Pitt-Hopkins Syndrome

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Key Words

Apnea • Constipation • Deletion of 18q • Epilepsy • Happy disposition • Hyperpnea • Myopia • Pitt-Hopkins syndrome • Stereotypic movements • *TCF4*

Abstract

Pitt-Hopkins syndrome (PTHS, MIM #610954) is characterized by severe intellectual disability, typical facial features and tendency to epilepsy, panting-and-holding breathing anomaly, stereotypic movements, constipation, and high myopia. Growth is normal or only mildly retarded, but half of the patients have postnatal microcephaly. Remarkably, congenital malformations are practically nonexistent. The cause of PTHS is de novo haploinsufficiency of the *TCF4* gene (MIM *602272) at 18q21.2. Altogether 78 PTHS patients with abnormalities of the *TCF4* gene have been published since 2007 when the etiology of PTHS was revealed. In addition, 27 patients with 18q deletion encompassing the *TCF4* gene but without given PTHS diagnosis have been published, and thus, the number of reported patients with a *TCF4* abnormality exceeds 100. The mutational spectrum includes large chromosomal deletions encompassing the whole *TCF4* gene, partial gene deletions, frameshift (including premature stop codon), nonsense, splice site, and missense mutations. So far, almost all patients have a private mutation and only 2 recurrent mutations are known. There is no evident genotype-phenotype correlation. No familial cases have been reported. Diagnosis of PTHS is based on the molecular confirmation of the characteristic clinical features. Recently,

a Pitt-Hopkins-like phenotype has been assigned to autosomal recessive mutations of the *CNTNAP2* gene at 7q33q36 and the *NRXN1* gene at 2p16.3. Copyright © 2011 S. Karger AG, Basel

History of the Syndrome

In 1978, pediatrician David Pitt and pediatric neurologist Ian Hopkins at the Royal Children's Hospital in Melbourne described 2 unrelated children with severe intellectual disability who while awake had spells of rapid overbreathing followed by holding of breath until cyanosis [Pitt and Hopkins, 1978]. Both patients had almost identical facial features with a wide mouth and palate, thick fleshy lips and a broad beaked nose with flared nostrils, and both also had clubbing of fingers and toes. The patients had been ascertained as a part of an etiological survey of 782 intellectually disabled individuals [Pitt and Roboz, 1965]. Over the next 28 years, only 4 sporadic patients [Singh 1993; Van Balkom et al., 1998; Peippo et al., 2006] and 1 pair of sibs [Orrico et al., 2001] supposed to have the same syndrome were published.

In 2007, 2 independent groups using molecular karyotyping identified a microdeletion at 18q21.2 in 2 Pitt-Hopkins syndrome (PTHS) patients and subsequently haploinsufficiency of the *TCF4* gene was found to cause the syndrome [Amiel et al., 2007; Zweier et al., 2007]. The original patients of Pitt and Hopkins [1978] and the one described by Singh [1993] were no longer available for

Table 1. Age distribution, clinical features and principle types of reported mutations among the *TCF4*-related Pitt-Hopkins syndrome patients published in the literature

Characteristic	Number of reported patients
Age distribution	
≤12 months	1
>1 year–<5 years	22
>5 years–<18 years	33
≥18 years	8
Frequent features	
Constipation (Hirschsprung)	36 (1)
Strabismus	35
Epilepsy	34
Myopia	30
Single palmar crease	18
Hypoplastic/small genitalia	13
Scoliosis	11
Supernumerary nipple	9
Rare features	
Pes planus/valgus	4
II–III toe syndactyly	4
Nystagmus	3
Gastroesophageal reflux	3
Hyperconvex nails	2
Sacral dimple	2
Prominent fetal pads	2
Anterior anus	1
Pyloric stenosis	1
Hypospadias	1
Absent clitoris	1
Dislocated hips	1
Locked knee	1
V finger clinodactyly	1
Behavior	
Stereotypic head/hand movements	54
Happy disposition	51
Sleeping disorders	12
Self-aggression	5
Anxiety	3
Growth	
Birth OFC < –2 SD	2 (of 22)
Postnatal OFC < –2 SD	31 (of 60)
Birth length < –2 SD	1 (of 26)
Postnatal height < –2 SD	14 (of 53)
Birth weight < –2 SD	6 (of 29)
Postnatal weight < –2 SD	4 (of 19)
Type of mutation	
Point mutation	54
Intragenic deletion (sizes from 63 to 101 kb)	6
Perigenic deletion (sizes from 0.5 to 11.9 Mb)	18
Disruption by translocation	1

