

Molecular Results of Congénital Multiple Pituitary Déficits in Algeria.

Soumeya Fedala¹, Ali El Mahdi Haddam², Akila Zenati³,
Rhadia Si Youcef⁴,

¹Department Of Endocrinology, Bab El Oued Hospital,

²Department Diabetology, Bab El Oued Hospital,

³Department Of Biochemistry And Molecular Biology Unit, , Bab El Oued Hospital,

⁴Department Of Biochemistry, Bologhine Hospital, Algiers, Algeria.

Abstract: The genetic study of 53 Algerian children with congenital multiple hypopituitarism found a mutation of transcription factors in 20.7% of cases (n = 11). Mutations in *Prop1* are familial (90% of cases) and predominant (n = 10 /11). Among them, the R73C mutation is majority (07 out of 10) and appears to be specific to the Maghreb. No genotype / phenotype correlation was found. In one case a *Pit1* mutation is identified: exon 1 PAL 32 Fsx18. This has not been previously reported. No molecular abnormalities were found in the case of ectopic posterior pituitary (n = 29).

Keywords: GH deficiency congenital - multiple congenital hypopituitarism - *prop1* - *Pit1* - post ectopic pituitary.

I. Introduction

Great strides have been made in recent years in the exploration of genetic deficiency of growth hormone. However, despite the discovery of several genetic mutations associated with this disease, only 10% of molecular defects have been identified to date. In this context we report the results of a genetic study in the Algerian children presenting a combined pituitary insufficiency.

II. Materials And Methods

53 patients (40 M and 13 G) with a growth hormone deficiency associated with other pituitary deficits were followed between 1997 and 2006. The mean age was 8.4 ± 4.3 years for males and 6.9 ± 3.8 years for girls. The diagnosis of GHD was based on a clinical and paraclinical beam arguments : slower growth rate , delayed bone age , failure to thrive > -2 SD / M (*sempé*) characteristic morphotype , IGF1 lowered and lack of GH response to two pharmacological stimulation tests: propranolol - glucagon and insulin tolerance test . GH deficiency was associated with thyrotropin insufficiency in 45,3%(n: 24 /53), hypocorticism insufficiency in 28,3%(n = 15/53) deficit, thyrotropin and corticotropin insufficiency in 28,4% (n = 14/53) and in sipidusdiabetes in n = 4.Neuroradiological exploration in magnetic resonance of hypothalamic-pituitary region (MRI H-H)revealed an ectopic posterior pituitary in 29 cases, pituitary hypoplasia in n = 07 cases and a normal imaging in 15 cases. In 02 cases there was a pseudo- tumoral pituitary hyperplasia. All patients underwent a genetic investigation. Besides a probing looking for familial cases, extraction of genomic DNA was carried out and fed to genetics department specializing in the design of GH deficits.

After amplification by polymerase chain reaction (PCR), direct sequencing of PCR chains was performed using an automatic process. The search for molecular abnormalities is then performed according to the phenotypic and neuroradiological characteristics. The genes studied were: *prop1*, *Pit1*, *HESX1*, *LHX3*, and *LHX4*.

III. Results

Among patients with normal post pituitary (n: 24), 11 (45.8 %) had a molecular abnormality. In two - thirds of them: n = 8/11 (72.7%) , there was a familial form. In most cases, n = 10/11 (90.09 %) a *prop1* mutation was identified. One patient had a *Pit1* mutation: exon1 P Ala 32FS x 18. It has never been described before.

The *prop1* molecular abnormalities are represented in 80% of cases by mutations of R73 exon 2. Among these, the R73C mutation is majority (75%) (Table I). All patients are homozygous for the mutation which is autosomal recessive. The genotype and phenotype study of patients with the same mutation, including those in the same family did not find any correlations (Table I).

Table I : Phenotype-genotype of patients with congénital multiple pituitary deficit

