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SHORT REPORT

The recurrent *TCF4* missense variant p.(Arg389Cys) causes a neurodevelopmental disorder overlapping with but not typical for Pitt-Hopkins syndrome

Bernt Popp^{1,2} | Thierry Bienvenu³  | Irina Giurgea⁴ | Julia Metreau⁵ |
 Cornelia Kraus⁶ | André Reis⁶ | Jan Fischer⁷ | María Palomares Bralo^{8,9,10} |
 Jair Tenorio-Castaño^{8,9,10}  | Pablo Lapunzina^{8,9,10}  | Berta Almoguera^{10,11} |
 Fermina Lopez-Grondona^{10,11} | Heinrich Sticht¹² | Christiane Zweier^{6,13} 

¹Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany

²Center of Functional Genomics, Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Berlin, Germany

³INSERM U1266, Institut de Psychiatrie et de Neurosciences de Paris, Université de Paris, Paris, France

⁴Département de Génétique Médicale, INSERM Childhood Genetic Diseases, AP-HP, Sorbonne Université, Hôpital Trousseau, Paris, France

⁵APHP, Service de Neurologie Pédiatrique, Hôpital Universitaire Bicetre, Le Kremlin-Bicetre, France

⁶Institute of Human Genetics, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

⁷Institute for Clinical Genetics, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Dresden, Germany

⁸INGEMM, Institute of Medical and Molecular Genetics, Hospital Universitario La Paz, IDIPAZ, Madrid, Spain

⁹ITHACA European Reference Network, Spain

¹⁰CIBERER, Centro de Investigación Biomédica en Red de Enfermedades Raras, ISCIII, Madrid, Spain

¹¹Department of Genetics and Genomics, Fundación Jiménez Díaz University Hospital, Madrid, Spain

¹²Institute of Biochemistry, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

¹³Department of Human Genetics, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

Correspondence

Christiane Zweier, Institute of Human Genetics, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany.
 Email: christiane.zweier@insel.ch

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Abstract

TCF4 haploinsufficiency by deletions, truncating variants or loss-of-function missense variants within the DNA-binding and protein interacting bHLH domain causes Pitt-Hopkins syndrome (PTHS). This neurodevelopmental disorder (NDD) is characterized by severe intellectual disability (ID), epilepsy, hyperbreathing and a typical facial gestalt. Only few aberrations of the N-terminus of *TCF4* were associated with milder or atypical phenotypes. By personal communication and searching databases we assembled six cases with the novel, recurrent, *de novo* missense variant c.1165C > T, p.(Arg389Cys) in *TCF4*. This variant was identified by diagnostic exome or panel sequencing and is located upstream of the bHLH domain. All six individuals presented with moderate to severe ID with language impairment. Microcephaly occurred in two individuals, epilepsy only in one, and no breathing anomalies or myopia were reported. Facial gestalt showed some aspects of PTHS but was rather non-specific in

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most individuals. Interestingly, the variant is located within the AD2 activation domain next to a highly conserved coactivator-recruitment motif and might alter interaction with coactivator proteins independently from the bHLH domain. Our findings of a recurrent missense variant outside the bHLH domain in six individuals with an ID phenotype overlapping with but not typical for PTHS delineate a novel genotype–phenotype correlation for *TCF4*-related NDDs.

KEYWORDS

intellectual disability, missense variant, neurodevelopmental disorder, Pitt-Hopkins syndrome, PTHS, *TCF4*

1 | INTRODUCTION

Pitt-Hopkins syndrome (PTHS, MIM #610954) was described in two unrelated individuals in 1978.¹ In 2007, haploinsufficiency of *TCF4*, encoding a basic helix–loop–helix (bHLH) transcription factor binding to E-box consensus motifs, was identified as the underlying molecular cause.^{2,3}

