Int J Clin Exp Pathol 2014;7(5):1842-1848
www.ijcep.com /ISSN:1936-2625/IJCEP1402051

Review Article

Clinical and genetic characteristics for the Urofacial Syndrome (UFS)

Yaqin Tu[1*], Ping Yang[1*], Jia Yang[2], Yuchen Xu[3], Fei Xiong[1], Qilin Yu[1], Weikuan Gu[4], Dinel Pond[5], Nancy Mendelsohn[5], Guus AMA Lachmeijer[6], Shu Zhang[1], Cong-Yi Wang[1]

[1]The Center for Biomedical Research, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Ave., Wuhan 430030, China; [2]Second Clinical College, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Ave., Wuhan 430030, China; [3]First Clinical College, Tongji Medical College, Huazhong University of Science and Technology, 1277 Jiefang Ave., Wuhan 430030, China; [4]Department of Orthopedic Surgery and BioMedical Engineering, Campbell-Clinic, University of Tennessee Health Science Center, Memphis, Tennessee, United States of America; [5]Genetics Clinic, Children's Hospitals & Clinics of Minnesota, MN 55404, USA; [6]VU University Medical Center, Amsterdam, Netherlands. [*]Equal contributors.

Received February 14, 2014; Accepted March 31, 2014; Epub April 15, 2014; Published May 1, 2014

Abstract: The Urofacial (Ochoa) Syndrome (UFS) is a rare autosomal recessive disorder and over 100 patients have been reported thus far. UFS is characterized by the abnormal facial expression and dysfunctional voiding. The patients show a peculiar distortion of the facial expression (grimacing as if in pain or sadness when they tried to smile or laugh) along with urinary tract infection, enuresis, vesicoureteral reflux and hydronephrosis without any underlying neurological lesion and previous urinary obstruction. Some patients are also noted with nocturnal lagophthalmos. Until 2010, *HPSE2*, the gene encodes Heparanse 2 on chromosome 10, was thought to be the only culprit gene for this syndrome. However, another criminal gene, LRIG2, which encodes leucine-rich repeats and immunoglobulin-like domains 2, was also come into the light in 2012. Studies for dissecting the biological functions of HPSE2 and LRIG2 in urinary abnormalities are ongoing. In this minireview, we will update the discovery of novel clinical manifestations relevant to this syndrome and discuss with focus for the impact of HPSE2 on voiding dysfunction.

Keywords: Urofacial Syndrome, dysfunctional voiding, HPSE2, LRIG2, facial expression

Introduction

In the early 1960s, Dr. Bernardo Ochoa followed a group of children manifested with urinary abnormalities (dysuria or incontinence, frequency, urgency, or enuresis) but without obvious neurological or obstructive lesions, in an isolated mountain area of Colombia, South America [1]. The patients usually had a history of recurrent urinary tract infections, frequent urination, dysuresia, enuresis, constipation, and vesicoureteral reflux. In addition, these children displayed a particular "inverted" facial expression when they tried to smile or laugh. Description of this disease was not included in the medical literature at that time, which attracted many researchers for conduction of studies to figure out how facial expression links to voiding dysfunction. In 1979, "Ochoa syndrome" was firstly proposed by Dr. Rafael Elejalde based on genetic analysis of three unrelated families [2], and the disease was next characterized with autosomal recessive inheritance [3]. Given that patients with similar clinical manifestations were noted in other areas, the disease was later officially named as Urofacial Syndrome (UFS). Ten years later, the causative gene was localized to chromosome 10 in a region of 10q23-q24 by homozygosity mapping [4]. The disease gene was finally characterized in 2010 as HPSE2, a gene encodes Heparanse 2 [5]. However, another culprit gene named LRIG2 was also identified two years later [6]. The functional relevance of the above defective genes in dysfunctional voiding is yet to be elucidated. This minireview intends to

update the clinical manifestations of this syndrome and discuss with focus for the impact of HPSE2 on voiding dysfunction.

Abnormal facial expression

Distorted facial expression is the major characteristics noted in UFS patients other than the dysfunctional voiding. Facial expression is not only an indication of a person's mental state, but also an indication of illness [7]. In 1946, Edith Potter initially described the relationship between congenital flat ear and renal hypoplasia [8]. Since then, many studies have noted a significant association between malformations of the face and renal anomalies. Wang reported that 29% of patients with ear anomalies also displayed renal anomalies [9], and facial structural defects were also noted in other congenital diseases such as CHARGE association [10], Townes-Brocks syndrome [11], and OAVS (Goldenhar syndrome) [12]. Nevertheless, the distorted facial expression in UFS patients is not caused by the facial structural defects; rather these patients are likely manifested as a dysfunctional expression.