Patient/ Gender/ number	Mutation	Déficit motivate d the first Consult	Age at the first Consult	Delay stature (DS)	Morpho type	GH Basale Peak mu/l	Hormonal Déficiés at diagnosis	H-HRMI
S (1)	Prop1 R120c Homozygous	GH	13	-7	Typical	0,43 - 1,02	GH + TSH	Hypoplasia
S -2	Prop1 R73H Homozygouse (F)	GH	04,3	-3	Typical	0,3 - 1	GH	Hypoplasia
? -3	Prop1 R73H Homozygous (F)	GH	14,3	-7	Typical	0,3 - 0,5	GH + TSH + ACTH	Hypoplasia
S -4	Prop1 R120c Homozygous (F)	GH	17,16	-7	Typical	0,19 - 0,24	GH + TSH	Hypoplasia
? -5	Prop1 R120c Homozygous (F)	GH	12,3	-6	Typical	0,16 - 0,67	GH + TSH	Hypoplasia
S -6	Prop1 R73c Homozygous (F)	GH	04	-3	Typical	0,18 - 0,10	GH + TSH	Hypoplasia
? -7	Prop1 R73c Homozygous (F)	GH	12	-7	Typical	0,24 - 0,31	GH + TSH	Hypoplasia
? -8 (77)	Prop1 R73c Homozygous (F)	GH	10	-6	Typical	0,23 - 0,35	GH + TSH + ACTH de réserve réserve	Hyperplasia
0 C 10	Prop1 R73c Homozygote (F)	GH	09	-6	Typical	0,05 - 0,26	GH + TSH + ACTH de Réserve	Hypoplasia
S -10 (42)	Prop1 R73c Homozygote (S)	GH	04	-4,5	Typical	0,31 - 0,42	GH + TSH + ACTH de Réserve	Hypoplasia
S -11 (19)	Pit 1 exon 1 p.Ala 32fs X 18 Homozygote (S)	TSH	01 month	-12	Typical	0,21 - 0,21	GH + TSH	Hypoplasia

11 GHD patients had a typical clinical picture and a complète and severe GH deficiency (peak GH < 1 mIU / l). thyrotropin insufficiency is constant during the investigation of patients. The corticotropin deficiency is present in just under half of the cases (n = 4/10, 40%). This deficit is part three of four times and completed secondary.

The Appearance Of The Pituitary Gland Is Variable From One Patient To Another. Two Aspects Are Found:

Hypoplasia objectified in 8/10 and hyperplasia transient tumor nickname in both cases (fig 1 and 2). It should be noted that no genotype phenotype correlation is found On the patient carrying the mutation Pit1, the GH deficiency is severe and GHD morphotype is typical. Congenital hypothyroidism was diagnosed early at 01 month life associated with prolactin deficiency.

Corticotropin function is maintained against until the age of 19 or has installed a partial ACTH deficiency that has completed secondary 03 years later. Puberty is developed later at the age of 20 years. H-H MRI revealed severe pituitary hypoplasia. The mutation Pit1 involved is homozygous and autosomal recessive. The genetic survey conducted in 29 patients with post ectopic pituitary showed no molecular abnormalities even in patients with a complex phenotype or other extra- pituitary malformations (n = 8).

IV. Discussion

Congenital multiple pituitary deficits are most often associated with malformations abnormalities of hypothalamic pituitary region . They are detected by magnetic resonance imaging.^{1,2} More rarely molecular ontogenesis abnormalities of pituitary genes are identified.^{3,4} However, despite the discovery of the involvement of several genes in association with growth hormone deficiency in humans , the molecular mechanisms responsible for the vast majority of congenital somatotropes deficiencies remain to be elucidated.^{4,5,6} The molecular abnormalities identified so far are mainly 04 genes (HESX1, LHX3, Pou 1F1 and Prop1).^{7,8} Some of them have an ethnic specificity.

The growth hormone deficiency is most often sporadique. Familial forms are rare and account for 3-30 % of cases.⁹ In our study the proportion of familial cases is higher (72.07 %) and was due to inbreeding. Mutations in Prop1 are currently the leading cause of multiple pituitary deficiencies.^{10,11} This finding was confirmed in our patients since prop1 mutations are almost exclusive. Familial forms are found in more than half of cases.^{9,11,12,13} This was verified in our patients (81.8 % in our study)

Most molecular anomalies are located in exon 2. It encodes the homeodomain transcription factor 1 which comprises two sites prop high mutability at codons 73 and 99.^{10,14,15,16} The R73 mutation is predominant in our series (72.7 %). Prop1 mutations most frequently described in the literature are mostly 301 del AG and 150 del a.^{10,12,16,17,18} Our patients prop1 mutations are represented by changing R73 and R120 C. Dusque Noy et

al¹⁹ were the first to report the mutation R73C. Thereafter, this mutation has been identified in patients mostly from the Maghreb.^{10,16,19}

Considering these genetic results, it is possible to consider that this mutation is specific to North Africa and it would be a founder effect. In these cases, The growth hormone deficiency is always complete and is accompanied by severe dwarfism.¹⁰

Patients' carriers of this prop 1 mutation, have a hypopituitarism characterized by gradual onset and variable different hypothalamic- pituitary deficits.^{10,15} Reaching, the prop1 causes abnormal development of somatotropin, gonadotropin and thyrotropin lines.^{3,4,10} Adrenocorticotrophic insufficiency is the only deficit considered inconsistent in the literature. Partial or complete, it can be initially diagnosed or occur secondarily.¹⁰ The severity of any acute decompensation requiring as it does for other potential deficits regular reevaluations. This is very important because there is no correlation of genotype / phenotype.^{3,4,10,15} The study of pituitary morphology in patients with a mutation of Prop 1 shows that three aspects can be observed: hypoplastic, hyperplastic or normal anterior pituitary size. Pituitary hypoplasia is the most common injury.^{17,18,19} The pathogenesis of pituitary hyperplasia remains unknown. It seems as Voutetakis²⁰ that the process causing this aspect, nickname tumor of the pituitary, would arise in the intermediate lobe. Its late regression due to the loss of function of prop1 would reduce this hyperplasia. However, the spontaneous evolution is generally favorable. Surgery is not indicated and simple neuro- ophthalmological controls are necessary because of the risk of chiasmatic compression.