PTHS is characterized by severe intellectual disability (ID), lack of or significantly impaired speech development, behavioral issues, seizures, constipation, early-onset myopia as well as a recognizable facial gestalt.⁴ The latter is characterized by a prominent nasal bridge, a beaked nose, prominence of the lower face and a wide mouth with a Cupid's bow shaped upper lip.^{4,5} Additionally, a narrow forehead, thin lateral eyebrows, flared nasal alae, full cheeks and thickened/overfolded helices were delineated as distinctive.⁶

The majority of PTHS related aberrations affect exons 9–19 of *TCF4* (NM_003199.2; NM_001083962.2; 20 exons) and comprise large deletions, intragenic deletions, truncating and few elongating variants. About 20% of causative variants are missense variants located in the highly conserved bHLH domain, encoded by exon 18 (overview in Zollino et al.⁶).

Of note, several (likely) pathogenic *TCF4* variants have been associated with milder or non-specific ID. While variants within the very N-terminal exons 1–4 were found in individuals with mild ID,⁷ in some instances even inherited within a family,^{8,9} loss-of-function variants in exons 7 and 8 seem to be associated with severe ID with or without typical features of PTHS.^{7,10}

We now report on six individuals harboring a recurrent missense variant (confirmed to be *de novo* in five individuals) upstream of the bHLH domain and presenting with rather non-specific moderate to severe ID without or with only few facial aspects of PTHS.

2 | METHODS AND RESULTS

By personal communication among colleagues, being contacted by one of the families, and by searching ClinVar,¹¹ Varsome,¹² and Decipher,¹³ we assembled six cases with the novel recurrent missense variant c.1165C > T, p.(Arg389Cys) in exon 15 (present in 46 isoforms in the human genome browser) of *TCF4* (NM_003199.3 or

NM_001083962). The variant occurred *de novo* in five individuals. In one case, the father was not available. Consent to publish clinical and molecular data with or without clinical photographs was obtained from the parents or legal representatives.

Genetic analysis was performed by either diagnostic trio exome sequencing in three cases or sequencing clinical exome or panels of 56 or nine ID genes, respectively, in the others (Table 1). The p.(Arg389Cys) variant is not reported in gnomAD.¹⁴ It occurs three times in ClinVar¹¹ with conflicting interpretation of pathogenicity (2× likely pathogenic, 1× with uncertain significance). One of the ClinVar cases is included in this study (SCV002011164.1). The variant affects a highly conserved amino acid and is predicted to be pathogenic by multiple *in silico* predictors (e.g., SIFT, Polyphen-2, CADD).

Clinical details of the six affected individuals are summarized in Table 1. All had moderate to severe ID with lack of or severely impaired speech in four of them. Two individuals could speak in (simple) sentences and had good comprehension. Birth measurements and growth were unremarkable apart from microcephaly in two and mild growth delay in three individuals, of which one had a positive family history for short stature. Muscular hypotonia and/or an unstable gait were reported in four individuals, respectively, and behavioral issues in two individuals. Epilepsy occurred in one individual with an onset at age 12 years. The facial gestalt of I6 resembled PTHS most with deep-set eyes, furrowing in the frontonasal angle, a beaked nose with downturned nasal tip and a wide mouth. The other individuals showed single or few facial aspects of PTHS such as the beaked nose in I4 or overfolded helices in several and overall a rather unspecific facial gestalt. None of the individuals has a protruding lower face or a Cupid's bow-shaped upper lip, both typical for PTHS (Table 1, Figure 1).

