

BRIEF COMMUNICATION



A human paradigm of *LHX4* and *NR5A1* developmental gene interaction in the pituitary gland and ovary?

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The pituitary gland, as a nodal component of the endocrine system, is responsible for the regulation of growth, reproduction, metabolism, and homeostasis. Although pituitary formation through the hierarchical action of different transcription factors is well studied in mouse models, there is little evidence of the analogous developmental processes in humans. Herein, we present a female patient with a phenotype that includes blepharoptosis–ptosis–epicanthus syndrome and premature ovarian failure. Clinical exome sequencing revealed two heterozygous variants in two genes, *LHX4* (pathogenic) and *NR5A1* (VUS) genes and no mutation in *FOXL2* gene. We propose a model of genetic interaction between *LHX4* and *NR5A1* during pituitary and ovarian development that may lead to a similar phenotype mediated by reduced *FOXL2* expression.

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INTRODUCTION

The pituitary gland is an integral part of the endocrine system for the regulation of growth, reproduction, metabolism, and homeostasis. Although the development of the pituitary gland in mouse models is well characterized, there is little evidence of the analogous developmental processes in humans. In this context, clinical cases with the specific characterization of the genotype–phenotype duality and mode of inheritance may give an insight for underlying interactions among human genes. Herein, we present a patient with premature ovarian failure (POF) and features of blepharophimosis–ptosis–epicanthus syndrome (BPES) (OMIM #110100) harboring two heterozygous variants in two genes, *LHX4* and *NR5A1*. We propose the hypothesis of how genetic interaction between *LHX4* and *NR5A1* may reduce *FOXL2* gene expression and lead to BPES.

CLINICAL REPORT AND GENETIC ANALYSIS

A 16-year-old girl was presented to our department with primary amenorrhea and short stature. Her height was 150.6 cm (<5th percentile, 2 SD below from her target mid-parental height) and her weight 37 kg (<3rd percentile). She had bilateral blepharophimosis, ptosis of the eyelids, and cubitus valgus. Both breast and pubic hair were Tanner III stage without any signs of further pubertal progression. She was the second child of a family of non-consanguineous parents, born full term but small for gestational age.

Her hormonal evaluation revealed hypergonadotropic hypogonadism with normal thyroid and adrenal function (Table 1). Her bone age was delayed by 3 years. A full panel of several autoantibodies was done, which was negative for autoimmunity. A pelvic ultrasound showed a uterus of early-pubertal size and small ovaries (2.5 cm³) containing a few follicles. The anti-Müllerian

hormone (AMH) was almost undetectable (0.01 ng/mL). Magnetic resonance imaging of the pituitary gland showed normal morphology and size (6 mm height and 10 mm depth).

Estrogen replacement therapy with transdermal patches was initiated and during the pubertal induction period, her growth rate was increased substantially from the 3rd percentile to 20th percentile of height trajectory.

Upon taking written and signed informed consent from the patient and her parents, she underwent a full genetic analysis. Her karyotype was normal, while microarray-based copy number screening was normal for *FOXL2* gene. Clinical exome sequencing (CES) was carried out employing the Clinical Exome Solution kit (4900 genes) by Sophia Genetics[®] carried out on an Illumina NextSeq 500. Computational analysis included read alignment to the human genome reference sequence (GRCh37/hg19), base quality score recalibration, indel realignment, and variant calling, using the SOPHiA DDM[®] platform (Sophia Genetics, SA) as previously described [1]. Variants were annotated with the ANNOVAR algorithm and filtered using VarAFT v2.16 (<http://varaft.eu>) application. Variant classification was based on the American College of Medical Genetics and Genomics (ACMG) criteria [2] and evaluated with the in silico analysis tools by the VarSome platform.

Although *FOXL2* gene was included in the CES panel employed, it was also Sanger-sequenced. Specifically, the single exon of the *FOXL2* gene (NM_023067.4) was PCR amplified and sequenced using the Big Dye Terminator cycle sequencing kit on an ABI 3500 genetic analyzer (Applied Biosystems, CA, USA). *FOXL2* gene PCR and sequencing primers are available on request. The CES findings were verified by Sanger-sequencing the coding regions and the intron-exon boundaries of exon 3 of the *LHX4* and exon 4 of the *NR5A1* genes.