Growth measures not below –2 SD (< –2 SD) are within normal limits.

molecular study, but one of the patients published by Peippo et al. [2006] had a microdeletion at 18q21.2, while the other had a missense mutation at the conserved helix-loop-helix region of the gene [Zweier et al., 2007]. The patient of Van Balkom et al. [1998] and the sib pair described by Orrico et al. [2001] were found to have an intact *TCF4* gene [Zweier et al., 2007]. Instead, compound heterozygosity of *CNTNAP2* mutations at 7q35q36 were later shown to cause the phenotype of the sibs described by Orrico et al. [2001] and this entity was named PTHSL1 [Zweier et al., 2009, this issue].

Interestingly, several patients with a cytogenetically visible deletion at 18q have been published in the literature since the 1960ies, and many of these must have included the *TCF4* gene. With hindsight, for example, patient 1-JL described already by Wilson et al. [1979] carrying a terminal deletion of 18q21.3 had the very typical facial features of PTHS.

Epidemiology

No population-based prevalence or incidence figures for PTHS are available at present. Until 2007, PTHS was rarely reported, but increasing numbers of patients have been published since molecular testing became available. At present, almost 80 patients with molecular genetic confirmation have been published mainly from Europe, the USA and Canada. PTHS is caused by new dominant mutations, and thus, it is expected to be equally prevalent all over the world.

PTHS is not extremely rare among patients with severe intellectual disability. During a study period of 17 months, Rosenfeld et al. [2009] screened 13,186 samples from intellectually disabled individuals by array-CGH, and they found 7 persons carrying a deletion which included the whole of or a part of the *TCF4* gene. From these results, the authors estimated the population frequency of PTHS caused by microdeletion to be 1/34,000–1/41,000 in Washington, USA. The true prevalence of PTHS is obviously higher, as many cases are caused by point mutations.

Clinical Description

PTHS is a dysmorphic syndrome characterized by severe and early onset developmental disability, characteristic facial features, epilepsy, and intermittent overbreathing while awake. Table 1 summarizes the main clinical

Fig. 1. Facial features of patients with *TCF4*-related Pitt-Hopkins syndrome. Patient 1 in **A** (6 months), **B** (18 months) and **C** (14 years). Patient 6 in **D** and **H** (29 years). Patient 2 in **E**, **F** (6 months) and **G** (11 years). Patient 3 in **I** (3 years), **J** (6 years) and **K** (8.75 years). Patient 4 in **L** and **M** (12.5 years). Note the deep-set eyes,

broad and beaked nasal bridge with down-turned, pointed nasal tip, and flaring nostrils; the wide mouth with widely spaced teeth, and Cupid-bowed and everted lower lip; the mildly cup-shaped, fleshy ears; as well as increased coarsening of the facial features with age (by courtesy of [Zweier et al., 2007]).

features and major types of *TCF4* gene mutations found in 77 molecularly confirmed patients aged 11 months to 29 years [Peippo et al., 2006; Amiel et al., 2007; Brockschmidt et al., 2007; Zweier et al., 2007, 2008, 2009; Andrieux et al., 2008; Giurgea et al., 2008; de Pontual et al., 2009; Rosenfeld et al., 2009; Kato et al., 2010; Taddeucci et al., 2010; Takano et al., 2010; Stavropoulos et al., 2010; Lehalle et al., 2011; Marangi et al., 2011].

