

Molecular Results of Familial Isolated Gh Deficiencies in Algeria

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Abstract: Genetic research in familial forms of isolated GH deficiencies found a molecular abnormality in 50% of cases (n = 5/10). The search for a mutation in the GH1 gene was negative. Involvement of the GHRH-R gene is exclusive. Three mutations were identified : PL 144 Histamine (-C-431 T> A); ALA5 ASoFsx 22 and C465-91-1105-119 del 5291 bp. No genotype / phenotype correlation has been found for these mutations.

Keywords: Isolated GH deficiency - GH1 gene - pituitary hypoplasia - GHRH-R gene - delay stature

I. Introduction

The study of molecular biology has identified several genetic abnormalities in patients with GH deficits. However only a small number of them were listed at present. Indeed, genetic abnormalities have been identified in less than 10%^{1,2,3}. Abnormalities responsible for isolated GH deficiency have defects in the genes coding for the GH1 and especially the GH HR receptor.^{4,5}

II. Aim:

Report molecular abnormalities found among Algerians children with familial isolated GH deficiency.

Population methodology:

A study in molecular biology was performed in 10 Algerian families with isolated GH deficiency (at least two index cases within the same family).

The diagnosis of GH deficiency (GHD) was placed on a beam of clinical and paraclinical arguments: slow growth rate, late stature-weight < -2 SD / M (sempé), abdominal adiposity, the delayed bone age and lack of GH response (peak GH <20 mU/ l) in two pharmacological stimulation tests: glucagon /propranolol and insulin tolerance.

Besides questioning and physical examination led to the search for other familial cases, a hormonal balance (hypophysioigramme: FT4, TSHus, insulin test on plasma cortisol, FSH, LH, testosterone or estradiol) and a neuroradiology exploration in magnetic resonance imaging (MRI HH) has been made. The paraclinical exploration was supplemented by a study in molecular biology. After DNA extraction, the samples were sent to a specialized genetics department in GH deficiency. DNA was processed and amplified by PCR (polymerase chain reaction). Thereafter direct sequencing of the PCR products is carried out using an automated sequencing. The search for a mutation in the GH1 gene is carried out first. In the absence of abnormalities of the latter, the search for a mutation of the GHRH receptor (GHRH -R) is then performed.

III. Results

Half of the familial forms of GH deficiencies isolated has a genetic defect (n = 5/10 families, 50 %). Molecular analysis of the GH1 gene performed first came back negative. Involvement of the GHRH -R gene is exclusive. Three mutations of the GHRH - R were identified (Table 1):

- PL 144 Histamine (C- 431T > A) mutation.
- The mutation ALA 8 ASOFsx 22.
- The mutation C 465-91-1105-119 del 5291 bp.

All patients are homozygous for the mutations found. Parents and siblings of patients have not been studied. Inbreeding is present in all cases (100%). The reason for consultation was the short stature in the majority of cases (90 %). The average age at diagnosis is late and short stature is severe (> - 3 SDS / M) in all patients. Two patients had a frontal prominence and an exaggeration of the nasal bridge (Table I). Means GH peaks were severely reduced (< 1 mU / l) in 04 cases. The pituitary MRI exploration revealed two radiological aspects: a pituitary hypoplasia and normal pituitary (Table I). There is no correlation of phenotype, genotype among patients with the same mutation including those who are from the same family (Table I)