3 | DISCUSSION

The phenotype in the herewith reported six individuals with the recurrent c.1165C > T, p.(Arg389Cys) missense variant in *TCF4* includes moderate to severe ID, lack of or severely impaired speech, muscular hypotonia and minor facial dysmorphism. In none of these individuals, Pitt-Hopkins syndrome was suspected before genetic testing. While severe ID with severely limited speech, muscular hypotonia and unstable gait overlap with PTHS, other typical and/or frequent

TABLE 1 Clinical and molecular details of individuals with the TCF4 variant c.1165C > T, p.(Arg389Cys) in exon 15 and comparison to typical Pitt-Hopkins syndrome (PTHS) **PTHS n = 150⁶**

| | 1 | 2 | 3 | 4 | 5 | 6 | PTHS n = 150 ⁶ |
|-----------------------------------|--|--|---|--------------------------------------|---|-------------------------------|--|
| Test | Trio exome | 56 genes ID panel | Sequencing of 9 ID genes | Trio exome | Trio exome | Clinical exome (4900 genes) | |
| Variant de novo | Yes | Yes | Yes | Yes | Yes | Unknown | |
| Gender | Female | Female | Male | Male | Female | Female | |
| Age last investigation | 6 years 8 months | 9 years 7 months | 9 years 6 months | 5 years | 11 years 6 months | 32 years 11 months | |
| Family history | Unremarkable | Parental consanguinity, otherwise unremarkable | Unremarkable | Short stature in the mother | Unremarkable | Unremarkable | |
| Normal birth measurements | Yes | Yes | Yes | Yes | NA | Yes | Usually normal |
| Growth/height | 113 cm (P3-P10) | Normal | 150 cm (P90-P97) | P2 at age 5.5 years | 147.5 cm (P25-P50) | 156 cm (P3) | 25% slow postnatal growth ⁴ |
| Weight | 21.1 kg (P25-P50) | Normal | 33 kg (P50-P75) | P15 at age 5.5 years | 34.5 kg (P3-P25) | 45 kg | |
| OFC | 52 cm (P50-P75) | Normal | 53 cm (P25-P50) | P5 at age 5.5 years | 52.5 cm (P25) | 53 cm (P3) | 19% microcephaly (up to 60% ⁴) |
| Age of walking | 31 months | 26 months | 20 months | 24 months | 20 months | 26 months | 95% gross motor delay |
| Age of first words | Not with 8 years | NA | 5 years | Not with 5 years | 3 years 6 months | Single word at 4 years | |
| Speech abilities | One single word, better perception | No sentences at 8 years, limited perception | Sentences, articulation problems, good perception | Undirected monosyllabic vocalization | Simple sentences, relatively good understanding | Absent speech | 90% absent or very limited speech |
| ID | Severe | Moderate to severe (WPSI-III: QIV, QIP, QIT 42-60) | Moderate | Severe | Moderate-severe (CI 46) at age 6 years | Severe | 97% severe ID |
| Seizures | No | No | No | No | No | Yes, 12 years (carbamazepine) | 37% |
| MRI | Normal | Normal | 2 × normal | Dysplastic corpus callosum | Normal | Normal | 37.5% small corpus callosum, 29% wide ventricles |
| Muscular hypotonia | Yes | Yes, upper limbs and bucco-facial with hypersalivation | No | No | Yes, first 3 years | Yes, 3-4 years | 73% infantile axial hypotonia |
| Unstable gait | Yes | Yes | No | No | Until age 3 years | Yes | 69% gait ataxia |
| Breathing anomalies | No | No | No | No | No | No | 56% |
| Sleeping issues | No | No | No | No | No | No | |
| Behavioral issues | Little interaction, aggressive outbursts | Cheerful, attention deficit | No | Cheerful | No | No | 60% |
| Stereotypic hand movements | No | No | No | No | No | No | 59.5% |
| PTHS facial gestalt | No | No | No | No | No | partially | Yes |
| Narrow forehead | Mild | No | No | No | No | No | 83% |

(Continues)