Pregnancy and perinatal period have been uneventful in the majority of cases. Thickened nuchal translucency was detected in only 2 cases who later were shown to carry a 1.2-Mb and a 1.8-Mb deletion including *TCF4* [Amiel et al., 2007; Zweier et al., 2007]. Measures at birth and neonatal growth are usually within normal limits. Post-natal microcephaly develops in half of the patients, and, to our own experience, it always starts before 1.5 years

and often during the first 12 months. Only one patient with congenital microcephaly with OFC <3rd centile at birth has been published [Marangi et al., 2011]. Growth retardation is later seen in a quarter of the patients.

Major congenital malformations have not been described. Minor anomalies occasionally seen are accessory nipples, hypoplastic genitalia (small penis in boys) and undescended testes. One patient had absent clitoris [Rosenfeld et al., 2009]. Wide-spaced nipples and sacral dimple have been described in rare cases. Mild anomalies of the extremities have commonly been reported and include slender and small hands and feet, clubbing of fingers, single palmar crease, limited flexion of the P1-P2 thumb joint with absent flexion crease, clinodactyly, overlapping fingers and toes, and pes planus. Prominent fetal finger pads in several PTHS individuals are a recent

Fig. 2. The boy's Pitt-Hopkins syndrome is due to a splice-site mutation in the *TCF4* gene. In photo 1, he is half-a-year old, in photos 2–4 three years old, and in photo 5 five years old. Note the broad nose, flared and anteverted nostrils, protruding philtrum, large mouth, bow-shape upper lip, broad lips, broad ear helices, and the development of myopia by the age of 5.

observation and this might turn out to be a useful additional diagnostic sign [Lehalle et al., 2011].

A constant feature of PTHS is severe intellectual disability. Totally absent or severely impaired speech is a major feature. The maximum vocabulary reported is 2 words [Zweier et al., 2007; Takano et al., 2010]. Drooling is an almost constant feature, but eating problems are rare. Interaction with others is limited, and, in infancy, the PTHS children are often described as quiet and good babies who may sleep in excess. In childhood, they appear sociable

and happy but often withdrawn in their own world. However, in none of the reports are the children considered autistic.

Motor milestones are grossly delayed, and less than half of the children learn to walk independently. If achieved, walking is unsteady and ataxic and usually begins at the age of 5–7 years (range 2–14 years). Head control is also poor.

Stereotypic movements of the hands and sometimes also of the head from side to side are common, and these may be present in over 80% of the patients. The hand stereotypies have been described as repeated hand-to-mouth movements, hand clapping, flapping, wringing, swaying, or lateral movements, and they may be triggered by attempts to interact with the environment. Aggression, anxiety, disruptiveness and sleep disturbances are occasional.

Detailed analysis on facial features of PTHS patients with *TCF4* mutations have been given in 11 reports [Peippo et al., 2006; Amiel et al., 2007; Brockschmidt et al., 2007; Zweier et al., 2007, 2008; Giurgea et al., 2008; de Pontual et al., 2009; Rosenfeld et al., 2009; Taddeucci et al., 2010; Takano et al., 2010; Marangi et al., 2011]. Typical features (see figs. 1 and 2) include a broad and beaked nose with broad nasal bridge and flared nostrils, a wide mouth with bow-shaped and tented or protruding upper lip, a full lower lip with thick vermillion border, widely spaced teeth, and ears with thick helices. The patients also show bitemporal narrowing, deep-set eyes, thin midline eyebrows, and full cheeks. With age the patients develop coarse facies [Brockschmidt et al., 2007; Zweier et al., 2008] and protruding lower face or prognathism [Amiel et al., 2007; Giurgea et al., 2008; Zweier et al., 2008; Taddeucci et al., 2010]. An experienced dysmorphologist may recognize the distinctive facial gestalt already in infancy, but usually it becomes evident after 3 years of age. All features, however, may be subtle [Marangi et al., 2011]. The emerging characteristic behavioral phenotype with happy disposition and stereotypic movements of head and hands, as well as constipation, are often clues that aid with the diagnosis.