Table I: Phenotypic and genotypic results of Isolated GH deficiency patients

Patients	Mutation in question of GHRH-1 st	Age at the 1 st	Reason for	R.S (DS)	clinical appearance	GH Base [^] Pic	Other pituitary	MRI pituitary
?	ALA8Aspfs	29	RSP	- 3,8	typical	0,11- 3,23		Hypoplasia
â	ALA8Aspfs	25	family survey	- 4	Typical	1 ^ 8,23		Normal
101	PLeu 144 Histamine (C-431 T>A)	15	family survey	-4,2	Typical	0,27-0,32		Normal
8	PLeu 144 Histamine (C-431 T>A)	12	family survey	- 6	Typical	0,31- 0,52		Normal
* A	PLeu 144 Histamine (C-431 T>A)	12	family survey	- 6	Typical	0,31- 0,52		Normal
?	PLeu 144 Histamine (C-431 T>A)	04	RSP	-11	Typical	0,38-0,40		Hypoplasia
(10)	PLeu 144 Histamine (C-431 T>A)	05	RSP	-4,1	Typical	0,15-2,37		Normal
?	PLeu 144 Histamine (C-431 T>A)	07	RSP	- 3	Typical	0,5 - 1,75		Normal
(37)	PLeu 144 Histamine (C-431 T>A)	05	RSP	-4,1	Typical	0,15-2,37		Normal
?	PLeu 144 Histamine (C-431 T>A)	07	RSP	- 3	Typical	0,5 - 1,75		Normal
(38)	PLeu 144 Histamine (C-431 T>A)	07	RSP	- 3	Typical	0,5 - 1,75		Normal
?	PLeu 144 Histamine (C-431 T>A)	04 %	RSP	- 4	Typical	0,32 -2,02		Normal
(83)	PLeu 144 Histamine (C-431 T>A)	04 %	RSP	- 4	Typical	0,32 -2,02		Normal
?	PLeu 144 Histamine (C-431 T>A)	08	RSP	- 3,5	Typical	0,30 -2,08		Normal
(99)	PLeu 144 Histamine (C-431 T>A)	08	RSP	- 3,5	Typical	0,30 -2,08		Normal
?	PLeu 144 Histamine (C-431 T>A)	10	RSP	- 4	Typical	0,20 - 3,8		Normal
(98)	PLeu 144 Histamine (C-431 T>A)	10	RSP	- 4	Typical	0,20 - 3,8		Normal

(-) Absent. _[The same family

Patients sex N°	Age at the last visit. (years)	Other hormonal deficits appeared (onset time (year) /	Evolution MRI pituitary
?	16	- (7)	Hypoplasia / Hypoplasia
â	17	- (2)	Normal / Normal
â * (8)	7	- (2)	Normal / Normal
â *	15	- (3)	Normal / Normal
?(10)	7	- (3)	Hypoplasia / Hypoplasia
? 1 (37)	18	- (13)	Normal / Normal
? ^ (83) J	20	- (15,5)	Normal / Normal
?(38)	13	- (4)	Normal / Normal
? "(99) ►	13	- (5)	Normal / Normal
? (98)	15	- (5)	Normal / Normal

Hormonal and neuroradiological réévaluations after a mean of ten years were stationary in all patients (Table II). The hormonal balance sheet did not show the appearance of additional pituitary deficits in patients with pituitary hypoplasia (Table II).

IV. Discussion

Great strides have been made over the past 20 years in genetic deficits associated with isolated GH or other pituitary deficits. However, despite the discovery of several mutations, the vast majority of somatotrophic congenital deficiencies remain to be elucidated⁶. These are often sporadic. Indeed, familial forms are rare and represent 3-30 % of cases. This frequency is higher in areas with high inbreeding as our country.

The first molecular studies in family isolated somatotrophic deficits, related to the GH1 gene which contain the most extensive studies^{7,8,9,10,11}. But now various publications tend to show that the genetic anomalies of GHRH-R gene are very frequent and increasingly implicated in the isolated GH deficiency^{12,13,14}. Our results are in agreement with the literature. Molecular abnormalities found in our patients are represented exclusively by changes in the GHRH -R gene.

The first mutation of the GHRH -R gene: the E72X was reported by Wajnrajch et al¹⁵. This mutation was subsequently found in other families of South India^{16,17} and a family from Sirilanca^{18,19}. The initial hypothesis of a founder effect in the Indian subcontinent has been verified by Wajnrajch¹⁵ and Kamijo²⁰. Currently several anomalies were identified.

PL 144 His mutation found in three families in our series was described for the first time by Salvatori et al^{21,22}. It is the fourth anomaly described of GHRH-R gene.

