Genetic Overlap in Kallmann Syndrome, Combined Pituitary Hormone Deficiency, and Septo-Optic Dysplasia

Taneli Raivio,* Magdalena Avbelj,* Mark J. McCabe, Christopher J. Romero, Andrew A. Dwyer, Johanna Tornmiska, Gerasimos P. Sykiotis, Louise C. Gregory, Daniel Dicazik, Vaita Tziaferi, Mariet W. Elting, Raja Padidela, Lacey Plummer, Cecilia Martin, Bihua Feng, Chengkang Zhang, Qun-Yong Zhou, Huaibin Chen, Moosa Mohammadi, Richard Quinton, Yisrael Sidis, Sally Radovick, Mehu T. Dattani, and Nelly Pitteloud

Children’s Hospital (T.R., J.T.), Helsinki University Central Hospital, Institute of Biomedicine/Physiology, University of Helsinki, 00290 Helsinki, Finland; University Children’s Hospital (M.A.), University Medical Centre Ljubljana, 1000 Ljubljana, Slovenia; Developmental Endocrinology Research Group Clinical and Molecular Genetics Unit (M.J.M., L.C.G., V.T., M.T.D.), University College London-Institute of Child Health, London EC1V 9EL, United Kingdom; Division of Pediatric Endocrinology (C.J.R., D.D., S.R.), The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205; Endocrinology, Diabetes, and Metabolism Service of the Centre Hospitalier Universitaire Vaudois and University of Lausanne (A.A.D., Y.S., N.P.), 1011 Lausanne, Switzerland; Department of Internal Medicine, Division of Endocrinology, and Department of Pharmacology (G.P.S.), University of Patras Medical School, 26500 Patras, Greece; Department of Clinical Genetics and Human Genetics (M.W.E.), VU Medical Centre, 1081 HL Amsterdam, Netherlands; Department of Paediatric Endocrinology (R.P.), Royal Manchester Children’s Hospital, Manchester M13 9WL, United Kingdom; Harvard Reproductive Endocrine Sciences Center and the Reproductive Endocrine Unit of the Department of Medicine (L.P., C.M., B.F.), Massachusetts General Hospital, Boston, Massachusetts 02114; Department of Pharmacology (C.Z., Q.-Y.Z.), University of California, Irvine, Irvine, California 92697; Department of Pharmacology (H.C., M.M.), New York University School of Medicine, New York, New York; and Institute for Genetic Medicine (R.Q.), Newcastle University, and Department of Endocrinology, Newcastle upon Tyne Hospitals, Newcastle upon Tyne NE1 7RU, United Kingdom

Context: Kallmann syndrome (KS), combined pituitary hormone deficiency (CPHD), and septo-optic dysplasia (SOD) all result from development defects of the anterior midline in the human forebrain.

Objective: The objective of the study was to investigate whether KS, CPHD, and SOD have shared genetic origins.

Design and Participants: A total of 103 patients with either CPHD (n = 35) or SOD (n = 68) were investigated for mutations in genes implicated in the etiology of KS (FGFR1, FGF8, PROKR2, PROK2, and KAL1). Consequences of identified FGFR1, FGF8, and PROKR2 mutations were investigated in vitro.

Results: Three patients with SOD had heterozygous mutations in FGFR1; these were either shown to alter receptor signaling (p.S450F, p.P483S) or predicted to affect splicing (c.336C>T, p.T112T). One patient had a synonymous change in FGF8 (c.216G>A, p.T72T) that was shown to affect splicing and ligand signaling activity. Four patients with CPHD/SOD were found to harbor heterozygous rare loss-of-function variants in PROKR2 (p.R85G, p.R85H, p.R268C).