Epilepsy is a problem in more than 40% of PTHS patients. It usually starts before school age, but onset may be during the first year of life and even as late as a young adult. Two babies had infantile spasms starting at ages 4 and 6 months, respectively, but enteroviral meningitis may have been the causative factor in one of them [de Pontual et al., 2009]. Clinically both staring spells and tonic-clonic seizures have been described. Rosenfeld et al. [2009] found 7 of 8 patients in their series with a missense

mutation to have epilepsy, while other types of mutations were associated with epilepsy in only 16–18% of cases. This suggestion of genotype-phenotype correlation remains, however, to be confirmed, and epilepsy appears frequent also in patients with loss-of-function mutations [Marangi et al., 2011].

Detailed descriptions of EEG findings in PTHS are scanty. EEG may be normal [Andrieux et al., 2008; Takano et al., 2010] or change from being normal at an early age to significantly abnormal later on [Peippo et al., 2006]. Both focal and generalized abnormality in EEG recordings can be seen. Frontal pseudoperiodic delta waves originally described in 2 patients reported by Peippo et al. [2006] have also been observed in other series [Amiel et al., 2009; de Pontual et al., 2009]. This finding may be quite pronounced and sometimes mimics nonconvulsive status epilepticus. Focal spikes slow/slow-and-sharp-wave activity can be seen in central and occipital areas. The EEG discharges are not related to the patient's breathing abnormality [Peippo et al., 2006; Amiel et al., 2007; de Pontual et al., 2009; Taddeucci et al., 2010].

Brain imaging shows no major malformations. MRI findings have been reported from 67 patients and of these 24 (36%) were normal [Zweier et al., 2007, 2008; Giurgea et al., 2008; de Pontual et al., 2009; Rosenfeld et al., 2009; Kato et al., 2010; Taddeucci et al., 2010; Takano et al., 2010; Brockschmidt et al., 2011; Marangi et al., 2011]. Most common abnormal findings reported are hypoplastic/thin corpus callosum, often more pronounced in the rostral parts, and enlarged ventricles. Bulging caudate nuclei have been described in 4 individuals [Peippo et al., 2006; Zweier et al., 2007, 2009]. Additional findings reported include small hippocampi, frontal lobe hypoplasia, thin hindbrain, and delayed myelination. Follow-up of brain MRI findings are reported of one patient who at 1 year showed marked delay of myelination with ventriculomegaly, but no alterations were seen 8 year later [Brockschmidt et al., 2007, 2011].

One hallmark of the PTHS is the breathing abnormality. The characteristic symptoms are paroxysms of hyperventilation followed by breath-holding until cyanosis. These paroxysms appear only while awake. The age at onset is usually close to that of epilepsy and usually before school age (range 2–19 years). The breathing abnormality has been observed in 56% of the reported PTHS patients. In addition to the typical hyperventilation-apnea disturbance, also apneas without evident hyperventilation episodes have been described, and these have been observed also in small infants aged 1–2 months [Zweier et al., 2008; Marangi et al., 2011]. Three patients were reported to have

nocturnal irregular breathing [Giurgea et al., 2008]. The breathing anomaly was described to show 'extreme fluctuations' already in the original description [Pitt and Hopkins, 1978] and, indeed, it is variable. In some patients, it has been described transitory and lasting a few months independently from the age at onset [Marangi et al., 2011]. To our personal experience, however, the breathing anomaly may be present for several years, and in 2 patients with onset at 6 years, the daily occurring paroxysms have lasted for nearly 15 years although fading and becoming more subtle.

At present, the natural history, intraindividual variability and the cause of the breathing abnormality in PTHS is unknown. Only a few patients have been studied using EEG-videotelemetry, and the breathing anomaly shows no relation to epilepsy [Peippo et al., 2006; Amiel et al., 2007; de Pontual et al., 2009; Taddeucci et al., 2010].

