Pituitary function and glucose tolerance in a family with a PAX6 mutation

Fonction hypophysaire et tolérance glucidique dans une famille atteinte d’une mutation de PAX6

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Abstract

Background. – PAX6 is a transcription factor involved in the regulation of eye and islet cell development in humans and has also been shown to be an early marker of the pituitary gland in mice. While some subjects with PAX6 mutations were found to have impaired glucose tolerance or diabetes in two previous studies, there has been no report of systematic pituitary function assessment in these patients. Aim. – The objective of this report was to assess pituitary function and glucose tolerance in five related patients with a heterozygous PAX6 mutation and an unusual ocular and neurological phenotype. Subjects and methods. – Pituitary function (static and dynamic exploration of the five ante-pituitary axes) and glucose tolerance (oral glucose tolerance test) were explored in all patients. Results. – Glucose tolerance was normal in all patients. We found no obvious pituitary deficiency in four of the five patients. However, borderline cortisol levels were observed in three out of these patients, with subnormal values, at baseline and/or after stimulation test. Basal and stimulated cortisol levels were both more clearly diminished in one subject. Conclusions. – We report here the first complete pituitary function assessment, together with glucose tolerance evaluations, in five related patients with a PAX6 mutation. We cannot rule out subtle corticotrope deficiency induced by PAX6 mutation. The conflicting results with the literature about glucose tolerance could be explained by genotype/phenotype correlations.

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Résumé

Contexte. – PAX6 est un facteur de transcription impliqué dans le développement embryonnaire de l’œil et des îlots pancréatiques chez l’homme et a également été mis en évidence dans les cellules embryonnaires hypophysaires de souris. Alors qu’intolérance au glucose ou diabète ont été identifiés chez certains patients atteints de mutations de PAX6 dans deux études, il n’a jamais été rapporté d’exploration systématique de la fonction hypophysaire chez ces patients. Objectif. – L’objectif de notre étude était d’évaluer fonction hypophysaire et tolérance au glucose chez cinq patients apparentés et hétérozygotes pour une mutation du gène PAX6, présentant un phénotype ophtalmologique et neurologique inhabituel. Patients et méthodes. – La fonction antéhypophysaire (dossages statiques et tests dynamiques sur les cinq axes antéhypophysaires) et la tolérance au glucose (hyperglycémie provoquée par voie orale) ont été explorées chez tous les patients. Résultats. – La tolérance au glucose était normale chez tous les patients. Nous n’avons pas mis en évidence de déficit antéhypophysaire franc chez quatre des cinq patients. Cependant, un taux de cortisol limite était observé chez trois des cinq patients, avec des valeurs subnormales, de base et/ou après test de stimulation. Cortisolémies basale et après stimulation étaient plus clairement diminuées chez un patient. Conclusion. – Nous rapportons ici la première évaluation complète de la fonction hypophysaire, associée à l’évaluation de la tolérance au glucose chez cinq patients apparentés et porteurs d’une mutation du gène PAX6. On ne peut éliminer un déficit corticotrope a minima induit par la mutation de PAX6. De plus, la discordance entre les données de la littérature et nos résultats concernant la tolérance au glucose suggère une corrélation génotype/phenotype.

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1. Introduction

PAX6 is involved in the regulation of eye development [1]. Heterozygous mutations of the gene encoding this transcription factor in humans cause congenital eye abnormalities [2]. PAX6 plays an essential role in islet cell development. Disruption of the PAX6 gene in mice causes a marked decrease in the numbers of all four types of endocrine cells in the pancreas [3], resulting in an overt diabetic phenotype at birth [4]. In a PAX6-deficient model in rat primary β-cells, Gosmain et al. have showed that PAX6 is crucial for β-cells through transcriptional control of key genes coding for proteins that are involved in insulin biosynthesis and secretion as well as glucose and incretin actions on β-cells [5]. There have been several reports of PAX6 mutation carriers having abnormal glucose tolerance, and of type 2 diabetes co-segregating with aniridia in families with PAX6 mutations [6,7]. Four human patients with sporadic aniridia and PAX6 mutations were found to have impaired glucose tolerance (IGT) [6]. In another study, nine of 16 patients with aniridia caused by a PAX6 mutation from a Chinese family developed IGT or diabetes [7]. The authors investigated the underlying mechanism, in PAX6 mutant Small eye mice, which also have abnormal glucose metabolism, and showed that PAX6 mutations led to prohormone convertase (PC) 1/3 deficiency.