TABLE 1 (Continued)

| | 1 | 2 | 3 | 4 | 5 | 6 | PTHS n = 150 ⁶ |
|---|--|--------------------------------|--|------------------------------|---|--|------------------------------|
| Thin lateral eyebrows | No | No | No | No | No | No | 76% |
| Wide nasal bridge/ridge/ tip | No | No | Yes | Yes | Mild | Yes | 91% |
| Flared nasal alae | No | No | No | No | No | Yes | 79% |
| Full cheeks/prominent midface | No | No | No | No | No | No | 85% |
| Wide mouth/full lips/cupid bow upper lip | No | No | Yes | No | No | Yes | 95% |
| Thickened or overfolded helix | Yes | No | No | Yes | Simplified, ear pit | No | 68% |
| Constipation | No | No | Mild | No | NR | No | 81% |
| Myopia | No | NA | No, Mild hyperopia | No, hyperopia, strabismus | No | No | 54% myopia (early- onset) |
| Hand anomalies (slender fingers and/or abnormal palmar creases) | Slender fingers | NA | No | No | No | Slender fingers | 76% |
| Zollino et al. [1] PTHS score | 4 | 3 | 1 | 2 | 2 | 8 | >6 in all |
| Other anomalies | No | NR | No | No | Triangular face, diastemas of teeth, low-set, simplified ears, gastroesophageal reflux | Ear lobe hypoplasia, multiple cavities, uterine myoma, early wrinkled skin | |
| Other exome results | No | No, only panel | No, only single genes | No | NR | No | |
| Other testing | Karyotyping, array and FraX normal | Karyotyping and FraX normal | Array normal, FraX normal, ALDH5A1, AHI1, TMEM67, CEP290, MED13, MED13L, NSUN2, TRAPPC9 sequencing normal, CFTR sequencing normal, metabolic screening normal | Karyotyping and array | karyotype, Fragile X, arrayCGH, metabolic work- up all normal | Karyotype and metabolic work-up, EEG, bone age. All normal | |

Notes: TCF4 (NM_003199.2): Shaded cells indicate aspects used for the clinical score as described by Zollino et al., ($\geq 3/7$ facial features: 4 points; severe ID with < 5 words; 2 points; breathing anomalies: 2 points; myopia, constipation, hand anomalies, unstable gait: 1 point each; score ≥ 9 clinical confirmation of PTHS; score ≥ 6 PTHS possible; score < 6 : insufficient evidence). Abbreviations: NA, not available or not applicable; NR, not reported; P, percentile

