Genetic Overlap between Holoprosencephaly and Kallmann Syndrome

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Key Words

Holoprosencephaly • Kallmann syndrome • Septo-optic dysplasia

Abstract

Patients with Kallmann syndrome (KS; congenital hypogonadotropic hypogonadism and decreased/absent sense of smell), septo-optic dysplasia (SOD), or holoprosencephaly (HPE) reportedly have midline defects. In this study, we investigate a genetic overlap between KS, SOD, and HPE. Nineteen subjects (18 males, 1 female) with KS and without mutations in the known KS genes were screened for mutations in *SOX2*, *SHH*, *SIX3*, *TGIF1*, *TDGF1*, *FOXH1*, *GLI2*, and *GLI3*. One male carried 2 heterozygous missense changes, one in *SIX3* (c.428G>A, p.G143D) and the other in *GLI2* (c.2509G>A, p.E837K). Both of these genes have been implicated in the etiology of HPE and neither of these changes were present in 200 control subjects. Other variants found among the subjects were known polymorphisms. KS and HPE may display a genetic overlap. The involvement of genes implicated in the etiology of midline defects in patients with KS warrants further studies.

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Kallmann syndrome (KS; MIM 147950), a combination of congenital hypogonadotropic hypogonadism (HH; MIM 146110) and decreased/absent sense of smell, has traditionally been thought to result from disturbed intrauterine migration of gonadotropin releasing hormone (GnRH) neurons from the olfactory placode to the hypothalamus [Schwanzel-Fukuda et al., 1989; Cariboni and Maggi, 2006]. Anatomically, KS can be defined by olfactory bulb aplasia and accumulation of GnRH neurons outside the brain, together with the premature termination of olfactory and terminal nerve fibers. It has been recently shown that deficient migration of GnRH neurons is also a feature in forebrain formation defects [Texeira et al., 2010]. Patients with KS usually lack puberty and associated phenotypic features include midline defects such as cleft lip/palate, renal agenesis, agenesis of the corpus callosum, and absent olfactory bulbs [Seminara et al., 1998]. The genes involved in the etiology of KS are *KAL1* [Franco et al., 1991; Legouis, 1991], *FGFR1* [Dode et al., 2003], *FGF8* [Falardeau et al., 2008], *PROK2* [Dode et al., 2006; Pitteloud et al., 2007a], *PROKR2* [Dode et al., 2006; Pitteloud et al., 2007a], *CHD7* [Kim et al., 2008] and *WDR11* [Kim et al., 2010]. Midline defects are also encountered in septo-optic dysplasia (SOD; MIM 182230) [Dattani, 1998] and holoprosencephaly (HPE;

MIM 236100), a complex brain malformation affecting both the forebrain and the face [Demyer and Zeman, 1963].

SOD is a highly heterogeneous condition with variable phenotypes including midline and forebrain abnormalities, and optic nerve and pituitary hypoplasia [Dattani, 1998]. Most instances of SOD are sporadic, and several etiologies, including alcohol abuse of the mother during pregnancy, have been suggested to account for the pathogenesis of the condition. However, an increasing number of familial cases have been described with mutations identified in transcription factor genes such as *SOX2*, *HESX1*, *SOX3*, and *OTX2*. These transcription factors are essential for normal forebrain development, and defects in these genes could account for the features observed in SOD and other midline disorders [Webb and Dattani, 2010; McCabe et al., 2011a]. In fact, genetic overlap between SOD and congenital HH has been suggested previously [Sato et al., 2007; Stark et al., 2011].

HPE results from incomplete cleavage of the prosencephalon that occurs between the 18th and the 28th day of gestation. Three ranges of increasing severity are described: lobar, semilobar, and alobar HPE [Demyer and Zeman, 1963; Cohen, 2006]. Children with HPE may have endocrine disorders such as diabetes insipidus, adrenal hypoplasia, hypogonadism, thyroid hypoplasia, and growth hormone deficiency. Midline defects observed in HPE include cyclopia, proboscis, median or bilateral cleft lip/palate in severe forms, ocular hypotelorism, or solitary median maxillary central incisor in minor forms. As in KS, many genes associated with the condition have been identified so far (including *SHH*, *SIX3*, *TGIF1*, *TDGF1*, *FOXH1*, and *GLI2*) [Dubourg et al., 2007].

In addition to midline defects, highly variable phenotype spectrum with incomplete penetrance and variable expressivity of the mutations are observed in all 3 syndromes (KS, SOD, and HPE) [Dode et al., 2003; Lazaro et al., 2006; Raivio et al., 2009; McCabe et al., 2011a]. Also, a molecular genetic diagnosis is attained only in approximately 30% of the KS and HPE patients [Dubourg et al., 2007; Bianco and Kaiser, 2009; Semple and Topaloglu, 2010] and in less than 1% of SOD cases [Webb and Dattani, 2010]. This suggests an existence of several other candidate genes or environmental factors underlying these syndromes. Recently, KS and HPE have been suggested as allelic syndromes, as at least mutations in *FGF8* appear to underlie both conditions [Arauz et al., 2010; McCabe et al., 2011b]. Accordingly, we have recently found mutations in *FGFR1* among patients with SOD or

combined pituitary hormone deficiencies [Raivio et al., 2012].