In addition to its role in endocrine pancreas development and function, PAX6 has also been shown to be an early dorsal marker of the pituitary gland and to control the established boundaries of somatotrope, lactotrope and thyrotrope cell types in mice [8]. In Small eye and PAX6-/- mouse mutants, the absence of PAX6 leads to a marked increase in the proportion of cells belonging to the thyrotrope lineage, with marked decreases in the proportions of somatotrope and lactotrope cells. Bentley et al. confirmed these findings, by showing that the numbers of growth hormone (GH) and prolactin (PRL)-immunopositive cells were much lower in PAX6 mutant mouse embryos than in the wild type [9]. These PAX6 homozygous mutant mice had serum concentrations of GH and PRL one fifth and one third, respectively, those of controls during the embryonic and neonatal stages.

Finally, in a recent case report, the only known surviving patient with mutations in both PAX6 alleles was described as having microophthalmia, neonatal diabetes mellitus, hypopituitarism and a complex structural abnormality of the brain [10]. These findings imply that PAX6 may be involved in pituitary development and function, but there has been no report of systematic pituitary function assessment in patients with heterozygous PAX6 mutations. Furthermore, the high frequency of glucose intolerance reported by Yasuda et al. [6] and Wen et al. [7] has not been confirmed by other studies. We assessed pituitary function and glucose tolerance in five related patients with a heterozygous PAX6 mutation.

2. Patients and methods

2.1. Patient recruitment

We recruited five related French patients from a family with a PAX6 mutation. Ocular signs, which led to screening for PAX6 gene mutation, other clinical symptoms and brain magnetic resonance imaging (MRI) findings for these patients have been described in detail elsewhere [11]. Briefly, patients presented either with minor or major bilateral foveal hypoplasia or, in some rare instances, either macular coloboma or a major posterior coloboma. All affected family members presented with congenital bilateral multidirectional nystagmus probably due to foveal or macular alterations. Moreover, they also displayed either a congenital cataract (rarely) or, more commonly, precocious infantile progressive bilateral cataracts with substantial phenotypic variability between the affected family members. Some of the patients had highly unusual ophthalmic phenotypes, which in a few cases differed bilaterally. This family extends over five generations and has 41 members, at least 14 of whom have a 655A>G (S74G) mutation in exon 6 of PAX6 (Fig. 1). The five patients recruited here belong to family F (patients IV-5, IV-8, V-6, V-10, and V-11 aged 50, 38, 27, 16, and 11 years, respectively) from the study by Dansault et al. [11]. Their weight and body mass index were, respectively, 66, 102, 47, 64, and 45 kg, and 28.9, 36.1, 15.9, 22.1, and 20.9 kg/m².

All participants gave informed consent for participation in accordance with the Bioethics Laws of the European Union and France and the Helsinki Declaration. The study was approved by the ethics committee of Pasteur-Necker and the Comité Consultatif pour la Protection des Personnes se prêtant à des Recherches Biomédicales, Advisory Committee for the Protection of Persons suitable for Biomedical Research (CCPRPB) of Pitié-Salpêtrière Hospital.

2.2. Endocrinological evaluation

All these evaluations have been performed in 2006. Pituitary function was explored in all patients.

Thyrotropin (TSH) (normal range [NR], 0.1–4 mU/L) was determined by chemiluminescence immunoassay (ACS Bayer); free tetraiodothyronine (T4) (NR, 7–20 pmol/L) and free triiodothyronine (T3) (NR, 3.0–5.5 pmol/L) were determined by radioimmunoassay (RIA). A stimulation test was also performed with TSH releasing hormone (TRH) (0.2 mg TRH administered IV).

Serum GH and insulin-like growth factor 1 (IGF-1) concentrations (NR according to patient age, Table 1A) were determined by immunoradiometric assay (IRMA; Immunotech-Beckman Coulter).

Cortisol (NR, 220–630 nmol/L, 80–228 μg/L) was determined by electrochemiluminescence assay and adrenocorticotropin (ACTH) (NR, 10–50 ng/L) was determined by IRMA.

An insulin tolerance test (ITT; 5 U insulin/m² IV) or a synacthen (1 μg synacthen IV) test and a GH releasing hormone (GHRH) test in cases for which ITT was contraindicated, was performed. ACTH deficiency was excluded if the peak serum cortisol response was more than 200 μg/L (550 nmol/L). GH deficiency was excluded if peak GH concentration was higher than 20 mU/L.

