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RESEARCH LETTER

Two unrelated individuals carrying rare mosaic deletions in *TCF4* gene

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Pitt–Hopkins syndrome (PTHS; MIM #610954) is a rare neurodevelopmental disorder first described in 1978 (Pitt & Hopkins, 1978), with a distinctive phenotype including facial dysmorphias, global developmental delay, severe intellectual disability, and hyperventilation episodes (Marangi & Zollino, 2015; Sweatt, 2013; Whalen et al., 2012). PTHS has a relatively short history of clinical and genetic investigation: its rarity and similarity to other well recognized syndromes has hampered the efforts for a deeper understanding of the underlying pathological mechanisms. It is only in the last decade that PTHS has emerged as a clinically and genetically defined entity (Whalen et al., 2012; Zweier et al., 2007).

On the molecular level, PTHS is caused by mutations or variable size deletions involving the gene encoding basic helix–loop–helix transcription factor 4 (TCF4, OMIM 602272) located on 18q21 (Amiel et al., 2007; Brockschmidt et al., 2007; Hasi et al., 2011; Zweier et al., 2007). The involvement of *TCF4* was first demonstrated in 2006 (Peippo et al., 2006) and to this day more than 200 PTHS patients with *TCF4* point mutations or deletions have been reported (Marangi & Zollino, 2015) with the sizes of deletions ranging from 63 kb (Brockschmidt et al., 2007) to 13 Mb (Gustavsson, Kimber, Wahlstrom, & Anneren, 1999). Literature on mosaic *TCF4* deletions is limited because of their rarity and interpretation challenges (Giurgea et al., 2008; Rossi et al., 2012; Stavropoulos, MacGregor, & Yoon, 2010).

In this research letter we describe a unique mosaic *TCF4* deletion in a girl with phenotype highly suggestive of PTHS (Family 1). This new finding is discussed in the light of a previous publication by our group (Family 2), where the phenotypically normal father of a PTHS patient carries a mosaic *TCF4* deletion which is inherited in full by his affected son (Kousoulidou et al., 2013).

Family 1 consists of a female with PTHS phenotype and her non-affected parents. The patient was 13 months old at the time of assessment and was referred for array-comparative genomic hybridization (array-CGH). Clinical features included global developmental delay, happy predisposition, flapping hand movements, prominent forehead, deep-set eyes, thin eyebrows, up-slanting palpebral fissures, wide mouth, full lips, prominent nose, and cup-shaped ears. Microcephaly noted at birth at the fifth percentile, progressed to below second percentile by the time of referral. The patient also displayed fleshy hands and bilateral single palmar creases. At the age of 6 years the patient managed to walk unaided in an unstable manner, whereas cyanotic episodes with possible sleep apnea were reported.

Array-CGH analysis of the patient using Cytochip ISCA array (BlueGnome-version 1.0) revealed a mosaic deletion of 10.17 Mb in size on chromosomal region18q21.2q21.33 spanning from 50,150,502 bp to 60,317,102 bp (GRCh38/hg19) harbouring *TCF4* gene (Figure 1). Fluorescence in situ hybridization (FISH) analysis using locus specific

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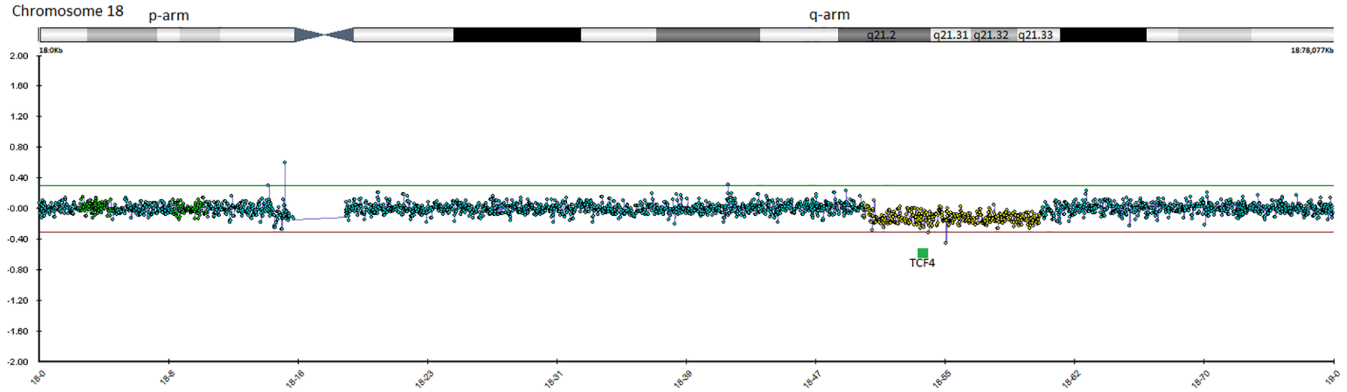

FIGURE 1 Array-CGH profile of Patient 1 shows a slight deviation of the deleted region within normal threshold, suggesting a mosaic deletion [Color figure can be viewed at wileyonlinelibrary.com]

probes confirmed this finding and determined the mosaicism level to be approximately 12%, with 6 abnormal out of 53 studied cells (Figure 2). FISH analysis of the parents was normal for all cells (n = 20), thus defining the patient's mosaic deletion as de novo.