Scoliosis is reported in more than one tenth of the individuals, but none of the published cases are known to have needed orthopedic measures to treat it. Only 2 individuals had gastroesophageal reflux [Giurgea et al., 2008] and one had pyloric stenosis [Zweier et al., 2008]. A frequent ailment is severe constipation, but only one patient had Hirschsprung disease [Peippo et al., 2006]. No hearing defects have been reported. Strabismus and myopia (even high myopia) is a frequent finding, but its complications have not been observed. One in-depth ophthalmological study of a PTHS patient is reported. The patient was highly myopic but had neither nystagmus nor strabismus. The ocular length was increased, but there was no fundus regeneration and the retinal layers were regular [Brockschmidt et al., 2011].

Diagnosis

The diagnosis of PTHS is based on the typical clinical presentation confirmed by molecular genetic methods. At present, no generally accepted diagnostic criteria have been published. The hallmarks of the syndrome are early onset severe developmental delay, typical facial gestalt and the characteristic phenotype. Lack of major congenital anomalies supports the diagnosis. Breathing abnormality may not be present or appears late.

In typical cases, the *TCF4* gene mutation analysis is performed using methods that are able to detect intra- and perigenic deletions. Whole gene deletions and large microdeletions spanning 18q21.3 are common, and they are best diagnosed using molecular karyotyping (array-CGH).

Differential Diagnosis

The main differential diagnostic syndromes are Angelman, Mowat-Wilson and Rett syndromes. Many PTHS patients have been ascertained through *TCF4* gene analysis in children with unsteady gait, lack of speech and happy disposition but who had normal results in Angelman syndrome 15q11.2 methylation test and UBE3A mutation analysis [Takano et al., 2010; Lehalle et al., 2011]. Takano et al. [2010] detected 2% PTHS patients among those who had a negative result in Angelman syndrome testing.

Mowat-Wilson syndrome resembles PTHS, and common features are severe mental retardation and some similarities in facial features. In Mowat-Wilson syndrome, however, Hirschsprung disease is seen in about 50% of the cases and cardiac and urogenital (hypospadias) malformations are common which may serve as differential diagnostic clues. Hand stereotypies and postnatal microcephaly may mimic Rett syndrome. All these syndromes have their distinguishing features and also molecular tests are available.

Recently, some patients with Pitt-Hopkins-like features were found to carry autosomal recessive mutations in the *CNTNAP2* gene at 7q35q36 or the *NRXN1* gene at 2p16.3 [Zweier et al., 2009; see Zweier et al., this issue]. These entities were subsequently named Pitt-Hopkins-Like Syndrome 1 (PTHSL1) and Pitt-Hopkins-Like Syndrome 2 (PTHSL2). Common features with PTHS are severe intellectual disability, lack of speech and breathing abnormality, but both in PTHSL1 and PTHSL2 the motor milestones are normal or only slightly delayed, and the children were described as 'hypermotoric' [Zweier et al., 2009]. Also the facial features are different: the nose in PTHSL1 being merely large and the upper lip lacking the curved shape. The only patient described with PTHSL2 walked at 2 years and, apart from a wide mouth, was said to have no dysmorphic facial features.

Other syndromes with severe intellectual disability, epilepsy, constipation, breathing abnormality, stereotypic movements, and/or dysmorphic features include Ondine syndrome, *MECP2* duplication syndrome and Alpha thalassemia/mental retardation syndrome. They each have their distinguishing features which will aid to direct diagnostic efforts.

Natural History and Management

The natural history of PTHS and morbidity in adult age remains to be investigated; the life expectancy is unknown. One of the 2 patients described by Pitt and Hop-

kins [1978] died of pneumonia at the age of 19 and one patient was diagnosed with Hodgkin lymphoma at the age of 29 years [Zweier et al., 2007]. The oldest living patient published in the literature is 29 years old [de Pontual et al., 2009].

Congenital malformations have not been described in PTHS. However, the customary thorough clinical survey of special babies is recommended. Postnatal growth retardation and microcephaly are common. Genital hypoplasia is frequent and a check-up for undescended testes is necessary. So far, very little information is known about the pubertal development in PTHS. The authors have observed initial stages of physical puberty in 1 teenage boy.

