genetic
130 abnormalities in patients with prolactinomas.

131

132 **PATIENTS AND METHODS**

133 **Inclusion**

134 Patients who underwent genetic testing in the context of an isolated prolactinoma, without
135 other endocrine lesions, at the molecular laboratory of Marseille Conception Hospital
136 between November 2003 and December 2020, were included in the study. Patient blood
137 samples were provided by various French clinical reference and tertiary centers. We
138 included only index cases with isolated prolactinomas, either sporadic or with a family
139 history of PitNET, regardless of the histological subtype. A familial history of PitNETs was
140 defined as having either first and/or second-degree relatives (considering the incomplete
141 penetrance of the disease in *AIP* families (7, 8, 19)) diagnosed with PitNETs, regardless of the
142 PitNET subtype. Exclusion criteria were: i) index cases with somatolactotroph and
143 plurihormonal PitNET at the time of genetic diagnosis, and ii) index cases with other lesions
144 suggestive of syndromic features at the time when the prolactinoma was diagnosed. The
145 diagnosis of macroprolactinoma was established by the combination of pituitary tumor size
146 where the maximal diameter exceeded 10 mm, and a plasma prolactin level above 200
147 ng/ml. Microprolactinoma was diagnosed based on the presence of a smaller than 10 mm
148 pituitary lesion on MRI and with prolactin levels above 100 ng/ml. Written informed consent
149 from all patients for genetic analyses was obtained during one-on-one genetic counseling.
150 This study was approved by the ethics committee of Aix-Marseille University (approval
151 number: 2018-13-12-004).

152 **Genetic analyses**

153 Genetic analyses were performed on genomic DNA extracted from whole blood. The
154 techniques used for genetic testing changed over the course of the study in line with
155 technological developments in genomic analysis. Exons and intron-exon junctions of the *AIP*
156 (NM_003977), *MEN1* (NM_130799), and *CDKN1B* (NM_004064) genes were sequenced by
157 Sanger sequencing or by next generation sequencing (NGS) according to the procedures

158 used in the laboratory at the time to detect point mutation (1, 20). *MEN1*, *AIP*, and *CDKN1B*
159 copy number variation (CNV) analyses were performed by multiplex ligation-dependent
160 probe amplification (MLPA, MRC-Holland, Amsterdam, the Netherlands), or by comparing
161 the coverage of depth on NGS by using Covcopcan software (21) or CLCGenomic Software
162 (Qiagen, Hilden, Germany).

163 ***SF3B1* germline Sanger sequencing**

164 Germline DNAs were amplified using primers targeting the R625H *SF3B1* mutation on exon
165 14 (Primer F GCTGCTGGTCTGGCTACTAT, primer R CTTGCCAGGACTTCTTGCTT) using the
166 AmpliTaqGold 360 MasterMix kit (Applied Biosystem), and sequenced on a 3500XLDX
167 Genetic analyser (Applied biosystems). Sequences were aligned on the reference gene
168 NM_012433.3 using Variant Reporter software (ThermoFisher).

169 **Classification of variants**

170 Each variant was classified according to the guidelines of the American College of Medical
171 Genetics and Genomics (ACMG) into one of five classes of pathogenicity (22): class 1: benign
172 variant, class 2: likely benign variant, class 3: variant of undetermined significance, class 4:
173 likely pathogenic variant (LPV), or class 5: pathogenic variant (PV).

174 *In silico* predictions were aggregated using Alamut Visual software (Interactive Biosoftware,
175 Rouen, France), MobiDetails (23), and VarSome (24), and were notably based on UMD-
176 Predictor (25), SIFT, PolyPhen-2, Revel, HSF (<https://hsf.genomnis.com/>) and spliceAI. The
177 population data were collected from the normal population database (gnomAD database
178 v2.1, last access 04/08/2023) and from inherited disease databases (ClinVar, LOVD, UMD-
179 *MEN1* database and HGMD). Benign variants were not reported in this study.

180 **Statistical analysis**

181 The data were analyzed using Excel (Microsoft). Qualitative variables were expressed as a
182 percentage and analyzed using Fisher's exact test using Prism software v9 (GraphPad). For
183 the calculation of odds ratios, we restricted the analysis to the population of patients whose
184 phenotype was consistent with French recommendations for genetic testing.