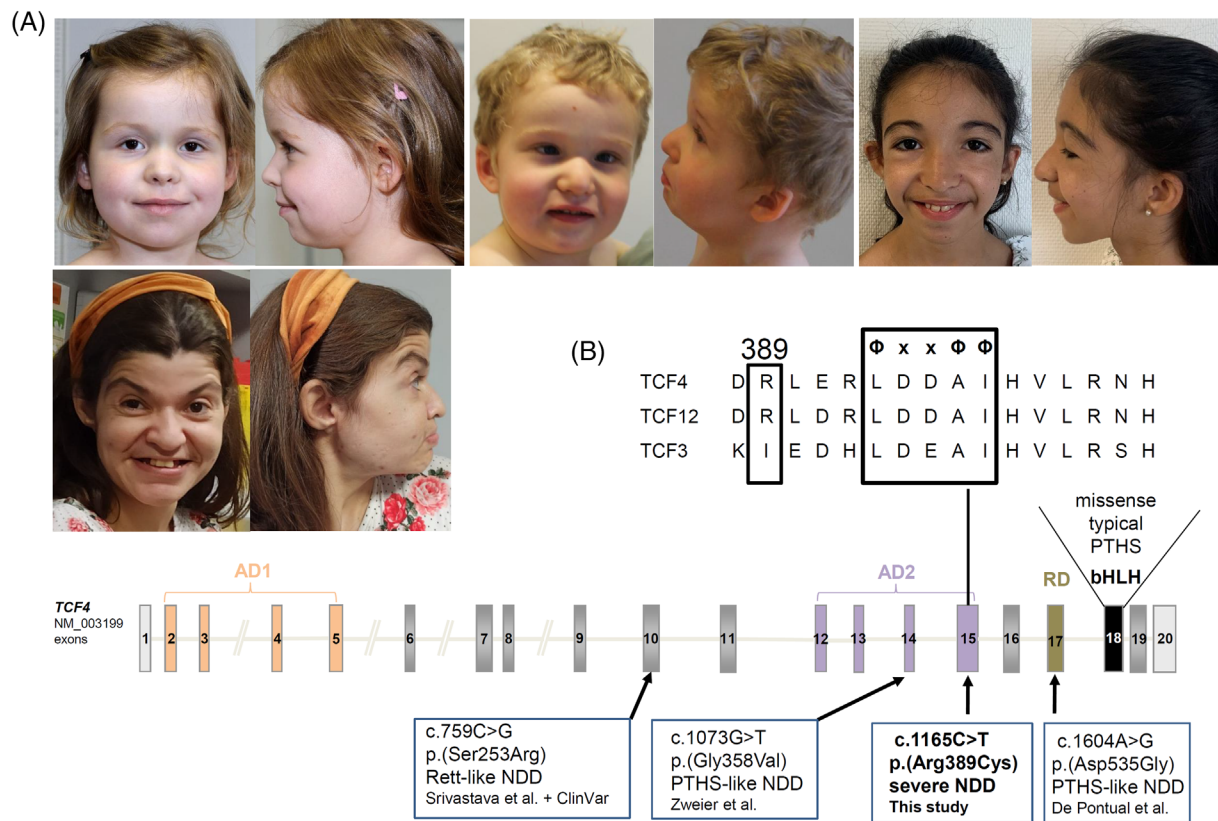


FIGURE 1 Missense variants in *TCF4*. (A) Facial gestalt of individuals 1, 4, 5, and 6, with 16 resembling Pitt-Hopkins syndrome most. Subtle aspects such as overfolded helices, bitemporal narrowing in individual 1, a mildly beaked nose in individuals 4 and 6, enophthalmia in individuals 1, 4, and 6 and hypertelorism in individual 5. (B) Schematic drawing of *TCF4* with encoded domains and location of published and the novel missense variants and location and sequence of the conserved Φ -x-x- Φ - Φ activator-recruitment motif within the AD2 domain. Non-coding exons are depicted in light gray, coding exons in dark gray, domain encoding exons in color or in black. AD1 and AD2, activation domain 1 or 2; RD, repression domain; bHLH, basic helix-loop-helix domain [Colour figure can be viewed at wileyonlinelibrary.com]

aspects of PTHS such as breathing anomalies, constipation and myopia were not present. Severity of ID and speech impairment appeared more variable compared to PTHS as two of the individuals could speak in sentences.

As reflected by three clinical scoring systems, the most distinctive clinical aspect of PTHS is the recognizable facial gestalt.^{6,15,16} Whereas one of the individuals clearly resembled PTHS, this was less or not apparent in the other individuals. While single or few dysmorphism such as overfolded helices and/or a beaked nose were noted in most of them and do overlap with PTHS, the overall facial gestalt would, in our opinion, not allow a “facial diagnosis” of PTHS. Utilizing the most recently published clinical scoring system,⁶ none of the six individuals would reach a score allowing clinical diagnosis of PTHS and only one would reach a score indicating possible PTHS (Table 1). Interestingly, the oldest individual in this cohort at the age of 32 years showed the largest resemblance to PTHS. However, for speculating on the commonalities and differences between natural histories of typical PTHS and p.(Arg389Cys)-associated NDD, a more prolonged observation and/or larger number of affected individuals would be required.

PTHS-causing missense variants are usually located within the bHLH domain of *TCF4*, encoded by exon 18. This domain is crucial to form homodimers or heterodimers with other bHLH or HLH transcription factors and to bind DNA. It has been demonstrated that missense variants in the bHLH domain result in decreased transcriptional activation,^{3,17,18} which is in accordance with a loss-of-function and haploinsufficiency mechanism comparable to deletions or truncating variants.