Luteinizing hormone (LH; IU/L) and follicle-stimulating hormone (FSH; IU/L), estradiol (nmol/L) and progesterone (nmol/L) (in female subjects only) were determined by
Table 1A
Results of biological assays: static and dynamic hormonal tests.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time</th>
<th>Thyrotrope axis</th>
<th>Somatotrope axis</th>
<th>Corticotrope axis</th>
<th>Gonadotrope axis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TSH after TRH test (mIU/L)</td>
<td>Free T3 (pmol/L)</td>
<td>Free T4 (pmol/L)</td>
<td>IGF1 (ng/ml) (NR for patient age)</td>
</tr>
<tr>
<td>IV-5</td>
<td>Basal</td>
<td>2</td>
<td>5.1</td>
<td>15.8</td>
<td>175 (107–310)</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>20.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV-8</td>
<td>Basal</td>
<td>1</td>
<td>4.6</td>
<td>14.5</td>
<td>169 (107–310)</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>14.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V-6</td>
<td>Basal</td>
<td>3.1</td>
<td>5.0</td>
<td>16.1</td>
<td>184 (107–310)</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>17.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V-10</td>
<td>Basal</td>
<td>1.8</td>
<td>5.3</td>
<td>14.4</td>
<td>530 (271–550)</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V-11</td>
<td>Basal</td>
<td>1.5</td>
<td>4.9</td>
<td>11.5</td>
<td>275 (76–499)</td>
</tr>
<tr>
<td></td>
<td>Peak</td>
<td>31.9</td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

TSH: thyrotropin; TRH: TSH releasing hormone; T3: triiodothyronine; T4: tetraiodothyronine; IGF-1: insulin-like growth factor 1; GH: growth hormone; ITT: insulin tolerance test; ACTH: adrenocorticotropic; GnRH: LH-releasing hormone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; NR: normal range.

a Basal cortisol concentration was determined for a blood sample taken at 08:00 h.
b GH concentration after GHRH administration.

3. Results

Static assays were carried out for all patients. Patient V-10 refused all dynamic tests other than the ITT. Patients V-11, aged 11 years, and V-6 did not undergo stimulation tests with GnRH. The somatotrope, thyrotrope and lactotrope axes were found to be normal in all patients (Table 1A).

The gonadotrope axis was normal in all patients, but previously undiagnosed Klinefelter syndrome was detected in one male patient (patient V-6) with low testosterone (15.6 nmol/L; normal values: 10–35) and high FSH (61 IU/L) and LH (27.2 IU/L) concentrations, and confirmed by a XXY karyotype. However, the only other male subject (patient V-11) had a total testosterone concentration in the lower half with low gonadotropins levels.

In corticotrope explorations, the results of both basal and stimulation tests were normal for only one patient (patient IV-8, Table 1A). In another patient (patient IV-5), basal cortisol concentration, determined for a sample taken at 08:00 h, was normal but the cortisol peak was subnormal (Table 1A). Two patients (patients V-10, and V-11) had subnormal or low basal serum cortisol concentrations and a subnormal response in the stimulation test (synacthen test for patient V-11, ITT being contraindicated because of a history of epilepsy; Table 1A). The final patient had a subnormal basal serum cortisol concentration for a sample taken at 08:00 h., but a normal cortisol peak in the ITT (patient V-6, Table 1A). In any case was the ACTH level increased in regard to 08:00 h basal serum cortisol. None of the patients had a low albumin concentration (45, 39, 49, 42, and 56 g/L, respectively).

Pituitary MRI findings were normal in all patients.

Table 1B

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>BMI (kg/m²)</th>
<th>Time</th>
<th>Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV-5</td>
<td>50</td>
<td>F</td>
<td>28.9</td>
<td>T0</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T+120 min</td>
<td>0.72</td>
</tr>
<tr>
<td>IV-8</td>
<td>37</td>
<td>F</td>
<td>36.1</td>
<td>T0</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T+120 min</td>
<td>1.13</td>
</tr>
<tr>
<td>V-6</td>
<td>27</td>
<td>M</td>
<td>15.9</td>
<td>T0</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T+120 min</td>
<td>1.22</td>
</tr>
<tr>
<td>V-10</td>
<td>16</td>
<td>F</td>
<td>22.1</td>
<td>T0</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T+120 min</td>
<td>ND</td>
</tr>
<tr>
<td>V-11</td>
<td>10.5</td>
<td>M</td>
<td>20.9</td>
<td>T0</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T+120 min</td>
<td>1.22</td>
</tr>
</tbody>
</table>

ND: not done.