Epilepsy is common, and usually it seems to debut at about 5–10 years of age. The seizure descriptions are scanty, but at least grand mal and staring spells have been observed. Also information about the treatment used for epilepsy in PTHS is almost nonexistent. Carbamazepine was used to treat 1 patient and a combination of lamotrigine and clobazam for another; these medications controlled the seizures relatively well [Taddeucci et al., 2010; Takano et al., 2010]. The authors have personal experience with an adult PTHS patient carrying a missense mutation who has myoclonic jerks occurring after falling asleep. These usually occur twice a month, but during infections they may appear daily. The EEG findings resemble Lennox-like nonconvulsive status epilepticus, but grand mal seizures are not present. He receives sodium valproate and clobazam for the myoclonic jerks with satisfactory results. Another adolescent patient carrying a microdeletion suffers from short absence seizures, and topiramate has been used with satisfactory results.

The breathing abnormality starts at about the same age as epilepsy and may cause a differential diagnostic problem. Spells of hyperventilation appear only when awake and serve as a diagnostic clue. A video-EEG recording may be needed, especially in small children and in diagnosis of the apneas. It is reassuring to know that although the patients frequently become cyanotic during the apneas, breathing restarts spontaneously, and no associated serious adverse effects have been described. There are no reports on respiratory function examinations or measurements of the PaO_2 and PaCO_2 in PTHS patients. No treatment attempts for the breathing anomaly have been published. Also the stereotypic movements may pose a differential diagnostic problem (e.g. head-rolling may be misdiagnosed as epilepsy). Knowledge about this frequent symptom in PTHS may save the patient from unnecessary examinations.

Knowledge of the musculoskeletal findings in PTHS is still limited. Scoliosis in present patients is frequent and appropriate surveillance is recommended. Physiotherapy to prevent contractures is important as the patients' own ability to move independently is very limited. Myopia is frequent in PTHS patients and might be underdiagnosed. Since it causes an additional handicap, all PTHS patients should be referred to ophthalmological examination. In several patients, high grade myopia has gradually developed and long-term follow-up is recommended. Hearing problems have not been reported in PTHS. Auditory testing, however, is recommended as a part of the clinical examination of all severely developmentally disabled patients.

Constipation is typical of PTHS syndrome, but little is known about its severity or of the success of the methods used to treat it. Only one patient has Hirschsprung disease [Peippo et al., 2006] with permanent ileostoma. All recommended treatment for constipation should be available for PTHS patients.

Oral findings have not been closer examined in PTHS patients. Patients have a shallow and wide palate, wide-set teeth and by age they develop prognathia. This may cause problems of mastication. Profuse drooling has been reported, but no treatment options have been published. The authors have personal experience of a patient with massive drooling to the extent of disturbing soaking of clothing, abnormally slow and defective swallowing and recurrent aspiration pneumonias. Consulting a dentist and a phoniatrix is important in order to learn more about oral pathology in PTHS and to find better treatment.

PTHS individuals are described to have happy disposition and aggression; anxiety and disruptiveness are rare problems. No reports include information on use of psychotropic drugs. Sleeping disturbances and eating problems have not been reported. The authors have personal experience of one adult PTHS patient who takes melatonin occasionally to help to fall asleep.

Genetics

PTHS is caused by haploinsufficiency of the *TCF4* gene at 18q21.2 due to deletions, stop, splice-site and, less frequent, missense mutations which occur de novo. The mode of inheritance is autosomal dominant.