It is worthy of note, facial expression is distinct from facial identity. Facial identity is reflected by the basic characteristics of face at rest, including the position and configuration of forehead, nose, chin, mouth and ears, as well as the visual image of the facial expressions. In contrast, facial expression refers to the slight face changes of emotions and feelings in communication with the outside world. The facial identity of patients with UFS is similar to normal individuals, whereas their facial expression is very different from others, in which they display a grimacing face when they smile as if expressing sadness or discomfort instead of joy and happiness seen in a normal individual. However, when they are sad or uncomfortable their facial expression looks normal. Therefore, the facial alterations in UFS patients constitute a real dysfunctional facial expression, probably caused by the defective coordination between facial muscles. Of importantly note, Cranial magnetic resonance imaging (MRI) analysis of UFS patients revealed negative for lesions in the brain, and MRI demonstrated a normal spine and conus medularis in the patients as well, which demonstrate a non-neurological origin for the disease pathogenesis [10, 13].

Urinary abnormalities

Dysfunctional voiding refers to an abnormality in either the storage or emptying phase of micturition. Repeated episodes of urinary tract infection, dysuria or incontinence, frequency, urgency, or enuresis relevant to dysfunctional voiding are the life-threatening components for the UFS patients. Due to high pressure in the bladder, the patients usually manifest vesicoureteral reflux along with hydronephrosis, and the patients would die from renal failure without appropriate treatment. Thickening and trabeculation of the bladder wall are also noted in UFS patients diagnosed by voiding cystourethrography [4]. Of important, urodynamic examination reveals a poor coordination of the sphincter and the detrusor as well as a high postvoid residual urine volume without precursor lesion of nerve or obstructive urinary tract abnormalities. In addition, almost two-thirds of UFS patients manifest constipation and 33% have encopresis [1]. Later-diagnosed patients generally exhibit more severe renal impairment, while some early-diagnosed patients can also develop a serious deterioration in renal function [14].

Detrusor overactivity is the most common voiding disorder in children [15], which is also known as overactive syndrome, urge syndrome, hyperactive bladder syndrome, persistent infantile bladder and detrusor hypertonia. Hinman syndrome represents a rare extreme form of dysfunctional voiding [16-19]. It is also known as non-neurogenic neurogenic bladder, occurs when there is a habitual and voluntary tightening of the external sphincter during an overactive detrusor contraction resulting in a learned failure to relax the external sphincter during voluntary voiding [20]. Based on the definitions provided by the International Children's Continence Society [21], dysfunctional voiding can be categorized into neuropathic or nonneuropathic voiding disorders. Neuropathic voiding is associated with neurologic conditions. The most common cause of neurogenic bladder dysfunction in children is neurospinal dysraphism, primarily an open back lesion, but an occult or closed dysraphic state is being diagnosed with more frequency as neonatal spinal ultrasound, and MRI are used with increasing regularity to visualize any lower midline spinal cutaneous or gluteal cleft malformation. Other

causes of neurogenic dysfunction involving the spine include spina bifida, transverse myelitis, sacral agenesis, tethered spinal cord associated with imperforate anus, cloacal malformations or spinal cord trauma. Central nervous system abnormalities include spastic diplegia (cerebral palsy) and learning disabilities such as attention deficit hyperactivity disorder or attention deficit disorder [22]. In contrast, non-neuropathic voiding encompasses functional problems as manifested by the absence of apparent neurological or obstructive abnormalities. Given that UFS patients manifested a severe lack of synergic action between urinary detrusor and sphincter during micturition with significant residual urine after voiding [13], but negative for lesions in the brain with manifestations of a normal spine and conus medularis, UFS is probably developed due to the defective neuronal signaling in the peripheral muscles, rather than caused by the defective development of the micturation center located in the rostral pons in the brainstem [10, 13].

Nocturnal lagophthalmos

More recently, Mermerkaya and colleagues reported 15 UFS patients from Turkey. Interestingly, they noted eye symptoms in 12 out of 15 UFS patients [23]. Those 12 UFS children with manifestation of lagophthalmos were either noted by their parents or by doctors during the course of medical treatments. Typical symptoms relevant to lagophthalmos include pain, dryness, foreign body sensation and tearing. However, among 12 patients with lagophthalmos, 6 was normal by ocular exam, while corneal staining and punctate keratopathy were characterized in the rest 6 patients [23].