In the current work, we further investigated the genetic overlap between KS, HPE, and SOD by screening 19 KS subjects without mutations in the known KS genes for mutations in *SOX2*, *SHH*, *SIX3*, *TGIF1*, *TDGF1*, *FOXH1*, *GLI2*, and *GLI3*.

Subjects and Methods

Subjects

The clinical features of the 18 KS male subjects have been reported in Laitinen et al. [2011]. In short, all adult subjects were diagnosed based on (1) absent or incomplete pubertal development by the age of 18 years, (2) low-circulating basal sex steroid levels in association with inappropriately low or normal gonadotropin levels, and subnormal or normal response to GnRH stimulation test, (3) otherwise normal anterior pituitary function, (4) no organic cause for their condition, and (5) anosmia or hyposmia as assessed by 40-item smell testing (University of Pennsylvania Smell Identification Test, UPSIT, Sensonics Inc, Haddon Heights, N.J., USA) and/or absent or rudimentary olfactory bulbs visualized in MRI. The following MRI protocol was used to visualize the olfactory bulbs, sulci, and inner ears (corresponding sequences in 1.5 and 3 tesla units): axial 5 mm T2 FSE and FLAIR images of the whole brain, coronal T2 FSE with 3 mm slice thickness starting from the anterior surface of the frontal lobe, 3D MPR sagittal images (1 × 1 mm) covering the whole head with coronal reconstructions, and 3D T2-weighted thin slice axial images (CISS, DRIVE, voxel size 0.3–0.5 mm × 3) from the region of the inner ear. No contrast medium was used. In addition to adult patients, 3 12–18 year-old patients with unequivocal signs of severe congenital HH (history of cryptorchidism and/or micropenis), absent pubertal development, and anosmia/hyposmia were enrolled. One female KS patient with a history of spontaneous puberty followed by secondary amenorrhea, congenital anosmia, absent olfactory bulbs in the MRI, and low estradiol (E2) in the setting of normal gonadotropin levels (single measurements), thus representing mild GnRH deficiency [Shaw et al., 2011], was also included. Two male subjects had CHARGE syndrome-associated features (1: cleft lip and palate, unilateral microphthalmia and coloboma, bilateral hearing impairment, left facial nerve palsy, cup-shaped ears, upper body muscular atrophy, and hypoplastic semicircular canals; 2: cup-shaped ears and upper body muscular atrophy). None of the subjects have been found to carry mutations in the 7 genes known to underlie KS (*KAL1*, *FGFR1*, *FGF8*, *PROK2*, *PROKR2*, *CHD7*, and *WDR11*), and no intragenic deletions were found in *CHD7* or *FGFR1* multiplex ligation-dependent probe amplification assays (MLPAs, MRC-Holland, Amsterdam, The Netherlands). The Ethics Committee of the Helsinki University Central Hospital approved the study protocol, appropriate permissions were provided from each university hospital in Finland, and all subjects obtained and signed the written consent.

Mutation Screening

Genomic DNA from peripheral blood leukocytes was extracted, and the coding exons and exon-intron boundaries of 8 genes

[SOX2 (MIM 184429; RefSeq NM_003106.3, gi: 325651854) [Williamson et al., 2006], *SHH* (MIM 600725; RefSeq NM_000193.2, gi:21071042) [Belloni et al., 1996], *SIX3* (MIM 603714; RefSeq NM_005413.3, gi:261878476) [Wallis and Muenke, 1999], *TGIF1* (MIM 602630; RefSeq NM_170695.2, gi:28178842) [Gripp et al., 2000], *TDGF1* (MIM 187395; RefSeq NM_003212.3, gi:261878476) [de la Cruz et al., 2002], *FOXH1* (MIM 603621; RefSeq NM_003923.2, gi:208973255) [Roessler et al., 2008], *GLI2* (MIM 165230; RefSeq NM_005270.4, gi:190343019) [Roessler et al., 2003], and *GLI3* (MIM 165240; RefSeq NM_000168.5, gi:195947346) [Kang et al., 1997; Engelen et al., 2011] were PCR amplified and screened by direct sequencing. PCR products were purified with ExoSAP-IT treatment (Amersham Biosciences, Piscataway, N.J., USA), and sequenced using the ABI BigDye Terminator Cycle Sequencing Kit (v3.1) and ABI Prism 3730xl DNA Analyzer automated sequencer (Applied Biosystems, Foster City, Calif., USA). The sequences were aligned and read with Sequencher® 4.9 software (Gene Codes Corporation, Ann Arbor, Mich., USA). All primer sequences and PCR conditions are available upon request. Mutations were confirmed from a second PCR product. Mutation nomenclature is according to the guidelines of the Human Genome Variation Society [Wildeman et al., 2008] and was verified using the Mutalyzer software (<http://www.mutalyzer.nl/2.0/>).