Family 2 consists of a male with PTHS spectrum phenotype and his nonaffected parents who were referred to our laboratory for genetic testing. A detailed clinical description of the patient as well as the results of genetic investigation, are included in a previous publication by our group (Kousoulidou et al., 2013). Briefly, array-CGH of the affected child revealed a 263.4 kb deletion of chromosomal region 18q21.2 spanning from 51,095,520 bp to 51,358,929 bp (hg18) and removing exons 4–9 of *TCF4* gene. The exact same region exhibited a slight ratio shift towards lower values on the array-CGH profile of the patient's father, suggesting a possible mosaic deletion. Quantitative real-time PCR and FISH confirmed the deletion in the patient and estimated a ~20% mosaicism in the phenotypically normal father.

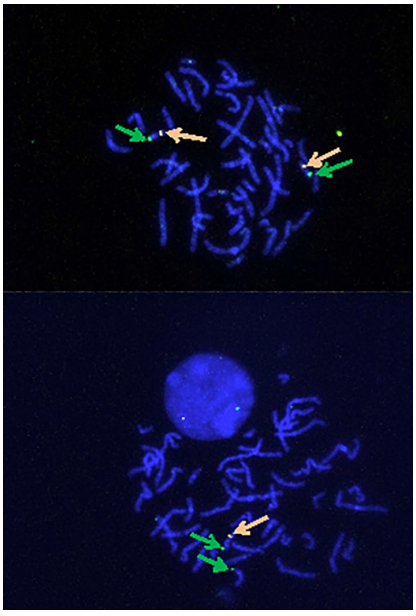

FIGURE 2 Metaphase FISH analysis of patient 1 using the locus specific probe RP11-7L24 located on 18q21.2 (red arrows) and the internal control probe RP11-288C17 located on 18p11.32 (green arrows). The mosaicism of *TCF4* deletion is confirmed by the presence of metaphases with normal copy number (above) along with metaphases carrying the *TCF4* deletion (below) [Color figure can be viewed at wileyonlinelibrary.com]

The rarity of mosaicism for *TCF4* deletions or mutations in PTHS patients adds a significant scientific and diagnostic value to any new findings of this type. A total of 11 individuals are currently found to carry various mosaic aberrations affecting *TCF4* and exhibit various phenotypes (de Pontual et al., 2009; Essaoui et al., 2013; Giurgea et al., 2008; Jehee et al., 2017; Rossi et al., 2012; Stavropoulos et al., 2010). A list of all patients with phenotypic data is presented on Table 1. The only mosaic *TCF4* aberrations with no clinical consequences are carried by the father of Family 2 (Kousoulidou et al., 2013) and the unaffected mother of twins with PTHS (Steinbusch et al., 2013).

Most PTHS occurrences so far are caused by de novo events; only in three cases the causative mutations/deletions were inherited from a mosaic parent, namely the father of Family 2, the unaffected mosaic female mentioned above (Steinbusch et al., 2013) and the mosaic female with depression presented on Table 1 (de Pontual et al., 2009). In these rare instances the detection of parental germline mosaicism is crucial for genetic counseling, as it defines the exact origin of the aberration and indicates a significantly increased recurrence risk requiring targeted prenatal diagnosis in future pregnancies. Novel genetic testing techniques, in combination with traditional cytogenetic approaches such as karyotype and FISH will enable accurate identification of mosaicism within the frame of routine genetic investigation. Mosaicism detection is vital not only for assessment of PTHS inheritance, but also for prediction of possible phenotypic outcomes in affected mosaic individuals or foetuses with prenatal ultrasound abnormalities, the latter being far more challenging for genetic counseling. The accumulation of clinical and genetic data on mosaic cases would enable deeper understanding of *TCF4*––PTHS correlation, which is currently unclear: because *TCF4* gene has important functions (Peippo & Ignatius, 2012), aberrations in the gene can affect several organs; consequently, some patients carrying *TCF4* abnormalities may not be classified as PTHS because of atypical phenotype, not resembling those described in original publications (Peippo et al., 2006; Pitt & Hopkins, 1978). Genotype–phenotype correlations are expected to create the background for predictions that are valuable to the families involved.