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RESULTS

CES achieved 25× coverage of the 99.6% of the targeted sequences and mean coverage rate of 70×. Particularly, 100% of the *FOXL2* gene was covered at least 50×. Neither CES, nor Sanger sequencing, found any pathogenic or likely pathogenic variant in *FOXL2* gene. CES revealed in our patient two heterozygous variants in two genes; the, p.Arg84Cys in *LHX4* gene (NM_033343.3: c.250C>T) and the p.Ile193Asn in *NR5A1* gene (NM_004959.5: c.578T>A). These variants were confirmed by Sanger-sequencing patient's and her parents' DNA and it was shown that the *LHX4* variant was paternal, whereas the *NR5A1* variant of maternal inheritance (Fig. 1).

The *LHX4* gene variant p. Arg84Cys, was classified according to the ACMG criteria as Pathogenic (criteria applied: PVS1 very strong, PP3 and PP5 supporting,). Criterion PVS1 has been applied since the *LHX4* variant c.250C>T is located within exon 3 acceptor splice site [acag|G(C/T)GC] and it is likely that disturbs normal splicing. It is a very rare variant with gnomAD exomes frequency 0.0000558 and gnomAD genomes frequency 0.0000131. Seventeen in silico tools of the VarSome platform as well as Polyphen 2, categorize this variant as damaging/pathogenic.

The *LHX4* gene variant p.Arg84Cys, has been previously described in a male patient with short stature, growth hormone deficiency and hypothyroidism [3]. This variant lies within the zinc-binding 1 region of the LHX4 protein, impairing activity on CGA,

POU1F1 and TSHB promoters. Interestingly, our patient's father had a normal puberty and attained a normal final height.

The *NR5A1* gene variant p.Ile193Asn is novel and it was classified according to the ACMG criteria as variant of unknown significance (criteria applied: PM2 moderate and PP2 and PP3 supporting). It is a highly conserved variant, not present in gnomAD exomes or gnomAD genomes databases. Her 56-year-old mother was a carrier of the *NR5A1* gene variant, p.Ile193Asn, with menarche at the age of 14 years and no history of POF. A specific variant at the adjacent position (p.Arg192Phe), located at the hinge region, results in a weakly conserved protein with predicted intermediate pathogenicity [4]. Eighteen in silico tools of the VarSome platform as well as Polyphen 2, categorize this variant as damaging/pathogenic.

DISCUSSION

Herein, we present a case of a girl with BPES phenotype, POF, and short stature. Because short stature is not a typical characteristic of the BPES phenotype we performed CES in addition to single *FOXL2* gene sequencing. No intragenic variants, deletions or cytogenetic rearrangements of *FOXL2*, accountable for the BPES [5] were found, but CES revealed two heterozygous variants, each in *LHX4* and *NR5A1* genes, indicating digenic inheritance.

Based on the existent data, we propose, within the functional framework of the *LHX4* and *NR5A1* genes, a model for the explanation of our patient's clinical phenotype. LHX3 and LHX4 proteins belong to the LIM-homeodomain transcription factors and play essential roles in the pituitary gland and nervous system development [6]. LHX3 binds to the promoter region of the alpha glycoprotein subunit (*aGSU*) and the beta subunits of *FSH*, *TSH*, and *Pit-1* genes [7, 8]. This multitargeting action is critical, for both the early pituitary gland formation and the development of lactotrope, somatotrope, gonadotrope, and thyrotrope cell lineages. A recent study, using single-cell RNA sequencing from human fetal pituitaries, assigned *LHX3* a major role in the early phase of pituitary developmental patterning [9]. The structurally related LHX4 protein is required for the proper expression of *LHX3* [6]. Gene targeting studies in mice have elucidated the overlapping, but distinct roles of *LHX3* and *LHX4* in development with the *LHX3* being essential for the emergence of all differentiated cell types in Rathke's pouch [10]. Recently, six disease-causing variants of *LHX4* gene in a heterozygous state were reported, in a large cohort of 417 unrelated patients with isolated growth hormone deficiency or combined pituitary hormone deficiency [11].

Table 1. Hormonal profile of the 16-year-old female patient.