Lagophthalmos is defined as a condition in which the eyelids do not close to cover the eye completely. Proper eyelid closure and a normal blink reflex are necessary for the maintenance of a stable tear film along with a healthy corneal surface. The facial frontalis muscle on both sides is innervated by the seventh cranial nerve, which raises the eyebrow [24]. On the other hand, the orbicularis oculi muscle is a sphincter muscle around the eye, and is in general responsible for narrowing the eye opening and closing the orbit of the eye [25]. This muscle plays an essential role in protecting and moistening the eye as well as in expressive displays. Unlike the facial frontalis muscle, the orbicularis oculi is innervated by both zygomatic and the seventh cranial nerve. Defective signaling for the facial nerve results in the inhibition of eyelid closure as well as the failure of the blink reflex and lacrimal pumping mechanism. Given that UFS patients manifest dyssynergia between urinary detrusor and sphincter, lagophthalmos is also probably caused by the defective coordination between the facial frontalis muscle and the orbicularis oculi muscle.

Defective genes for UFS

Initially, UFS was considered as a local or regional phenomenon, related perhaps to the consanguineous marriages in the rural areas of Colombia, South America. However, subsequent report of patients from Middle East confronted the initial assumption [7, 8]. Later on, patients with this syndrome have also been reported in different cities in Colombia [1], the United States [9], Spain [9, 10], France [9-11], South Arabia [7, 8] and Japan [26], as well as more recently Turkey [23]. We have also recruited patients from Germany, Brazil, the Netherlands, Ireland and Pakistan (unpublished data). The earliest genetic study of UFS was from seven patients in three unrelated families [2]. In 1987, more patients and their families were included in the study and the parents of affected children were found to be normal in all cases. Moreover, the corrected ratio (CR) obtained by the proband method clearly demonstrated an autosomal recessive inheritance for the disorder.

In collaboration with Dr. Bernardo Ochoa, we conducted our first genetic mapping by employing a combination of homozygosity-mapping and DNA-pooling strategies in 1997. We have interestingly noted that all patients were homozygous for the two closely linked markers (D10S198 and D10S1726) on chromosome 10. In sharp contrast, only 12% of the unaffected relatives were homozygous for these two particular markers, which allowed us to localize the UFS gene to chromosome 10q23-10q24 [27]. Our subsequent fine mappings using high density of polymorphic markers and patients collected from other countries further narrowed the disease gene to two overlapping BAC clones [5, 27]. Studies carried out by Chauve and colleagues confirmed our conclusion by analysis of a French family, the first European cases of UFS [28]. All of the above described studies

were in favor of the hypothesis of genetic homogeneity. In 2010, we and the UK research group reported the characterization of the defective gene as loss-of-function mutations in the HPSE2 gene [5, 29]. Different types of mutations with loss-of-function were characterized in HPSE2 of patients originated from Colombia, the United States, Spain, and France, as well as those patients with Irish and Pakistan heritage. Interestingly, a follow up study of a Pakistani UFS family with three affected siblings further demonstrated that a missense mutation (N543I) in HPSE2 also predisposes the children to the development of UFS [30], suggesting that functional alterations (expression levels or functional activity) in HPSE2 may also confer UFS susceptibility. Those data also support the notion that patients with UFS could be under diagnosed in the general population, particularly for those patients without typical distorted facial expression.

Genetic heterogeneity

Unexpectedly, HPSE2 mutation could not be detected in some of the UFS patients, suggesting the possibility of genetic heterogeneity. More recently, Stuart and colleagues conducted a whole-exon capture study followed by massively parallel sequencing, and by which they demonstrated that mutations in the LRIG2 gene could also cause UFS [6]. Specifically, they analyzed 14 families affected by classical UFS, and identified nine (64.3%) with mutations in HPSE2, three (21.4%) with mutations in LRIG2, and two (14.3%) with mutations in neither gene. For those three families with mutations in LRIG2, they characterized a homozygous single-base-pair deletion (c.1230delA) resulting in a frameshift in exon 10, a heterozygous frameshift mutation (c.2088delC) in exon 15 and a heterozygous insertion (c.1980_1981ins371) in exon 14, and a homozygous nonsense mutation (c.2125C>T) in exon 15, respectively [6]. All of these mutations are associated with loss-of-function for the LRIG2 gene. Follow up studies carried out by Mermerkaya and colleagues further confirmed genetic heterogeneity for some of the UFS patients as manifested by the mutations detected in LRIG2 [23]. The LRIG2 gene is localized on chromosome 1p13, and is immunodetected within the nerve fascicles located between muscle bundles and in smooth-muscle bundles themselves of bladder in the first trimester when detrusor muscle differentiates and innervation of autonomic nerves occurs [31], but how altered LRIG2 function leads to UFS is yet to be clarified.