Results

Mutation Screening

In 1 male patient, we found 2 heterozygous missense changes, one in *SIX3* (c.428G>A, p.G143D) and the other in *GLI2* (c.2509 G>A, p.E837K). This patient had a small testis volume (1.5 ml), absent sense of smell, no synkinesia, or detectable midline defects such as cleft lip or palate. The MRI revealed absent olfactory bulbs and partially hypoplastic olfactory sulci. These changes in *SIX3* and *GLI2* were not present in 200 control subjects from the same geographical region, but the *GLI2* E837K was reported in the dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP/>) with 1 entry from the 1000Genome project (rs193090538). According to MutPred (<http://mutpred.mutdb.org/>), a probability of a deleterious mutation is 0.5 for the *SIX3* variant and 0.284 for the *GLI2* variant [Li et al., 2009], but both of them were predicted to be possibly damaging by PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2/>) [Adzhubei et al., 2010]. Other variants found were known polymorphisms.

Discussion

KS, SOD, and HPE are genetically heterogeneous disorders with multiple genes involved identified so far. Yet, the majority of patients remain without molecular genet-

ic diagnosis. Although the phenotype spectrum of patients varies from severe to asymptomatic, midline defects are reported in most cases. We investigated the genetic overlap between KS, HPE, and SOD by screening 19 KS subjects without mutations in the known KS genes for mutations in *SOX2*, *SHH*, *SIX3*, *TGIF1*, *TDGF1*, *FOXH1*, *GLI2*, and *GLI3*.

We found 2 heterozygous missense changes, c.428G>A (p.G143D) in *SIX3* and c.2509G>A (p.E837K) in *GLI2*, not present in 200 controls, in 1 male KS patient. Although these rare variants were predicted possibly damaging by PolyPhen2, there is no evidence that they are disease causing. In addition, the *GLI2* E837K has also been reported in the dbSNP with 1 entry from the 1000Genome project. The patient had anosmia, absent olfactory bulbs and hypoplastic olfactory sulci bilaterally, severe HH with bilateral cryptorchidism and micropenis, but no other additional phenotypic features: he neither displayed midline anomalies (such as cleft lip and palate, high-arched palate, or dental agenesis), nor post axial polydactyly. A central incisor was not observed. The MRI of this patient did not reveal any signs consistent with HPE. Even truncating *GLI2* mutations have been described in families with variable pituitary hormone deficiencies, midline defects, and absence of signs of HPE [Franca et al., 2010]. Incomplete penetrance of the mutations and variable phenotype including subtle microform of HPE have also been reported in patients with *SIX3* defects [Solomon et al., 2010]. In addition, additive effects of the mutations and/or di/oligogenic inheritance have been shown in congenital HH [Pitteloud et al., 2007b; Sykiotis et al., 2010]. However, this has not been observed in HPE, and it is suggested that more subtle genetic and environmental interactions are more likely to contribute to the phenotype variability in HPE than mutations in more than 1 gene [Roessler et al., 2012]. Unfortunately, the patient's parents are deceased, so their DNA samples were not available for testing. The mother had had juvenile rheumatoid arthritis and delayed menarche at 16 years of age. The parents did not display any distinct syndromes, and a sibling (half-brother from the mother's side) was also devoid of midline defects. The pubertal development of the half-brother was normal. MRIs or other imaging studies of the relatives are not available.

Two of our KS patients have CHARGE syndrome-associated features, but no mutations in *CHD7*, shown to underlie both CHARGE and KS [Kim et al., 2008]. It has been recently shown that Chd7 and Sox2 interact physically and cooperate to regulate a set of common target genes mutated in several human syndromes [Engelen et

al., 2011]. In addition, very recently, a frameshift mutation in *SOX2* leading to anophthalmia/microphthalmia in siblings was also found in their mother who only manifested isolated HH [Stark et al., 2011]. To the best of our knowledge, this is the first study where *SOX2* has been analyzed in a series of patients with congenital HH. The fact that no mutations were found, however, suggests that *SOX2* is not a significant HH gene, at least when no family history of anophthalmia/microphthalmia is present. However, larger patient series, including patients with normosmic congenital HH, need to be investigated to further elucidate the role of *SOX2* in congenital HH.

Our conclusion is that mutations in *SOX2*, *SHH*, *SIX3*, *TGIF1*, *TDGF1*, *FOXH1*, *GLI2*, and *GLI3* are not a com-

mon cause for KS in Finland. However, 1 patient harbored a novel missense change in *SIX3* and another rare missense variant in *GLI2*, suggesting the possibility of a genetic overlap between KS and HPE. As our patient series is small, this finding needs to be confirmed by studies in different patient groups and populations.

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