TSH = 2.73 mIU/mL (0.270–4.2)	LH = 22.5 U/l (2.4–12.6)
FT4 = 1.35 ng/dL (0.8–2)	FSH = 64.4 U/l (3.5–12.5)
β-HCG = 0.4 mIU/mL (<5)	E2 = 8.91 pg/mL (26.7–1506)
17-OHP = 0.56 ng/mL (0.1–1.1)	SHBG = 148.5 nmol/l (26.1–110)
DHEAS = 162.7 μg/dL (148–407)	ACTH = 50.9 pg/mL (5–60)
IGF-1 = 243 ng/mL (183–792)	Δ4 = 1.6 ng/mL (0.47–3.40)
AMH = 0.01 ng/mL (1.52–9.95)	Cortisol = 22 μg/dL (6.2–19.4)

To convert to SI units: testosterone, nmol/L = ng/dL × 0.03467; FT4 pmol/L = pg/mL × 1.29; DHEAS, μmol/L = μg/dL × 0.027; Δ4, nmol/L = ng/mL × 3.479; 17-OHP, nmol/L = ng/mL × 3.026; ACTH, pmol/L = pg/mL × 0.2222; Cortisol, nmol/L = μg/dL × 27.58; AMH, pmol/L = ng/mL × 7.14; IGF-1, nmol/L = ng/mL × 0.13.

17-OHP 17-OH-progesterone, Δ4 Δ4-androstenedione.

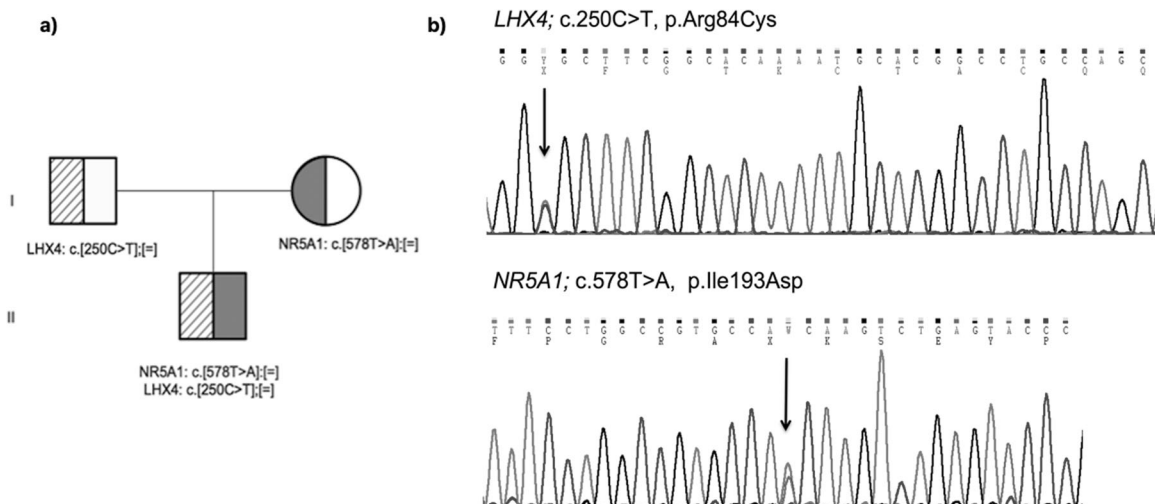


Fig. 1 Genetic analysis of the proband and her parents. **a** Pedigree of the patient's family and **b** parts of the sequencing chromatograms of *LHX4* gene showing the exon 3 c.250C>T, p.Arg84Cys and *NR5A1* gene showing the exon 4 c.578T>A, p.Ile193Asn variants detected in our patient.

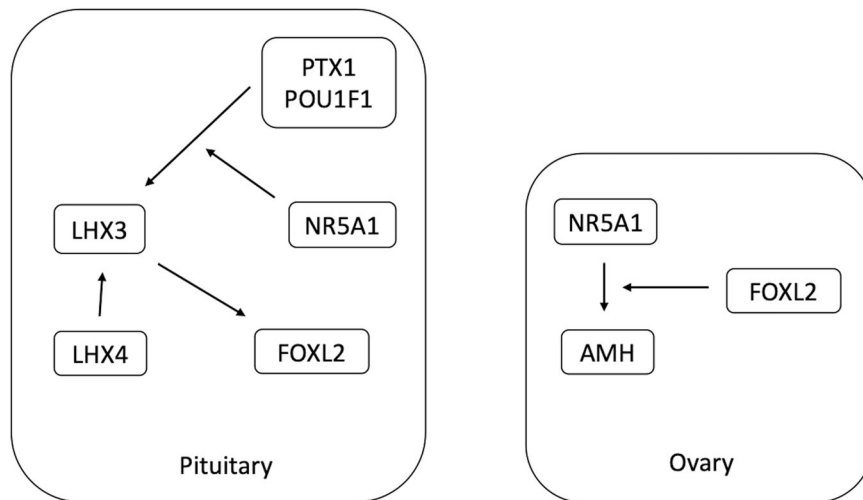


Fig. 2 Part of networks depicting gene interactions among *LHX4*, *NR5A1*, and *FOXL2* in pituitary and ovary during development. **Pituitary:** *NR5A1* co-regulates with *PITX1* and *POU1F1* the transcription of *LHX3*. *FOXL2* transcription needs *LHX3* and is enhanced by *LHX4* **Ovary:** SF-1 transactivates ovarian *AMH* in a *FOXL2*-dependent manner and *FOXL2* is required for the binding of SF-1 to the *AMH* promoter.