185

186 **RESULTS**

187 **Characteristics of the whole cohort**

188 A total of 506 patients were included, among whom 256 (50.6%) were women. The mean
189 age of patients at the time of pituitary adenoma diagnosis was 25.6 ± 11.2 (SD) years old

190 (median 23, min 2, max 77 years), A total of 378 out of 506 (74.7%) patients had a
191 macroprolactinoma, 15.8% (80/506) a microprolactinoma; information was not available for
192 the remaining 9.5% (48/506) (Table 1). Patients with microprolactinoma (n=80) were mainly
193 in a pediatric context (n=33), in a familial context (n=22) or analyzed before the French
194 recommendations (n=14). A total of 9.7% (49/506) of patients had a family history of PitNET,
195 among whom 22 had a microprolactinoma (22/49, 44.9%), 20 had a macroprolactinoma
196 (20/49, 40.8%), and information was lacking for 7 patients (7/49, 14.3%).

197

198 **Patients with pathogenic and likely pathogenic variants ((L)PV) in the whole cohort**

199 The DNA samples of 171 patients were sequenced by NGS (34%), and the remaining 335 by
200 Sanger sequencing (66%) to detect point mutations. CNV analysis was performed for the 49
201 patients with prolactinoma in a familial context and the 155 patients with prolactinoma in a
202 sporadic context were sequenced by NGS. No CNV was found. A total of 14 patients (2.8%)
203 presented with a (L)PV in either *MEN1* (n=7) or *AIP* (n=7). No (L)PV was found in *CDKN1B*
204 (Table 1). In addition, 10 variants of undetermined significance (*MEN1* (n = 5), *AIP* (n = 3) and
205 *CDKN1B* (n = 2)), and 12 likely benign variants (*MEN1* (n = 4), *AIP* (n = 5) and *CDKN1B* (n = 3)),
206 were identified in a total of 22 patients (Supplemental table 1 & 2). None of the patients
207 presenting with a microprolactinoma, regardless of age at diagnosis, presented with a (L)PV
208 variant. All patients with a (L)PV were younger than 30 years old (yo) at the time of diagnosis
209 of adenoma (mean age 15.3 years (min 10, max 27 years)), and all had a macroprolactinoma.
210 Five were women (5/14, 36%) and nine were men (9/14, 64%). Three patients had a family
211 history of PitNET (patients #2, #10, and #12).

212

213 **(L)PVs in patients with sporadic isolated macroprolactinomas before 30 years**

214 A total of 11 out of the 258 (4.3%) sporadic patients with an isolated macroprolactinoma
215 diagnosed \leq 30 yo carried a (L)PV: six in *MEN1* (6/258, 2.3%) and five in *AIP* (5/258, 1.9%)
216 (Table 1, 2 & 3). Seven were men and four were women (Table 3). The mean age at
217 diagnosis of macroprolactinoma was 13.5 yo (min 11, max 22 years) (Table 1). Among the
218 cohort of patients \leq 18 yo, the rate of germline mutation reached 10% (9/90) for both
219 genders, 15% in men (5/33), 7% in women (4/57). In patients older than 18 yo (n=168, 93
220 men, 75 women), the prevalence drastically decreased to 2.2% (2/93) only in the male
221 population, while no mutations were identified in the female group (Table 3). Patients

222 diagnosed with sporadic macroprolactinoma before 18 yo had a 9-fold higher risk of carrying
223 a germline mutation than patients aged between 18 and 30 (9/90 versus 2/168 patients; OR,
224 9; 95%CI: 2.3-43; p-value: 0.0016) (Table 3).