The human *TCF4* gene consists of 20 exons (exons 1 and 20 are noncoding), spans 360 kb and encodes several isoforms of the transcription factor-4 protein, a polypep-

tide with homology to other basic helix-loop-helix transcription factors [Corneliussen et al., 1991; Zweier et al., 2007]. It is widely expressed during embryo development particularly in brain and retina but also in peripheral organs and splanchnic mesenchyme. It is also expressed in adult brain, lymphocytes, fibroblasts, gut, muscle, and myenteric plexus [Pscherer et al., 1996; Amiel et al., 2007; Brockschmidt et al., 2007; de Pontual et al., 2009]. On expression of *Tcf4* in transgenic mice, see Brzózka et al. [2010]. So far the significance of *TCF4* has been examined in relation to ASCL1-PHOX-RET pathway, the expression of *MATH1* and the function of plasmacytoid dendritic cells.

TCF4 interacts with ASCL1-PHOX-RET pathway thoroughly discussed by Zweier et al. [2007] and Amiel et al. [2007]. *TCF4* forms a functional complex with ASCL1 which has been shown to be a rare cause of congenital central hypoventilation syndrome (MIM #209880) [de Pontual et al., 2003]. *RET* (MIM #164761) is the major gene for isolated Hirschsprung disease. Mutations affecting the ASCL1-PHOX developmental pathway have been shown to impair the development of neurons of the noradrenergic lineage, resulting in abnormal respiratory network in the brainstem and periphery [Guillemot et al., 1993; de Pontual et al., 2003]. The downstream targets of *TCF4* include *RNX* (MIM #604640). *Rnx*-deficient mice display an abnormal respiratory pattern, with alternating tachypnea and apnea [Shirasawa et al., 2000]. Thus malfunctioning *TCF4* would play a role in the breathing abnormality and also in the constipation and Hirschsprung disease in PTHS through defects of noradrenergic neuronal development.

TCF4 interacts also with *MATH1*. It is a proneural transcription factor essential in forming neural rhombic lip that gives rise to structures of the hindbrain and neural crest. Intellectual disability, breathing abnormalities and epilepsy could be due to aberrant migration of specific groups of cortical neurons and intestinal phenotype caused by partially defective differentiation of neural crest cells [Flora et al., 2007; de Pontual et al., 2009].

Knock-out of *Tcf4* in mice lead to embryonal lethality of unknown cause [Zhuang et al., 1996]. Both in men and mice it has been shown that an intact *TCF4* is required for a normal number of pro-B cells [Zhuang et al., 1996; Cisse et al., 2008]. Plasmacytoid dendritic cells are part of the innate immune system and have a special function in interferon type I response to viral nucleic acids. Cisse et al. [2008] showed that PTHS patients had reduced interferon alpha response although immune cell types were normal. However, PTHS patients have so far not been reported to

be inclined to infections or to have immunological problems. It is only known that one of the 2 original patients of Pitt and Hopkins died of pneumonia [Pitt and Hopkins, 1978] and 1 patient reported by Zweier et al. [2007] had Hodgkin lymphoma at the age of 29 years.

The microdeletion size has been reported in 18 patients and varies from 0.5 Mb [Brockschmidt et al., 2007] to 11.9 Mb [Giurgea et al., 2008]. No recurrent breakpoints are seen. Presently, PTHS can be diagnosed unequivocally in patients with intragenic deletions and mutations of *TCF4*, while the present experience of large deletions involving *TCF4* is insufficient to demarcate the diagnosis between PTHS and microdeletion 18q21 syndrome. For clinical purposes, refinement of diagnostic criteria will be necessary after more information has been acquired along with meticulous long-term follow-up of patients with different genotypes. One suggestion to set the inclusion criteria would be to provisionally use the clinical criteria proposed by Peippo et al. [2006].

Mutation Spectrum and Genotype-Phenotype Correlation

Altogether 78 PTHS patients with alterations in the *TCF4* gene have been published (summarized in Marangi et al. [2011]; in addition, 1 patient in Hasi et al. [2011]; and 2 patients in Lehalle et al. [2011]). Of these, 18 are chromosome deletions encompassing the whole *TCF4* gene, 6 partial gene deletions, 25 frameshift mutations including premature stop codons, 7 nonsense mutations, 5 splice site mutations, and 17 missense mutations.