Steroidogenic factor-1 (SF-1 or NR5A1) is an orphan nuclear receptor that has multiple functions in steroidogenesis, sexual differentiation, and reproduction. Variants in *NR5A1* gene have been found in a small percentage of women with POF, while many female carriers show no phenotype, indicating varying degrees of expression and incomplete penetrance [12]. At the hypophyseal level, *PITX1* (Pituitary Homeobox-1) regulates the *LHX3* gene expression [13] and this regulation is affected by *NR5A1* and *POU1F1* transcription factors.

Our patient's clinical phenotype, described as BPES with or without POF [14], is usually encountered in individuals with *FOXL2* gene mutations. *FOXL2* is expressed in the ovary, the pituitary gland and the eye during early development [15] and requires the transcription factors *LHX3* and *LHX4* [16]. Recently, in a large cohort of patients with BPES only 67% harbored pathogenic *FOXL2* variants [17] and some of them had coexistent hypopituitarism, supporting *FOXL2* involvement in human pituitary development [18]. In addition, data from single-cell RNA sequencing from human fetal pituitaries indicate that *FOXL2* acts with *NR5A1* for the gonadotrope cell lineage development.

Based on our findings, we postulate that the additive effect of heterozygous variants in *LHX4* and *NR5A1*, due to digenic inheritance, may lead to a BPES type I-like phenotype, by acting on the *FOXL2* expression levels during development (Fig. 2). Firstly, the transcriptional balance of *LHX3* and *LHX4* is affected by the *NR5A1* transcription levels because *NR5A1* is needed for *PITX1* and *POU1F1* to regulate *LHX3* gene expression [13]. Normal *FOXL2* expression requires the transcription factor *LHX3*, because it is an upstream activator of *FOXL2* [16]. *LHX4* is indispensable for the proper expression of *LHX3* and inductively affects *FOXL2*. These interactions may explain the BPES phenotype of our patient. Focusing on the ovaries, the inter-regulatory network of *AMH*, *FOXL2*, and *NR5A1* is essential for developing the ovarian reserve during development. Experimental evidence supports that *AMH* is a target gene of *NR5A1* and *FOXL2* acts as an important factor in this transcriptional regulation [19]. Consequently, low *AMH* expression during ovarian development due to reduced drive by *NR5A1* and *FOXL2* may lead to POF (Fig. 2). The limitation of our study is the lack of functional studies on *FOXL2* interaction with *NR5A1* and *LHX4* variants. However, we think that developmental tissue-specific and spatiotemporal gene interactions cannot be easily captured by in vitro cellular systems or the more informative transgenic mouse models due to the increased redundancy of gene networks. With an integrative approach of biological and

computational data [20], we consider our patient's phenotype to be the result of a natural gene perturbation experiment that provides valuable biologic data concerning the genes *LHX4* and *NR5A1* in the development of pituitary and ovary.

In conclusion, we present a patient with the phenotype of BPES type I syndrome and heterozygous variants in *LHX4* and *NR5A1* genes. We propose a model of interaction between *LHX4* and *NR5A1* during pituitary and ovarian development that results to this phenotype by reduced *FOXL2* expression.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available in the LOVD v.3 repository <https://www.lovd.nl/3.0/home>.