225

226 **(L)PVs in patients with macroprolactinomas in a familial context:**

227 Among the 49 patients with a prolactinoma in a familial context, 3 patients (6.1%) carried a
228 (L)PV: one (2%) in *MEN1* (patient #2) and two (4.1%) in *AIP* (patients #10 and #12). All three
229 patients presented with macroprolactinomas (12, 18, and 27, respectively; Table 2 & 3),
230 consequently, we have focused on the patients with macroprolactinomas in a familial
231 context for the remainder of the manuscript. All patients with macroprolactinoma in a
232 familial context were aged ≤ 30 yo at the time of diagnosis of the prolactinoma. Thus, the
233 prevalence of germline mutations in patients with macroprolactinomas in a familial context
234 of PitNET was 15% (3/20): 5% for *MEN1* (1/20) and 10% for *AIP* (2/20). The patients with
235 macroprolactinomas diagnosed before age 30 in a familial context had an almost 10-fold
236 higher risk of carrying mutations in predisposition genes as compared to their sporadic
237 counterparts (3/13 versus 11/258 patients; OR 6.7; 95% CI: 1.8 to 25.8; p=0.023). This
238 increase in risk concerned the cohort of patients between 18 and 30 yo (2/8 versus 2/168,
239 OR: 27.7; 95% CI: 3.6-186.4; p-value: 0.0104), but not the cohort of patients under 18 yo (1/5
240 versus 8/90, OR: 2.6; 95% CI: 0.2-19.7; p-value: 0.4).

241

242 **Germline R625H *SF3B1***

243 The germline DNAs of 264 genetically negative patients with macroprolactinomas were
244 screened for the recurrent R625H *SF3B1* mutation. No germline R625H *SF3B1* mutation was
245 identified in the 247 sporadic patients younger than 30 yo, nor in the 17 patients with
246 PitNETs in a familial context.

247

248 **DISCUSSION**

249 Genetic screening in PitNETs has evolved considerably in recent years as a
250 consequence of the progress made in molecular techniques for DNA sequencing and
251 amplification (26). Except for GH-secreting PitNETS, in which germline mutations in *AIP* are a
252 well-known cause of familial acromegaly, few studies have focused on germline alterations
253 that may occur in other subtypes of PitNETs. To the best of our knowledge, we present here

254 the first study specifically focusing on hereditary predisposition to prolactinomas. We
255 performed a retrospective study combining genetic and clinical data from 506 patients with
256 isolated prolactinomas, representing the largest reported series to date on this topic (Table
257 4). In the whole cohort, the sex ratio distribution was the same as in the French population,
258 despite the higher incidence of prolactinoma in young women (27). Moreover the age at
259 diagnosis of pituitary adenoma was younger than reported in the literature in patients with
260 pituitary adenoma (15, 16, 28). This is due to a distribution bias linked to the
261 recommendations for genetic testing in patients with pituitary adenoma, i.e. adenoma in a
262 familial context or isolated sporadic macroadenoma before 30 yo.

263 Compared to previous studies, the prevalence of *AIP* and *MEN1* germline mutations
264 discovered in our cohort appears lower in patients with macroprolactinomas (5% vs. 8% in
265 the literature), and in patients with microprolactinomas (0 vs. 2% in the literature), prior to
266 variant reclassification (Supplemental Table 3). Indeed, some earlier studies were biased due
267 to incorrect classification of allelic variants (Supplemental Table 3 and 4). Significant progress
268 has been made in classification of variants over recent years thanks to the huge genomic
269 database available for the “normal population”, the availability of *in silico* prediction
270 software, and the ACMG algorithm for classification (22), as is shown by numerous
271 publications. Therefore, some *AIP* and *MEN1* variants which were previously considered as
272 pathogenic are now classified as of undetermined significance or as likely benign variants
273 (see Supplemental Table 4 and below). After reclassification, prevalence rates are equivalent
274 in the present study and the literature. However, as previously pointed out by ourselves and
275 others, in the desire for robustness and universality its clinical application, the ACMG
276 algorithm can lead to over-classification of variants as VUS (29). In particular, patients
277 carrying certain rare missense variants of undetermined significance should be carefully
278 examined (i.e., *AIP*: c.166C>T, p.(Arg56Cys); *AIP* c.584T>C, p.(Val195Ala); *AIP*: c.174G>C,
279 p.(Lys58Asn), ... Supplemental Table 4). Indeed, these variants are suspicious considering the
280 reported phenotype in the literature, their low frequency in the general population, and
281 their localization in functional domains. Further investigations, including somatic analysis (in
282 the rare cases where patients undergo surgery), cosegregation studies, and functional
283 analysis are needed to definitively classify them as pathogenic.