Regarding the mutation Pit, it was found in only one patient. The rarity of this one identified the first has been highlighted in several studies.³ In addition, it should be noted that the mutation reported in our patient has not been previously described.

Mutation of LH X3, LHX4 and HESX1 have not been found in our serie, especially in patients with intra and extra cranial malformations. In the literature; these mutations are in fact extremely rare.^{3,5} The mutations of the transcription factor HESX1 is the first gene to have been implicated in the SITH. Several mutations have been described and reported primarily in families forms and in some patients with GH insufficiency and complex heterogeneous phenotype. Mutation LHX4 gene were also associated with SITH.^{3,4,5} In most cases like our patients, no genetic cause is found. However, the existence of familial forms and the combination of SITH with micropenia and congenital abnormalities, especially eye, suggests a prenatal origin. In fact, all molecular mechanisms behind the pituitary stalk interruption syndrome are unknown and remain to be elucidated.

V. Conclusion

Several transcription factors involved in pituitary ontogenesis were discovered and reported as the cause of congenital multiple pituitary deficits mutations. In agreement with the literature, reaching the prop1 (R730) seems to be the most common cause in our country. The mutation of Pit 1 is rare and should be considered when looking for a mutation prop1 reveal that it's negative.

International multicenter studies are needed to improve the knowledge and management of congenital pituitary deficits.

References

- [1]. Adams Baum. C, Gini Sty. D, Chaussain. JL, Kalifa. G, Houang. M, Andre. CH, Richard. I. Imagerie par résonance magnétique des hypopituitarismes non tumoraux de l'enfant. *Revue d'Imagerie Médicale* 1993 ; 5 : 251-257.
- [2]. Dinatale. B, De Angelis. R, Luke. Zic. M, Pellini. C, Chiu Mello. G. Neuro- Radiologic Diagnosis of the hypothalamo-hypophysical region in children with hypopituitarism. *Minerva Endocrinol* 1993 ; 18 : 48-52.
- [3]. Cohen. LE, Rado Vick. S. Molecular basis of combined pituitary hormone deficiencies. *Endocr Rev*, 2002 ; 23 (4) : 431-442.
- [4]. Cu Shman. LJ, Showalter. AD, Rhodes. SJ. Genetic deficits in the development and function of the anterior pituitary gland. *Ann Med* 2002 ; 34 : 179-191.
- [5]. Barlier. A, Vallette-Kasic. S, Ma Navela. M, Perez. M, Diaz. A, Pelle Grini- Bouillcri, Enjalbert. A, Brue. TH. Développement hypophysaire et pathologie des facteurs de transcription. *Ann d'Endocrin* 2000 ; 61 : 201-207.
- [6]. Enjalbert. A. Déficits pituitaires dans : diagnostic génétique en
- [7]. endocrinologie. Réunion du comté d'interface génétique. *Reproduction humaine et biologie clinique* 2 Octobre 2002. INSERM.
- [8]. Brue. T, Reynaud. R, Becker. A, Petrossians. P, Saveanu. A, Barlier. A, Enjalbert. A. Autogénèse de l'hypophyse du développement antéhypophysaire normal à la pathologie. *Medicine Clinique Endocrinologie et Diabète* 2004. (Numéro Hors Serie) : 4-7.
- [9]. Castinetti. F, Reynaud. R, Saveanu. A, Quentien. M.H, Albarel. F, Barlier. A, Enjalbert. A, Brue. T. Clinical and genetic aspects of combined pituitary hormones deficiency. *Ann Endocrinol* 2008 ; 69 : 7-17.
- [10]. Dattani. MT, Robinson. IC. The molecular basis for developmental disorders of the pituitary gland in man. *Clinical Genetics* 2000 ; 57 : 337-346.
- [11]. Deladoey. J, FLuck. C, Büyükgöbüz. A, Kuhlmann. B, Eble. A, Hindmarsh. P, Wu. W, Mullis. PE. Hot spot in the prop1 gene responsible for combined pituitary hormone deficiency. *J Clin Endocrinol Metab*, 1999 ; 84 : 1645-1650.
- [12]. Wu. W, Cogan. HD, Pfaffle. RW, Dasen. JS, Frish. H, Oconnell. SM. Mutations in prop1 cause familial combined pituitary hormone deficiency. *Nat Genet* 1998 ; 18 : 147-149.
- [13]. Cogan. J, Wu. W, Phillips. A III, Ivo. J, Arnhold. P, Agapito. A, Osorio. MGF, Bircan. I, Moreno. A, Mendonca. B. The prop 1-2 base pair deletion is a common cause of combined pituitary hormone deficiency. *J Clin Endocrinol Metab* 1998 ; 83 : 3346-33
- [14]. Fofanova. O, Takamura. N, Kinoshita. E, Parks. JS, Brown. MR, Peterkova. VA, Evgrafow. OV, Gon Charon. B, Bulatov. AA,