The second mutation found C465 -91- 1105-119 del 15291bp consists in a deletion of a region of 5291 base pairs comprising exons 6 to 11 which carries the receiver and making it non- functional. It was reported for the first time by Souza et al²³ in a very wide Brazilian family.

For the third mutation identified in our patients: Ala- 8AS PFsx22 has never been described before. It involves the insertion of an adenine between nucleotides 22 and 23, resulting in a shift of the frame reading. This change predicts the appearance of a stop codon of 22 aminoacid after Alanine 8 and production of a truncated non functional protein or a lack production.

It should be noted that the mutations found in our patients are different from those reported by Hilal et al²⁴ in Morocco. Based on these findings we can hypothesize a high frequency of mutations in the GHRH -R, whose exon 1 is probably the hypermutability region, and the lack of specificity characterizing the Maghreb. More extensive multicenter studies should be conducted to confirm or refute this hypothesis. All mutations reported to date are autosomal recessive¹. They are most often family^{15,25,26}. The family genetic study couldn't be performed at the time of the study, however, inbreeding prejudice the autosomal recessive transmission of the mutation.

Clinically, the phenotypic picture is generally characteristic. The "miniature" aspect with frontal prominence and exaggeration of the nasal saddle deformity classically reported^{26,27} has been objectified in one case in our study. The same applies to the very important short stature (> - 4.5 DS / M)^{5,10,12,26,27,28} which was found in only two of our patients. On the hormonal level, GH peaks after pharmacological test are higher than those of combined genetic somatotrophic deficits. Maheshwari¹⁷ demonstrated that patients with a homozygous mutation of a GHRH-R keep pulsatile GH secretion in contrast to patients with GH1 molecular abnormalities and to patients with genetic defects of the transcription factors. The number of peaks of secretion persists but their amplitude is very small. Frequency peak GH is controlled by the lower tone somatostatin inhibitor whereas the amplitude of these peaks is secondary to the action of GHRH¹⁷.

Furthermore, Netchine et al¹⁹ reported that prolactin levels can be lowered. This decrease is linked to the action of GHRH on lactotrope cell²⁹. Other authors³⁰ as we have not found this effect on prolactin.

Radiologically two aspects have been objectified, the pituitary hypoplasia (fig 1 and 2) and that a normal pituitary gland.

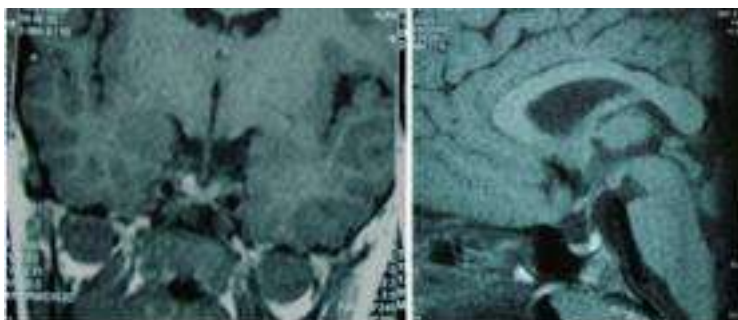


Fig 1 and 2: Coronal and sagittal section MRI : Severe pituitary hypoplasia in the child with Pit1 mutation

In the literature, mutations in the GHRH-R gene are usually associated with pituitary hypoplasia^{5,10,15,17,25,31,32}. This aspect can be found in early childhood. The size reduction of the pituitary is explained by the role of GHRH in the proliferation of somatotrophs cells and GH secretion³³. The phenotypic variability observed in patients attests to the lack of genotype/phenotype for the same mutation between patients and within the same family.

When the pituitary gland is normal, changes can be marked by a regression of the pituitary volume^{15,25}. This finding was not verified in our patients during the study. Due to pituitary hypoplasia other pituitary deficits can occur^{9,10,25,27,28} and should be monitored.

Conclusion:

The GH1 gene does not seem to be implicated in our isolated GH family deficits. Thus, genetic analysis of the gene of the GHRH receptor should be considered first.

The molecular study is needed in the family of an index case to allow early diagnosis and initiation of effective treatment. Conducting multicenter studies are needed for a better understanding and proper management of the disease.

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