TABLE 1 Genetic and phenotypic description of currently known individuals with mosaic *TCF4* aberrations

Publication		Mosaic aberration	Mosaicism %	Tissue tested	Clinical features
Giurgea et al. (2008)		arr 18q21.1q22.1(45572497_57471912) × 1~2	~80	Peripheral blood	Severe intellectual disability, absent speech, happy disposition, stereotypic hand movements, microcephaly, myopia, and typical facial features
de Pontual et al. (2009)		c.1725C=/>G	Not specified, stated as "low level"	Leucocytes, urethral cells, and buccal swab	Depression and epilepsy
Stavropoulos et al. (2010)		arr 18q21.2q21.32(48287265_55792077) × 1~2	~50	Peripheral blood	Happy disposition, stereotypic hand movements, microcephaly, seizures, ataxia, myopia and typical facial features
Rossi et al. (2012)	Patient 1	arr 18q21.2q22.3(48257324_8243893) × 1~2	4–9, ~30 in buccal swab	Peripheral blood and buccal swab	Severe developmental delay, typical facial features, microcephaly, ocular anomalies, hand anomalies, overriding toes and brain anomalies
	Patient 2	arr 18q21.2q22.2(50121562-68400438) × 1~2	28–42, 77 in bone marrow	Peripheral blood and bone marrow	Severe developmental delay, typical facial features, microcephaly, hand anomalies and brain anomalies
Essaoui et al. (2013)	Twin A	arr 18qter(51431696-77982186) × 1~2	~25	Amniotic fluid and fetal blood	Apparently normal foetus and no ultrasound findings
	Identical twin B			Amniotic fluid and fetal blood	Prenatal ultrasound abnormalities: Intrauterine growth restriction, unilateral cleft lip and palate
Steinbusch et al. (2013)		c.1901_1909=/delinsA	8–16	Peripheral blood, urine, and saliva	Normal
Kousoulidou et al. (2013) (Family 2, father)		arr 18q21.2(51095520_51358929) × 1~2	~20	Peripheral blood	Normal
Jehee et al. (2017)		c.145=/+1G>A	~26	Peripheral blood	Convulsions and panic disorder
Current patient (Family 1)		ish mos del(18)(q21.2q21.2)(RP11-7L24-)[6/53].arr 18q21.2q21.33(50150502_60317102) × 1~2	~12	Peripheral blood	Developmental delay, happy disposition, stereotypic hand movements, microcephaly, and typical facial features

The degree of mosaicism in the patient from Family 1 is lower than in the nonaffected father from Family 2 (~12% vs. ~20%), although the clinical consequences are more severe, probably because of tissue specificity: the nonaffected father of Family 2 may have a lower rate of mosaicism in the brain than in peripheral blood; in contrast it could be assumed that the patient from Family 1 exhibits a higher percentage of abnormal cells in the brain, causing PTHS phenotype. Brain tissue mosaicism can indirectly be estimated by buccal swab analysis, where gene expression correlates with brain tissue (de Hoon, Monkhorst, Riegman, Laven, & Gribnau, 2015; Smith et al., 2015); however neither Family 1 nor Family 2 were available for further testing therefore a buccal sample could not be obtained.

The size of deletions differs significantly between the two investigated families −10 Mb in Family 1 versus 263.4 kb in Family 2. The phenotype of the patient from Family 1 was consistent with PTHS, given the wide variability found in different patients (Marangi & Zollino, 2015; Whalen et al., 2012). Despite the small size of the deletion in Family 2, it removes some of the critical exons (Kalscheuer et al., 2008) and affects all 47 transcripts of *TCF4* (Sepp, Kannike, Eesmaa, Urb, & Timmusk, 2011), hence the affected child carries the key clinical features of PTHS. When comparing the affected individuals from the two families, it is difficult to determine whether or not the size of deletion is an important factor in the phenotype severity, since one of the patients carries a larger deletion but in a mosaic state. Some studies suggest that larger deletions including contiguous genes add to the phenotype severity (Kato, Morimoto, Kimura, Matsushima, & Kondo, 2010; Marangi & Zollino, 2015), whereas other studies show no significant phenotypic differences between various size deletions and even point mutations, confirming TCF4 haploinsufficiency as the main pathogenic mechanism of PTHS (Giurgea et al., 2008). Currently the phenotypic variation among PTHS patients is not fully understood and not always correlates with the size of deletions detected in different patients. For this reason, epigenetic modifications, variable expressivity and genetic background are among the factors that should be taken into account.

In our research letter we have once again highlighted that accurate diagnosis can only be achieved by combining clinical evaluation with detailed genetic profiling, especially for syndromes with phenotypic and genetic variability such as PTHS. Current and future developments in genetic testing will lead to more PTHS patients being diagnosed, thereby increasing the variability of PTHS phenotype, further defining the boundaries of PTHS spectrum. In addition, we have demonstrated the wide range of possible phenotypic outcomes in individuals carrying mosaic *TCF4* mutations––from severe PTHS to a completely normal phenotype. For these rare and challenging cases, tissue specificity would be an exciting new focus for further studies. This information is vital for a more accurate diagnosis, prognosis and management. Clinical and molecular characterization of carriers of *TCF4* mosaic deletions and/or mutations contributes to our understanding of the pathogenic mechanisms leading to PTHS.

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CONFLICT OF INTEREST

None.

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