In addition, 27 patients not designated as PTHS cases but with 18q deletions of various sizes and encompassing the *TCF4* gene have been published [Hasi et al., 2011]. Of these, 13 individuals had large terminal deletions (24–30 Mb), 8 had small interstitial deletions (5–16 Mb) and 6 had large interstitial deletions (24–27 Mb). Each patient had their unique deletion with different breakpoints. Obviously, some but not all of them have the PTHS facial phenotype. Thus, the present number of published individuals with abnormalities of the *TCF4* gene exceeds 100.

The reported *TCF4* abnormalities in PTHS patients vary from point mutations to intragenic and perigenic deletions (range from less than 100 kb to 12 Mb). The intragenic mutations either create a stop codon or cause malfunction by deranging the conserved loop-helix-loop receptor region of the protein [Rosenfeld et al., 2009]. A detailed analysis of the mutations with relation to the phenotype has been given by Rosenfeld et al. [2009] and

a recent mutation summary by Marangi et al. [2011]. Recurrent mutations have been observed in exon 18. The deletions are each unique and no clustering of breakpoints has been observed, which is in accordance with what is known of 18q deletions in general [Heard et al., 2009; Hasi et al., 2011].

The parental origin of the deleted chromosome is reported only in 2 individuals, who both harbor a paternal de novo deletion [Amiel et al., 2007; Brockschmidt et al., 2007].

At present, there is no evident phenotype-genotype correlation as regards the intragenic deletions or point mutations of the *TCF4* gene. As to the 18q deletion patients, hemizygosity for *TCF4* appears to confer a major impact with regard to motor and cognitive development: in one study, children with larger regions of hemizygosity, including many other genes, were not more developmentally delayed than children with hemizygosity for the *TCF4* gene alone. In contrast, patients with large deletions but haplosufficient for *TCF4* had milder symptoms and longer survival [Hasi et al., 2011].

TCF4 gene alterations have been associated also with milder phenotypes than PTHS. A de novo translocation disrupted its exon 4, and the patient had mild to moderate mental retardation without any of the other PTHS characteristics [Kalscheuer et al., 2008]. In addition, genome-wide association studies for schizophrenia risk factors have pinpointed all PTHS-, PTHSL1- and PTHSL2-causing genes to be linked to the disease [Blake et al., 2010]. In particular, allele C of *TCF4* has been of interest suggesting schizophrenia to be another *TCF4*-related phenotype. Carriership of allele C has shown to influence prepulse inhibition of the acoustic startle response and learning and memory functions in similar way as in patients [Lennertz et al., 2011; Quednow et al., 2011].

Genetic Counseling and Prenatal Diagnosis

PTHS in *TCF4*-related cases is almost invariably due to a new mutation and risk of recurrence is low. One case of inherited somatic mutation is known to have caused PTHS in the descendant. The patient's mother carried the same missense mutation in her blood leucocytes and urethral cells, but not in her buccal cells. She was treated for epilepsy and chronic depression but was otherwise without pathological findings [de Pontual et al., 2009]. Although rare, the possibility of gonadal mosaicism should be considered when discussing the need for prenatal diagnosis.

A chromosome translocation has involved *TCF4* only twice [Kalscheuer et al., 2008; Marangi et al., 2011]. In the first case, the patient had a de novo t(18;20)(q21.1;q11.2) which disrupted exon 4 of *TCF4*. This did not, however, cause PTHS, but instead a considerably milder clinical phenotype [Kalscheuer et al., 2008]. In another case, a de novo t(14;18)(q13.1;q22.2) disrupted *TCF4* and caused a full PTHS phenotype [Marangi et al., 2011]. No inherited translocations have been published. If a chromosome (micro)deletion is found, parental samples should be studied according to current general practice.

For a PTHS person, the recurrence risk for offspring would be 50%. Obviously due to severe intellectual dis-

ability, no PTHS individual is known to have reproduced; even the 2 patients with mosaic microdeletion including *TCF4* (80 and 50%, respectively, in peripheral blood) were severely disabled [Giurgea et al., 2008; Stavropoulos et al., 2010].

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