The OGTT revealed no diabetes or glucose intolerance. Only blood glucose concentrations at 0 and 120 minutes are shown in Table 1B.

4. Discussion

While we found no glucose intolerance in any of the five patients from the same family, we cannot rule out subtle corticotrope deficiency induced by PAX6 mutation.

Even if normal OGTT does not allow to rule out subtle abnormalities in terms of glucose tolerance, these results clearly conflict with those of Yasuda et al. [6] and Wen et al. [7], who reported a high prevalence of glucose intolerance or diabetes in patients with PAX6 mutation (four of four unrelated patients, and nine of 16 patients from the same family, respectively). Interestingly, Wen et al. observed a correlation between glucose tolerance status and the age of the patient. The seven patients with normal glucose tolerance were aged from 11 to 33 years, whereas the five patients with IGT were 17 to 70 years old, and the four patients with diabetes were 36 to 68 years old. However, glucose tolerance test results were normal for the two oldest patients in our study, aged 50 and 37 years.

These discrepancies may also result from differences in the location of the mutations and their consequences for PAX6.
function. All the heterozygous mutations described by Wen et al. and Yasuda et al. were stop mutations leading to aniridia and the production of truncated polypeptides, terminating in the paired domain, linker region or homeodomain, whereas the mutation in the family studied here was an S74G missense mutation in exon 6. Dansault et al. reported this mutation to be associated with unusual ocular phenotypes without aniridia, a permanent cerebellar syndrome and cognitive impairments, with a low intelligence quotient [11]. Genotype/phenotype correlations have also been described in mice. Dames et al. showed that inactivation of the PAX6 paired domain resulted in a more severe pancreatic α-cell differentiation phenotype than loss of the transactivation domain [12]. These results suggest a hierarchy in the roles of the three domains of PAX6 in pancreatic α-cell development. Explorations of genotype/phenotype correlations for the glucose metabolism abnormalities induced by PAX6 mutations would require assessments of glucose tolerance in mutant mice with a similar missense mutation in exon 6.

PAX6 may play a role in pituitary development and function, but only one study has reported hypopituitarism in a patient with compound heterozygous mutations that is usually lethal. No other study has included a full investigation of pituitary function in patients with heterozygous PAX6 mutations. Wen et al. reported lower concentrations of ACTH in the peripheral blood of nine related patients with a PAX6 mutation than in the peripheral blood of control patients [7]. They suggested that this low ACTH concentration was due to a PC1/3 deficiency induced by the PAX6 mutation, resulting in both defective proinsulin processing (leading to IGT or diabetes) and pro-opiolanocortin conversion to ACTH. Interestingly, even though there is a significant cortisol increase in all patients after ITT (the lowest stimulated cortisol concentration in patient V-11 was seen after synacthen administration), four of our five patients had suboptimal basal and/or stimulated cortisol levels while displaying no increase in basal ACTH levels. These findings are consistent with the hypothesis put forward by Wen et al. While we did not assess serum free or salivary cortisol concentrations, we do not have any reason to suspect that the corticosteroid-binding globulin levels are low in these patients. However, Wen et al. did not determine cortisol levels and we did not assess corticotrope function in a control group of related subjects from the same family but without the PAX6 gene mutation. Finally, if our PAX6 gene mutation causes PC1/3 deficiency, it would also be expected to lead to a proinsulin processing defect and IGT or diabetes.

We identified no pituitary abnormality induced by this heterozygous PAX6 gene mutation other than these subnormal cortisol levels.

In conclusion, we report here the first complete pituitary function assessment, together with glucose tolerance evaluation, in five related patients with a PAX6 mutation and an unusual ocular and neurological phenotype. In this limited number of patients, we found no clear abnormalities of pituitary function due to this heterozygous mutation but we cannot rule out subtle corticotrope deficiency induced by PAX6 mutation. Pituitary function, and particularly corticotrope axis, should be assessed in other families with PAX6 mutations. In addition, further studies in families or mouse models with different mutations and functional studies would be required to determine whether the absence of glucose intolerance observed here is due to a particular genotype.

**Disclosure of interest**

The authors declare that they have no conflicts of interest concerning this article.

**References**