Only few missense variants outside the bHLH domain of *TCF4* have been reported. The *de novo* c.1073G > T, p.(Gly358Val) variant in exon 14 was identified in a 20-year-old individual within a PTHS cohort, presenting with severe ID, breathing anomalies, constipation, but without seizures and with a less typical facial gestalt.⁵ This variant did not result in significantly decreased activity in a transcriptional assay⁵ and also behaved similar to the wildtype in other assays.¹⁸ Another *de novo* missense variant, c.1604A > G, p.(Asp535Gly) in exon 17 was also identified within a PTHS cohort but clinical details were not provided.¹⁷ This variant was shown to have only minor functional effects *in vitro* but to change the ASCL1 and *TCF4* heterodimer versus *TCF4* homodimer binding

preference.¹⁸ The variant c.759C > G, p.(Ser253Arg) in exon 10 was reported in a male individual with Rett-like features but without clinical photographs.¹⁹ The same variant is contained three times in ClinVar as likely pathogenic and was shown to result in slightly reduced DNA binding and transactivation.²⁰ In summary, so far reported missense variants outside the bHLH domain are associated with severe NDDs not completely typical for PTHS and behave more like the wildtype and different from PTHS-related bHLH missense variants in *in vitro* assays.

Consequently, in at least three of the six individuals, the c.1165C > T, p.(Arg389Cys) variant was initially classified as of unknown significance due to its location outside the bHLH domain and to a rather unspecific neurodevelopmental phenotype not suggestive for PTHS. Only the recurrence of the identical variant in several individuals allowed its reclassification as pathogenic. This underlines the importance of careful interpretation and clinical follow-up of exome sequencing results.

Interestingly, the c.1165C > T, p.(Arg389Cys) variant is located in the AD2 activation domain next to a highly conserved Φ -x-x- Φ motif (amino acid residues 393–397), which consists of three hydrophobic amino acids (Φ) that are spaced by two variable amino acids (x). This motif is conserved between TCF4 and TCF12 and mediates binding to the KIX domain of the transcriptional coactivator CBP/p300.²¹ This variant might therefore impair interaction with coactivator proteins independently from the bHLH domain and in a less specific way as was demonstrated for the bHLH domain.²² Impaired heterodimer formation with HLH transcription factor ASCL1 and therefore impaired interaction with the ASCL1-Phox-Ret pathway was previously discussed to possibly underlie the autonomic dysregulation in PTHS in terms of breathing anomalies and constipation or Hirschsprung disease.^{3,17} Indeed, only recently, loss of Phox2b-expressing parafacial neurons have been reported in a *Tcf4* truncation (*Tcf4*^{tr/+}) mouse model which displayed hyperventilation and apnoea.²³ Leaving the bHLH domain function and this specific interaction intact might explain the lack of breathing anomalies and constipation in the six individuals. Other clinical aspects that might be specifically related to the bHLH domain might include craniofacial abnormalities, seizures, and myopia.

To conclude, our findings of a recurrent missense variant upstream of the bHLH domain in six individuals with rather non-specific, severe ID overlapping with but not typical for PTHS delineates a novel genotype–phenotype correlation for *TCF4*-related NDDs.

AUTHOR CONTRIBUTIONS

Bernt Popp, Thierry Bienvenu, Irina Giurgea, Julia Metreau, Cornelia Kraus, André Reis, Jan Fischer, María Palomares Bralo, Jair Tenorio Castano, Pablo Lapunzina, Berta Almoquera, Fermina Lopez-Grondona, and Christiane Zweier collected mutational and clinical data. Heinrich Sticht performed structural modeling. Bernt Popp and Christiane Zweier wrote the manuscript, which was read and revised by all co-authors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Thierry Bienvenu  <https://orcid.org/0000-0002-5953-2728>

Jair Tenorio-Castaño  <https://orcid.org/0000-0002-5308-2316>

Pablo Lapunzina  <https://orcid.org/0000-0002-6324-4825>

Christiane Zweier  <https://orcid.org/0000-0001-8002-2020>

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