- Dedov. II, Yamashita. S. Compound Heterozygous Deletion of the prop1 gene in children with combined pituitary hormone deficiency. *J Clin Endocrinol Metab* 1998 ; 83 : 2601-2604.
- [15]. Takamura. N, Fofanova. OV, Noshita. E, Yamashita. S. Gene analysis of prop 1 in DWAR fism with combined pituitary hormone deficiency. *Growth horm IGF. Res* 1999 ; 9 : 12-17.
- [16]. REYNAUD.R, CHADLI-CHAIEB.M, VALLETTE-KASIC.S, BARLIER.A, SARLES. J, PELLEGRINI-BOUILLER.I, ENJALBERT. A, CHAIEB. L, And BRUE. T. A Familial Form of Congenital Hypopituitarism Due to a PROP1 Mutation in a Large Kindred : Phenotypic and in Vitro Functional Studies. *J. Clin. Endocrinol. Metab.* 2004 ; 89 (11) = 5779-5786.
- [17]. REYNAUD.R, BARLIER.A, VALLETTE-ASIC.S, SAVEAUNU. A, GUILLET. M.P, SIMONIN. G, ENJALBERT. A, VALENSI. P, And BRUE.T. An Uncommon Phenotype with Familial Central Hypogonadism Caused by a Novel PROP1 Gene Mutant Truncated in the Transactivation Domain. *J. Clin. Endocrinol. Metab.* 2005 ; 90 (8) = 4880-4887.
- [18]. DELADOEY. J, FLUCK. C, BÜYÜKGEBİZ. A, KUHLMANN. B, EBLE.A, HIN D MARSH.P, WU. W and MULLIS. P.E. Hot Spot in the Prop 1 Gene Responsible For Combined Pituitary Hormone Deficiency. *J. Clin. Endocrinol. Metab.* 1999 ; 84 = 1645-1650.
- [19]. GUIGNAT. L, AMSELEM. S, NETCHINE. I, THOMOPOULOUS. P. Hypopituitarisme Par Mutation du Gène Prop1 dans une Famille. *Ann. Endocrinol.* 2001 , 62 = 400.
- [20]. DUQUESNOY.P, ROY. A, DASTOT. F, GHALI. I, TEINTURIER. C, NETCHINE. I, CACHEUX. V, HAFEZ. M, SALAH. N, CHAUSSAIN. J.L, GOOSENS. M, BOUGNERES. P, AMSELEM. S. Human Prop1 : Cloning, Mapping, Genomic Structure, Mutations in Familial Combined Pituitary Hormone Deficiency. *FEBS. Lett.* 1998 ; 437 = 216 - 220.
- [21]. VOUTETAKIS.A, ARGYROPOULOU. M, SERTEDAKI. A, LIVADAS.S, XEKOUKI. P, MANIATI-CHRISTIDIM, BOSSIS. N, THALASSINO, PATRONAS. N, DACOUVOUTETAKIS. C. Pituitary Magnetic Resonance Imaging in 15 Patients with Prop1 Gene Mutation = Pituitary Enlargement May. Originate from the Intermediate Lobe. *J. Clin. Endocrinol. Metab.* 2004, 89 = 4520-4856.

Figures

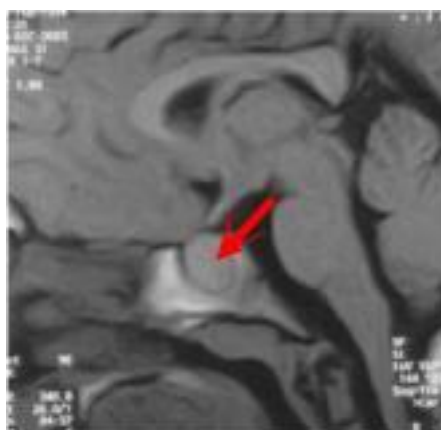


Fig. 1: Sagittal section in MRI : Expansive homogeneous Pituitary process in a child with prop1 mutation



Fig 2: Sagittal section MRI : Severe pituitary hypoplasia in the child with Pit1 mutation