HPSE2 in voiding disorders

The full-length HPSE2 gene encodes a 592aa protein containing a glycosyl hydrolase motif which is homologous to the heparanase 1 (HPSE1) protein [32], an endoglycosidase that can promote the remodeling of the extracellular matrix via cleaving heparan sulfate chains [33]. Therefore, it is presumed that HPSE2 may exert similar functions as that HPSE1 [34], but functional evidence supporting this assumption is lacking. Currently, the biological functions for HPSE2 and how alterations in HPSE2 function lead to UFS are completely unclear.

Previously, we demonstrated that the HPSE2 gene is highly expressed in the urinary detrusor and sphincter as well as facial muscle [5]. Daly and colleagues also reported HPSE2 expression both in the mature and nascent human brain and spinal cord [29]. However, Western blot analysis of mouse tissues revealed that HPSE2 is highly expressed in the urethral sphincter, anal sphincter, bladder and urinary tract, and high levels of HPSE2 are also detected in the uterus, blood vessels and facial muscle, while HPSE2 is almost undetectable in the colon, stomach, intestine, skeletal muscle, brain and spinal cord (unpublished data). These results support the notion that HPSE2 may regulate neuronal signaling specifically in the peripheral muscles such as facial muscles, urinary detrusor and sphincter. This assumption is further supported by the observations in some sporadic patients collected from Colombia, in which those patients experienced similar voiding disorders such as urinary tract infection, frequency, urgency and so on as UFS patients, but absent of abnormal facial expression, and this category of patients were characterized carrying a defective HPSE2 allele (UFS carriers) (unpublished data). Indeed, many parents of UFS patients or sibling carriers experienced dysfunctional voiding as that of UFS patients. These data further suggest that HPSE2 could contribute to UFS pathogenesis in a dose-dependent manner, in which complete loss of HPSE2 function would develop severe dysfunctional voiding along with distorted facial expres-

sion (UFS), while reduced HPSE2 function would predispose to increased susceptibility for developing voiding disorders.

Summary and perspectives

In summary, UFS is an autosomal recessive disorder that shares many clinical features as other non-neurogenic voiding disorders other than the distorted facial expression. Genetic studies demonstrated that HPSE2 and LRIG2 are the culprit genes responsible for the syndrome. Although the biological functions for HPSE2 and LRIG2 are yet to be elucidated, there is suggestive evidence that HPSE2 may contribute to the disease phenotype in a dose-dependent manner, in which complete loss of HPSE2 function is likely associated with both facial (distorted facial expression and/or nocturnal lagophthalmos) and urinary abnormalities, while reduced HPSE2 function could confer higher risk for developing voiding disorders. In line with this notion, a missense mutation associated with altered HPSE2 function rather than the classical loss-of-function mutations was characterized in UFS patients, and UFS patients with voiding disorders but absent of abnormal facial expression or with distorted facial expression but absent of uropathology were also noted [23]. Based on these observations, the disease frequency for UFS in the general population could be higher than what we estimated, and therefore, UFS could be underdiagnosed.

Current available data suggest that altered HPSE2 function may impact the synergic action between urinary detrusor and sphincter or facial muscles, which may be related to the dysregulation of neuronal signaling in these peripheral muscles rather than defects occurred in the brain or urinary system during development. Studies in adult animals with induction of HPSE2 deficiency would be necessary to demonstrate this assumption. Dysfunctional voiding in children encompasses a wide spectrum of clinical entities, accounting for approximately 40% visits in the office of the pediatric urologists. It is estimated that 5 to 10% of school-age children experience daytime wetting. This prevalence includes a range of urinary incontinence from a few times per week to multiple episodes daily. Given that UFS patients share voiding features as those patients with dysfunctional voiding in the general population, it could be an excellent model for dissecting the underlying pathoetiology of voiding disorders such as frequency, urgency, enuresis, dysuria or incontinence.