Conclusions: Mutations in FGFR1/FGF8/PROKR2 contributed to 7.8% of our patients with CPHD/SOD. These data suggest a significant genetic overlap between conditions affecting the development of anterior midline in the human forebrain. (J Clin Endocrinol Metab 97: E694–E699, 2012)
In the vertebrate embryo, the preplacodal field arises at the edge of the neural plate adjacent to the neural crest, and its derivatives give rise to neuronal and nonneuronal head structures (1). Cells within the preplacodal field separate into individual placodes, of which the most anterior are the adenohypophyseal, lens, and olfactory placodes (1). The adenohypophyseal placode gives rise to the intermediate and anterior pituitary lobes (2). The olfactory placode gives rise to different cell types, including vomeronasal neurons, support cells, mucous-producing cells, and GnRH neurons (1). These developmental processes are orchestrated by multiple transcription factors and signaling molecules (2).

Mutations in the transcription factors SOX2, SOX3, HESX1, and OTX2 have been implicated in septo-optic dysplasia (SOD), a disorder characterized by pituitary hormone deficiencies, optic nerve hypoplasia, and midline defects including agenesis of the septum pellucidum and/or corpus callosum (3). Furthermore, mutations in transcription factors PROP1, POU1F1, LHX3, and LHX4 underlie combined pituitary hormone deficiency (CPHD) (4). However, such mutations only account for a small percentage of all CPHD/SOD cases.

A different set of genes have been implicated in Kallmann syndrome [KS; defined as idiopathic hypogonadotropic hypogonadism (IHH) and anosmia/hyposmia]. These genes, KAL1, PROK2, PROKR2, FGFR1, and FGF8, play critical roles in the development of olfactory system and GnRH neuron ontogeny (5). KS manifests as absent or incomplete puberty, sexual immaturity, and infertility, and additional phenotypes include midline defects (5).

FGFR1 and FGF8 are expressed in Rathke’s pouch and in the ventral diencephalon, respectively (6), and murine transcriptome data have identified members of the fibroblast growth factor (FGF)-8 signaling network during pituitary development (7). We therefore hypothesized that mutations in genes underlying KS could also underlie CPHD and/or SOD.

**Subjects and Methods**

**Patients and control subjects**

A total of 103 patients with either sporadic CPHD (n = 35) or sporadic SOD (n = 68) were included. Patients were recruited at four medical centers in the United States and the United Kingdom. Patients with SOD exhibited optic nerve hypoplasia, agenesis of the corpus callosum, and/or septum pellucidum on radiologic examination with or without pituitary hormone deficiencies (4). CPHD was diagnosed as a deficiency of at least two pituitary hormones. Unaffected control subjects (n = 268) were also studied. The ethics committees of participating institutions approved the study, and written informed consent was obtained before participation from subjects/parents/guardians.

The description of DNA sequencing of KAL1, FGFR1, FGF8, PROK2, and PROKR2 and the assessment of the functional consequences of FGFR-1, FGF8, and prokineticin receptor 2 (PROKR2) mutations have been previously reported. Detailed descriptions of these methods as well as a description of Prokr2 promoter reporter gene expression in the mouse pituitary are provided in the Supplemental Data, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org.

**Results**

Among 103 patients with either CPHD (n = 35) or SOD (n = 68), four unrelated probands (3.9%) harbor rare sequence variants in FGFR1 or FGF8, and four (3.9%) have PROKR2 variants (Table 1). All mutations are heterozygous, and, in most cases, DNA from the parents was not available. The probands’ phenotypic data are detailed in Table 1 and case descriptions are provided in the Supplemental Data. Notably, a number of probands exhibited a reproductive phenotype consistent with hypogonadotropic hypogonadism based on their neonatal presentation because they are yet prepubertal.