284

285

286 Previously, van den Broek et al. concluded that genetic analysis should not be
287 performed routinely in patients with sporadic pituitary adenomas (4). They recommended
288 screening for germline *AIP* mutations in young (≤ 30 yo at diagnosis) patients with sporadic
289 pituitary adenomas, especially in the presence of gigantism and macroadenoma, and for
290 germline *MEN1* mutations in young patients (≤ 30 yo at diagnosis) with sporadic pituitary
291 adenomas (mainly prolactinomas). Microprolactinomas were not excluded in these
292 recommendations; however, in our cohort, no mutations were identified in 52 patients with
293 sporadic microprolactinoma found before 30 yo (data not shown). In the literature, five
294 patients with isolated sporadic microprolactinoma have been reported carrying four
295 different *AIP* variants. One patient was a young man with microprolactinoma diagnosed at
296 16 yo, harboring a truncating pathogenic *AIP* variant c.344del, p.Leu115Argfs*41 (30). We
297 reevaluated the classification of the three other *AIP* variants according to the ACMG-AMP
298 recommendations ((31, 32), Supplemental table 4). Finally, we classified these variants as
299 follows: i) the missense variant c.911G>A, p.Arg304Gln as benign, ii) the missense variant
300 c.563G>A, p.(Arg188Gln) as variant of undetermined significance, and iii) the isosemantic
301 variant c.591G>A, p.(Glu197Glu), as likely benign. In this way, to our knowledge, there is only
302 one patient with an isolated microprolactinoma in a sporadic context with an *AIP* pathogenic
303 or likely pathogenic variant that has been reported in the literature, and none in *MEN1*.

304 On the other hand, in a familial context, five patients carrying an *AIP* pathogenic
305 variant with isolated microprolactinomas have been previously reported (7, 33, 34, 35). Four
306 patients were diagnosed with an *AIP* pathogenic variant after family genetic screening,
307 leading to the discovery of a microprolactinoma in a 19 yo man (34), and of a small pituitary
308 adenoma (3 mm) with slightly raised prolactin level in a 12 yo girl (35). Interestingly two
309 relatives with *AIP* mutations had developed microprolactinomas prior to a mutation being
310 discovered in their families: i) a woman, genetically screened at around 50 yo, who had
311 developed a microprolactinoma of 8 mm at the age of 36 (her younger sister had also
312 developed multiple microprolactinomas at the age of 36) (34), and ii) a woman genetically
313 screened at 65 yo, diagnosed with an 8 mm prolactinoma at the age of 30 following
314 investigation for secondary amenorrhea (33). The chronology of the genetic screening and
315 age at prolactinoma diagnosis was not available for the fourth patient, who was the
316 daughter of man who had developed acromegaly at 29 yo (7). In the present cohort, no

317 mutation was identified in 22 patients with microprolactinoma in a familial context (first
318 and/or second-degree relatives with PitNET, regardless of the subtype).

319 We conclude that, to date, there is no evidence to recommend systematic genetic
320 testing in patients with sporadic microprolactinoma, even before age 30, but it is important
321 to look for a family history and to request that they report any new clinical events in their
322 families, in order to propose genetic testing if the situation arises. In all cases, the indication
323 for genetic testing must be discussed on a case-by-case basis, taking into account any
324 missing data in the personal and family history, and any delay in diagnosis.

325

326 Moreover, we did not identify any (likely) pathogenic variant in the 96 individuals
327 with an isolated macroprolactinoma diagnosed after the age of 30, regardless of the family
328 context. This is consistent with the recommendations of van den Broek et al., who suggested
329 30 yo as a cut-off point for performing germline genetic analysis in index cases (4). In
330 patients carrying *AIP* mutations, the onset of PitNET is indeed earlier than in sporadic
331 patients (36). In patients with *MEN1* mutations, the strong penetrance of primary
332 hyperparathyroidism, which is the first lesion of the disease in 85% of patients, makes the
333 occurrence of an isolated macroprolactinoma after 30 yo unlikely (37, 38). In addition, we
334 did not find any *MEN1* or *AIP* mutation in women with prolactinoma over 18 yo in a sporadic
335 context, including 75 women with macroprolactinoma. To our knowledge, there are no
336 reported cases of women with isolated sporadic macroprolactinoma between the ages 18
337 and 30, in a sporadic context, due to *AIP* or *MEN1* pathogenic or likely pathogenic variants
338 reported in the literature, taking into account that a single case of a macroprolactinoma in a
339 29 yo woman was attributed to the c.100-18C>T, p.(?) *AIP* variant (39), here classified as a
340 benign variant according to ACMG recommendations (Table 5).