REFERENCES

- Marinakos NM, Svingou M, Veltra D, Kekou K, Sofocleous C, Tilemis F-N, et al. Phenotype-driven variant filtration strategy in exome sequencing toward a high diagnostic yield and identification of 85 novel variants in 400 patients with rare Mendelian disorders. *Am J Med Genet Part A*. 2021;185:2561–71.
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–24.
- Pfaeffle RW, Hunter CS, Savage JJ, Duran-Prado M, Mullen RD, Neeb ZP, et al. Three novel missense mutations within the *LHX4* gene are associated with variable pituitary hormone deficiencies. *J Clin Endocrinol Metab*. 2008;93:1062–71.
- Janse F, De With LM, Duran KJ, Kloosterman WP, Goverde AJ, Lambalk CB, et al. Limited contribution of *NR5A1* (SF-1) mutations in women with primary ovarian insufficiency (POI). *Fertil Steril*. 2012;97:141–6.e2.
- Beyens D, De Paeppe A, De Baere E. *FOXL2* mutations and genomic rearrangements in BPES. *Hum Mutat*. 2009;30:158–69.
- Mullen RD, Colvin SC, Hunter CS, Savage JJ, Walvoord EC, Bhangoo APS, et al. Roles of the *LHX3* and *LHX4* LIM-homeodomain factors in pituitary development. *Mol Cell Endocrinol*. 2007;265-6:190–5.
- Sloop KW, Meier BC, Bridwell JAL, Parker GE, Schiller AMC, Rhodes SJ. Differential activation of pituitary hormone genes by human *LHX3* isoforms with distinct DNA binding properties. *Mol Endocrinol*. 1999;13:2212–25.
- Bach I, Rhodes SJ, Pearse RV, Heinzl T, Gloss B, Scully KM, et al. P-Lim, a LIM homeodomain factor, is expressed during pituitary organ and cell commitment and synergizes with Pit-1. *Proc Natl Acad Sci USA*. 1995;92:2720–4.
- Zhang S, Cui Y, Ma X, Yong J, Yan L, Yang M, et al. Single-cell transcriptomics identifies divergent developmental lineage trajectories during human pituitary development. *Nat Commun*. 2020;11:5275.
- Sheng HZ, Moriyama K, Yamashita T, Li H, Potter SS, Mahon KA, et al. Multistep control of pituitary organogenesis. *Science*. 1997;278:1809–12.

11. Cohen E, Maghnie M, Collot N, Leger J, Dastot F, Polak M, et al. Contribution of LHX4 mutations to pituitary deficits in a cohort of 417 unrelated patients. *J Clin Endocrinol Metab.* 2017;102:290–301.
12. Philibert P, Paris F, Lakhil B, Audran F, Gaspari L, Saâd A, et al. NR5A1 (SF-1) gene variants in a group of 26 young women with XX primary ovarian insufficiency. *Fertil Steril.* 2013;99:484–9.
13. Tremblay JJ, Lanctôt C, Drouin J. The pan-pituitary activator of transcription, Ptx1 (Pituitary Homeobox 1), acts in synergy with SF-1 and Pit1 and is an upstream regulator of the LIM-homeodomain gene Lim3/Lhx3. *Mol Endocrinol.* 1998;12:428–41.
14. Crisponi L, Deiana M, Loi A, Chiappe F, Uda M, Amati P, et al. The putative forkhead transcription factor FOXL2 is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome. *Nat Genet.* 2001;27:159–66.
15. Cocquet J, Pailhoux E, Jaubert F, Servel N, Xia X, Pannetier M, et al. Evolution and expression of FOXL2 [1]. *J Med Genet.* 2002;39:916–21.
16. Ellsworth BS, Egashira N, Haller JL, Butts DL, Cocquet J, Clay CM, et al. FOXL2 in the pituitary: molecular, genetic, and developmental analysis. *Mol Endocrinol.* 2006;20:2796–805.
17. Bunyan DJ, Thomas NS. Screening of a large cohort of blepharophimosis, ptosis, and epicanthus inversus syndrome patients reveals a very strong paternal inheritance bias and a wide spectrum of novel FOXL2 mutations. *Eur J Med Genet.* 2019;62:103668.
18. Castets S, Roucher-Boulez F, Saveanu A, Mallet-Motak D, Chabre O, Amati-Bonneau P, et al. Hypopituitarism in patients with blepharophimosis and FOXL2 mutations. *Horm Res Paediatr.* 2020;93:30–9.
19. Jin H, Won M, Park SE, Lee S, Park M, Bae J. FOXL2 is an essential activator of SF-1-induced transcriptional regulation of anti-Müllerian hormone in human granulosa cells. *PLoS One.* 2016;11:e0159112.
20. Fernandez-Valverde SL, Aguilera F, Ramos-Díaz RA. Inference of developmental gene regulatory networks beyond classical model systems: new approaches in the post-genomic era. *Integr Comp Biol.* 2018;58:640–53.

AUTHOR CONTRIBUTIONS

AG conceived and drafted the manuscript. AS performed the genetic analysis and AS and DC critically revised the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL

The study is approved by the ethics committee of the University Hospital of Patras.

CONSENT FOR PUBLICATION

Full informed consent was obtained from the patient and her parents to publish the results of phenotyping and genetic studies.

ADDITIONAL INFORMATION

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