**FGFR1 and FGF8 mutations in patients with SOD**

Three FGFR1 heterozygous mutations were identified in SOD probands (Table 1). The FGFR1 variant c.1349C>T, p.S450F maps to the intracellular domain of the receptor upstream of the tyrosine kinase domain and the amino acid (S450) is highly conserved across vertebrates (Supplemental Fig. 1A). The S450F mutant FGFR1 exhibits total protein and receptor cell surface expression levels (Fig. 1A and Supplemental Fig. 1B) similar to wild type (WT), yet downstream signaling is severely compromised (Fig. 1B). The FGFR1 variant c.1447C>T, p.P483S maps to a highly conserved residue in the tyrosine kinase domain. P483S also exhibits normal expression levels (Fig. 1A) but disrupted downstream signaling (Fig. 1B). Of note, the affected amino acid residue is also mutated in a patient with KS (c.1447C>A, p.P483T, Pitteloud, N., unpublished data). Subject 1 harbors a synonymous change (c.336C>T, p.T112T) mapping to the C-terminal end of immunoglobulin-like domain 1 (Supplemental Fig. 1A). This change was not observed in the 268 healthy controls, in the single-nucleotide polymorphism database or in the 1000 genomes data set. The Human Splicing Finder software (8) predicts that this variant generates a new exonic splicing enhancer binding site (TTACTTC) for the SRp40 splicing factor [score 79.46 (0–100)] and/or disrupts an overlapping putative exonic splicing enhancer octamer (CCTACTTC) (score 31.53).
A synonymous change in FGF8 (c.216G>A, p.T72T) was identified in a CPHD proband. This variant (Supplemental Table 1) is predicted by the Human Splicing Finder program (8) to compromise an exonic splicing enhancer site for the serine/arginine-rich splicing factor SF2/ASF (9). We generated a minigene expression construct, which includes the entire FGF8 gene (exons 1a to 3) except for 2.4 kb of intron 1d sequence (Supplemental Fig. 2), to measure relative expression levels of the four FGF8 isoforms in transfected cells using quantitative RT-PCR. Consistent with the software prediction, the e and f isoform transcripts (which incorporate an alternatively spliced exon, 1c) expressed from the mutant construct were significantly elevated compared with WT (Fig. 1C). We further assessed the biological significance of the minigene-induced alterations in gene expression and found that the mutant construct displayed significantly higher activity in a luciferase transcription reporter assay compared with WT (Fig. 1D).

Three different PROKR2 mutations were found in four patients with SOD or CPHD. One Caucasian and one African SOD proband both harbor the identical heterozygous PROKR2 variant (c.802C>T, p.R268C) (Table 1 and Supplemental Fig. 3A), previously reported in association with both normosmic IHH and KS (10, 11) and shown to be loss of function in vitro (11). The other PROKR2 variant (c.253C>G, p.R85G) found in a SOD proband affects an amino acid conserved across vertebrates (Supplemental Fig. 3A) and is predicted to be loss of function (Supplemental Table 1). Western analysis indicates reduced total protein expression suggesting a defect in protein folding and stability (Fig. 1E). Accordingly, cell surface expression is significantly reduced (Fig. 1F) accompanied by a severe decrease in signaling via both calcium (Fig. 1G) and MAPK (Supplemental Fig. 3B) (log

### Table 1. Phenotypes of SOD and CPHD probands found to harbor gene mutations in either FGFR1, FGF8, or PROKR2

<table>
<thead>
<tr>
<th>Patient</th>
<th>SOD ± hormone deficiencies</th>
<th>CPHD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Rare variant</td>
<td>FGFR1 T112T</td>
<td>FGFR1 S450F</td>
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<tr>
<td>Gender</td>
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<td>Male</td>
</tr>
<tr>
<td>Abnormal pituitary MRI</td>
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<td>No</td>
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<tr>
<td>Hormone deficiencies</td>
<td>GH</td>
<td>X</td>
</tr>
<tr>
<td>ACTH</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>LHFSH</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Midline defects</td>
<td>Corpus callosum Agenesis</td>
<td>Septum pellucidum Agenesis</td>
</tr>
<tr>
<td>Midline defects</td>
<td>Other</td>
<td>Other</td>
</tr>
<tr>
<td>Reproductive phenotypes</td>
<td>Cryptorchidism</td>
<td>Microphallus</td>
</tr>
<tr>
<td>Reproductive phenotypes</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Reproductive phenotypes</td>
<td>Seizures</td>
<td>ASD and VSD, brachydactyly, brachycephaly, preauricular skin tags</td>
</tr>
<tr>
<td>MRI, Magnetic resonance imaging; AVP, arginine vasopressin; ASD, atrial septal defect; VSD, ventricular septal defect.</td>
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MRI. Hypogonadotropic hypogonadism is based on neonatal diagnosis (phenotype: cryptorchidism/microphallus or low serum gonadotropins or LHRH stimulation test).
Discussion