341 Patient age <18 years, as well as the presence of a family history, have been found to
342 be independently associated with an increased risk of carrying a genetic mutation. Although
343 men are more represented among the patients carrying mutations in our cohort, sex was not
344 a predictive factor for the occurrence of a genetic mutation in the pediatric
345 macroprolactinoma cohort (data not shown) (Table 3). It is therefore necessary to be
346 particularly vigilant in this group of patients.

347 No mutation in *CDKN1B* was found in the 506 patients included in the study. This is in
348 agreement with the very low level of evidence for a benefit of *CDKN1B* genetic screening in

349 patients with PitNETs (4). In the literature, only one patient with prolactinoma was
350 attributed to a *CDKN1B* variant. This case was a young patient (19 yo) harboring a
351 prolactinoma of 10 mm. She carried the *CDKN1B* c.272C>T, p.Pro91Leu variant (40). This
352 variant is rare in the general population (only one entry for 250442 alleles in gnomAD v2.1),
353 the amino acid is well conserved through evolution, but the nucleotide sequence is not, the
354 physicochemical distance between proline and leucine is mild (98/215 distance of
355 Grantham), and there are other missense variants at this position in the general population.
356 In the absence of supplementary data and according to the ACMG-AMP guidelines, we think
357 that it could be considered as a variant of undetermined significance (Table 5). However, we
358 have identified two other variants of undetermined significance which must be further
359 explored to be reclassified (Supplemental table 1).

360 We subsequently searched for new candidate genes. *SF3B1* was recently identified as
361 a driver somatic oncogenic gene in prolactinomas (18). *SF3B1* encodes the splicing factor 3
362 subunit B1, a component of the U2 small nuclear ribonucleoproteins (snRNP) complex,
363 involved in mRNA splicing. In prolactinomas, the recurrent R625H *SF3B1* mutation causes
364 aberrant splicing of estrogen related receptor gamma (ESRRG), which results in a stronger
365 affinity for binding to pituitary-specific positive transcription factor 1 (Pit-1) and leads to
366 excessive prolactin secretion (18). In the study by Li et al, the somatic R625H *SF3B1*
367 mutation was found in 45/227 prolactinomas (20%), and the germline DNA of 0/13 patients.
368 We looked for the R625H *SF3B1* at a germline level in 264 patients with macroprolactinomas
369 from our cohort. However, none of our patients carried this mutation. We can conclude that
370 *SF3B1* does not appear to be a good candidate gene for genetic predisposition to
371 prolactinoma, or alternatively we could hypothesize that R625H *SF3B1* heterozygous
372 germline mutation is non-viable, as is the case for *GNAS* activating mutations in McCune-
373 Albright syndrome (41). In fact, *SF3B1* is involved in fundamental cellular processes and
374 activating mutations cause aberrant splicing in numerous transcripts.

375
376 In conclusion, we have collected genetic and clinical data from a large cohort of 506
377 patients carrying an isolated prolactinoma and have updated the classification of variants
378 according to current knowledge and ACMG recommendations. We have shown that among
379 the patients with a macroprolactinoma between the ages of 18 and 30, those with a family
380 history of PitNET have a 10-fold greater risk of this having a genetic origin than those with a

381 sporadic context. In the pediatric cohort, 10% arise from a genetic origin. The major
382 remaining issue is the relevance of *MEN1* and *AIP* genetic screening in prolactinomas. We
383 did not identify any (likely) pathogenic *MEN1* or *AIP* variants in patients with prolactinoma
384 after 30 yo, either in a sporadic or a familial context. The lack of *AIP* and *MEN1* (likely)
385 pathogenic variants in isolated sporadic macroprolactinomas in women, both from our data
386 and the literature, suggest that systematic *AIP* and *MEN1* genetic screening can be
387 questioned in such cases. Nevertheless, the hypothesis of a diagnostic delay must be
388 considered on a case-by-case basis. Our data does not support the relevance of systematic
389 genetic screening in sporadic isolated microprolactinomas, even in the pediatric population.
390 However, patients should be well informed about the need to report new clinical findings,
391 both in themselves and their family, so that the relevance of genetic screening can be
392 reevaluated. Overall, this study emphasizes the need for reliable and good quality clinical
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418

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597 **Figure 1:** Flow chart of the study.