Acknowledgements

We are grateful to the affected subjects and their family members for providing us their DNA for genetic studies. This work was supported by the National Natural Science Foundation of China (81130014 and 81100538), the Chinese Ministry of Science & Technology (2012BAI39B05), and the European Foundation for the Study of Diabetes (EFSD)/Chinese Diabetes Society (CDS)/Lilly Program for Collaborative Diabetes Research between China and Europe.

Disclosure of conflict of interest

The authors declare no competing financial interests.

Address correspondence to: Dr. Cong-Yi Wang or Dr. Shu Zhang, The Center for Biomedical Research, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Ave., Wuhan 430030, China. Tel: 86-27-8366-3485; Fax: 86-27-8366-3486; E-mail: wangcy@tjh.tjmu.edu.cn (CYW); shuzhang1976@gmail.com (SZ)

References

[1] Ochoa B. Can a congenital dysfunctional bladder be diagnosed from a smile? The Ochoa syndrome updated. Pediatr Nephrol 2004; 19: 6-12.

[2] Elejalde BR. Genetic and diagnostic considerations in three families with abnormalities of facial expression and congenital urinary obstruction: "The Ochoa syndrome". Am J Med Genet 1979; 3: 97-108.

[3] Ochoa B and Gorlin RJ. Urofacial (ochoa) syndrome. Am J Med Genet 1987; 27: 661-667.

[4] Wang CY, Hawkins-Lee B, Ochoa B, Walker RD and She JX. Homozygosity and linkage-disequilibrium mapping of the urofacial (Ochoa) syndrome gene to a 1-cM interval on chromosome 10q23-q24. Am J Hum Genet 1997; 60: 1461-1467.

[5] Pang J, Zhang S, Yang P, Hawkins-Lee B, Zhong J, Zhang Y, Ochoa B, Agundez JA, Voelckel MA, Fisher RB, Gu W, Xiong WC, Mei L, She JX and Wang CY. Loss-of-function mutations in HPSE2 cause the autosomal recessive urofacial syndrome. Am J Hum Genet 2010; 86: 957-962.

[6] Stuart HM, Roberts NA, Burgu B, Daly SB, Urquhart JE, Bhaskar S, Dickerson JE, Mermer-

kaya M, Silay MS, Lewis MA, Olondriz MB, Gener B, Beetz C, Varga RE, Gulpinar O, Suer E, Soygur T, Ozcakar ZB, Yalcinkaya F, Kavaz A, Bulum B, Gucuk A, Yue WW, Erdogan F, Berry A, Hanley NA, McKenzie EA, Hilton EN, Woolf AS and Newman WG. LRIG2 mutations cause urofacial syndrome. Am J Hum Genet 2013; 92: 259-264.

[7] Teebi AS, Farag TI, el-Khalifa MY, Besisso MS and al-Ansari AG. Urofacial syndrome. Am J Med Genet 1989; 34: 608.

[8] Teebi AS and Hassoon MM. Urofacial syndrome associated with hydrocephalus due to aqueductal stenosis. Am J Med Genet 1991; 40: 199-200.

[9] Wang CY, Huang YQ, Shi JD, Marron MP, Ruan QG, Hawkins-Lee B, Ochoa B and She JX. Genetic homogeneity, high-resolution mapping, and mutation analysis of the urofacial (Ochoa) syndrome and exclusion of the glutamate oxaloacetate transaminase gene (GOT1) in the critical region as the disease gene. Am J Med Genet 1999; 84: 454-459.

[10] Garcia-Minaur S, Oliver F, Yanez JM, Soriano JR, Quinn F and Reardon W. Three new European cases of urofacial (Ochoa) syndrome. Clin Dysmorphol 2001; 10: 165-170.

[11] Chauve X, Missirian C, Malzac P, Girardot L, Guys JM, Louis C, Philip N and Voelckel MA. Genetic homogeneity of the urofacial (Ochoa) syndrome confirmed in a new French family. Am J Med Genet 2000; 95: 10-12.

[12] Skalova S, Rejtar I, Novak I and Juttnerova V. The urofacial (Ochoa) syndrome--first case in the central European population. Prague Med Rep 2006; 107: 125-129.

[13] Nicanor FA, Cook A and Pippi-Salle JL. Early diagnosis of the urofacial syndrome is essential to prevent irreversible renal failure. Int Braz J Urol 2005; 31: 477-481.

[14] Wang CY, Shi JD, Huang YQ, Cruz PE, Ochoa B, Hawkins-Lee B, Davoodi-Semiromi A and She JX. Construction of a physical and transcript map for a 1-Mb genomic region containing the urofacial (Ochoa) syndrome gene on 10q23-q24 and localization of the disease gene within two overlapping BAC clones (<360 kb). Genomics 1999; 60: 12-19.