We describe eight prepubertal patients with CPHD/SOD carrying a heterozygous mutation in FGFR1, FGF8, or PROKR2, associated with altered function. Thus, mutations in genes generally associated with IHH/KS may also be associated with CPHD/SOD, demonstrating a genetic overlap between these syndromes. Patients with KS often display midline defects such as cleft lip and/or palate and corpus callosum anomalies, and FGF8 mutations were recently found to be associated with recessive holoprosencephaly, craniofacial defects, and hypothalamic-pituitary dysfunction (6). Additionally, as early as in 1954, de Morsier described a syndrome of dysplasie olfacto-ge´nitale, which included agenesis of the olfactory bulbs, corpus callosum, and the anterior commissure as well as infantile genitalia (14). Although the hypogonadotropic hypogonadism observed in both CPHD and SOD is thought to be of pituitary origin, the verification of hypothalamic GnRH deficiency in these patients is difficult, if not impossible, because of the concomitant presence of a pituitary defect.
As with the FGFR1 mutations in IHH/KS patients, the mutations identified in SOD probands are also loss of function. In contrast, the FGFR8 mutation in the CPHD proband shows enhanced downstream receptor signaling. The rare synonymous change in FGFR8 leads to differential expression of FGFR8 isoforms and enhanced FGFR1 signaling in vitro. Thus far, gain-of-function FGFR1 mutations have been reported only in osteoglyphonic dysplasia and Pfeiffer syndrome. Of note, 40% of patients with Apert syndrome (caused by activating FGFR2 mutations) have partial or complete absence of the septum pellucidum and 23% have corpus callosum defects (15), phenotypes also observed in SOD. Thus, it remains unclear whether CPHD is caused by increased (and not decreased) FGF signaling or, more broadly, by any significant perturbation of FGF signaling.

There are some precedents of IHH/KS sharing the same genetic basis with another developmental disorder. Mutations in CHD7 occur in CHARGE syndrome (coloboma, heart defect, choanal atresia, retardation, genital hypoplasia, ear anomalies) and KS patients (16). Similarly, a frameshift mutation in SOX2 associated with anophthalmia/microphthalmia was also found in an IHH patient (17), suggesting that SOD and IHH share a genetic basis. Furthermore, deletion of Otx2, a locus for SOD, targeted to GnRH neurons results in hypogonadotropic hypogonadism in mice (18).

We also evaluated the PROKR2/PROKR2 pathway in patients with CPHD/SOD, identifying three loss-of-function mutations in PROKR2 in four unrelated CPHD/SOD probands. The PROKR2 R268C variant has been described in heterozygous state in IHH/KS patients, healthy first-degree relatives of KS probands, and in one of 250 healthy controls (10, 12). We therefore propose that these mutations do not cause major midline defects per se, but may act as modifier genes, or contribute to the phenotype through digenic inheritance, as previously demonstrated in IHH/KS (19, 20). Thus, further studies are needed to elucidate the role of PROKR2 signaling in pituitary and midline development. In conclusion, this report identified substantial genetic overlap between syndromes affecting the anterior midline and associated with the primitive placode.

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References


Address all correspondence and requests for reprints to: Nelly Pitteloud, M.D., Centre Hospitalier Universitaire Vaudois, Endocrinology, Diabetes, and Metabolism Service, BH 19-701, Rue du Bugon 46, 1011, Lausanne, Switzerland. E-mail: nelly.pitteloud@chuv.ch


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