[15] Franco I. Overactive bladder in children. Part 1: Pathophysiology. J Urol 2007; 178: 761-768; discussion 768.

[16] Paparel P, Mure PY, Garignon C and Mouriquand P. [Koff's urethral mobilization: report of 26 hypospadias presenting a distal division of the corpus spongiosum]. Prog Urol 2001; 11: 1327-1330.

[17] Sanchez OM, Childers NK, Fox L and Bradley E. Physicians' views on pediatric preventive dental care. Pediatr Dent 1997; 19: 377-383.

[18] Chaichanamongkol V, Ikeda M, Ishikura K, Hamasaki Y, Hataya H, Satoh H, Asanuma H, Shishido S and Honda M. An infantile case of Hinman syndrome with severe acute renal failure. Clin Exp Nephrol 2008; 12: 309-311.

[19] Hinman F Jr. Nonneurogenic neurogenic bladder (the Hinman syndrome)--15 years later. J Urol 1986; 136: 769-777.

[20] Feldman AS and Bauer SB. Diagnosis and management of dysfunctional voiding. Curr Opin Pediatr 2006; 18: 139-47.

[21] Norgaard JP, van Gool JD, Hjalmas K, Djurhuus JC and Hellstrom AL. Standardization and definitions in lower urinary tract dysfunction in children. International Children's Continence Society. Br J Urol 1998; 81 Suppl 3: 1-16.

[22] Bauer SB. Neurogenic bladder: etiology and assessment. Pediatr Nephrol 2008; 23: 541-551.

[23] Mermerkaya M, Suer E, Ozturk E, Gulpinar O, Gokce MI, Yalcindag FN, Soygur T and Burgu B. Nocturnal lagophthalmos in children with urofacial syndrome (Ochoa): A novel sign. Eur J Pediatr 2014; 173: 661-5.

[24] Kunz M, Lautenbacher S, LeBlanc N and Rainville P. Are both the sensory and the affective dimensions of pain encoded in the face? Pain 2012; 153: 350-358.

[25] Terzis JK and Karypidis D. Blink restoration in adult facial paralysis. Plast Reconstr Surg 2010; 126: 126-139.

[26] Masuno M. [Urofacial syndrome]. Ryoikibetsu Shokogun Shirizu 2001; 782-783.

[27] Wang CY, Davoodi-Semiromi A, Shi JD, Yang P, Huang YQ, Agundez JA, Moran JM, Ochoa B, Hawkins-Lee B and She JX. High resolution mapping and mutation analyses of candidate genes in the urofacial syndrome (UFS) critical region. Am J Med Genet A 2003; 119A: 9-14.

[28] Paul Y. Face of the child as an index of disease. Indian J Pediatr 1972; 39: 76-83.

[29] Daly SB, Urquhart JE, Hilton E, McKenzie EA, Kammerer RA, Lewis M, Kerr B, Stuart H, Donnai D, Long DA, Burgu B, Aydogdu O, Derbent M, Garcia-Minaur S, Reardon W, Gener B, Shalev S, Smith R, Woolf AS, Black GC and Newman WG. Mutations in HPSE2 cause urofacial syndrome. Am J Hum Genet 2010; 86: 963-969.

[30] Mahmood S, Beetz C, Tahir MM, Imran M, Mumtaz R, Bassmann I, Jahic A, Malik M, Nurnberg G, Hassan SA, Rana S, Nurnberg P and Hubner CA. First HPSE2 missense mutation in urofacial syndrome. Clin Genet 2012; 81: 88-92.

[31] Sanlaville D and Verloes A. CHARGE syndrome: an update. Eur J Hum Genet 2007; 15: 389-399.

[32] Powell CM and Michaelis RC. Townes-Brocks syndrome. J Med Genet 1999; 36: 89-93.

[33] Rollnick BR, Kaye CI, Nagatoshi K, Hauck W and Martin AO. Oculoauriculovertebral dyspla-

sia and variants: phenotypic characteristics of 294 patients. Am J Med Genet 1987; 26: 361-375.

[34] Parvizi J, Coburn KL, Shillcutt SD, Coffey CE, Lauterbach EC and Mendez MF. Neuroanatomy of pathological laughing and crying: a report of the American Neuropsychiatric Association Committee on Research. J Neuropsychiatry Clin Neurosci 2009; 21: 75-87.