Published in final edited form as:

Curr Genet Med Rep. 2017 March ; 5(1): 1–7. doi:10.1007/s40142-017-0110-0.

Molecular Mechanisms of Transcription Factor 4 in Pitt Hopkins Syndrome

Matthew D. Rannals¹ and Brady J. Maher^{2,3,4}

¹Department of Neurobiology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

²Lieber Institute for Brain Development, Johns Hopkins Medical Campus, Baltimore, MD, USA

³Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD, USA

⁴Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD, USA

Abstract

Purpose of Review—Pitt Hopkins syndrome (PTHS) is a rare neurodevelopmental disorder that results from mutations of the clinically pleiotropic Transcription Factor 4 (TCF4) gene. Mutations in the genomic locus of TCF4 on chromosome 18 have been linked to multiple disorders including 18q syndrome, schizophrenia, Fuch's corneal dystrophy, and sclerosing cholangitis. For PTHS, TCF4 mutation or deletion leads to the production of a dominant negative TCF4 protein and/or haploinsufficiency that results in abnormal brain development. The biology of TCF4 has been studied for several years in regards to its role in immune cell differentiation, although its role in neurodevelopment and the mechanisms resulting in the severe symptoms of PTHS are not well studied.

Recent Findings—Here, we summarize the current understanding of PTHS and recent findings that have begun to describe the biological implications of TCF4 deficiency during brain development and into adulthood. In particular, we focus on recent work that has looked at the role of TCF4 biology within the context of PTHS and highlight the potential for identification of therapeutic targets for PTHS.

Summary—PTHS research continues to uncover mutations in TCF4 that underlie the genetic cause of this rare disease, and emerging evidence for molecular mechanisms that TCF4 regulates in brain development and neuronal function is contributing to a more complete picture of how pathology arises from this genetic basis, with important implications for the potential of future clinical care.

Keywords

Pitt Hopkins Syndrome; Transcription Factor 4; TCF4; ITF2; SEF2; E2-2; Autism Spectrum

Corresponding Author: Matthew D. Rannals, mrannals@pitt.edu, Department of Neurobiology, University of Pittsburgh School of Medicine, E1440 BSTWR, 200 Lothrop Street, Pittsburgh, PA 15213-2536, (Fax) 412-648-1441.

Conflict of Interest

Matthew D. Rannals declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction

The rare developmental disorder, Pitt Hopkins Syndrome (PTHS), arises from haploinsufficiency of *TCF4* (*ITF2*, *SEF2*, *E2-2*) as a result of autosomal dominant mutations at this gene locus on chromosome 18 [1–5]. Unfortunately in addition to having multiple names in the literature, *TCF4* (GeneID:6925, OMIM:602272) shares its acronym with *T cell factor 4* that is an alternate name for transcription factor 7-like 2 (*TCF7L2*), a protein implicated in type-2 diabetes through its role in the Wnt signaling pathway and whose gene is located on chromosome 10 (10q25.3)[6]. This confusion has been thoughtfully addressed in many PTHS manuscripts and reviews, but should continue to be a point to recognize for clarity[7, 8].

Since the first description of Pitt-Hopkins syndrome as a condition of severe intellectual disability (ID) manifesting with breathing abnormalities and characteristic facial features[9], proposals have been suggested to standardize a set of clinical phenotypes of the condition in order to efficiently diagnose and identify patients with this rare disorder[5, 10]. Cognition and behavioral phenotypes of PTHS patients has been reported and found to strongly correlate to behavior criteria for autism spectrum disorders (ASD), including severe impairments in communication and language, difficulties in social engagement, fascinations with specific objects, and stereotypical repetitive motor behaviors[11]. Many patient studies confirm reports that PTHS children have happy dispositions[10, 11], though this characteristic is not completely universal to all patient cohorts[12]. This discrepancy may be explained by additional observations that happy dispositions are also described as contentment, thus not directly a result of social or environmental interactions and strengthening the argument that PTHS behavioral deficits fall into the spectrum of ASD behavioral and social deficits[11].

Due to phenotypes of PTHS, such as epilepsy, gastrointestinal complications, distinct facial characteristics, severe intellectual disability (ID), and language deficits that highly overlap with similar encephalopathies and autism spectrum disorders (ASD), there is an immediate need for patients and families to more readily achieve a differential diagnosis rather than cycle through clinical testing for other more common conditions, thus delaying important and accurate answers[10]. Patients are commonly only referred for possible PTHS screening after other tests find that they do not carry a *MECP2* mutation of Rett syndrome, a *UBE3A* mutation of Angelman syndrome, or a *ZFHX1B* mutation of Mowat-Wilson syndrome[5, 10, 12]. A complication for early diagnosis of PTHS is that normal growth parameters are often observed at birth, however, recent studies have suggested that stereotypical facial gestalt and signs of delayed mental development can allow for PTHS diagnosis within the first year of life [5, 12]. For instance, hypotonia which is a commonly identified trait among PTHS patients was noted to be observed in many of the patients during the neonatal period in one set of case reports[5]. The emerging description of a specific set of phenotypic traits associated with PTHS provides an opportunity to uncover the direct genetic and molecular mechanisms that drive these particular traits, hopefully illuminating potential early-intervention therapies for PTHS and related disorders.

The diagnosis of PTHS is currently based upon severe ID and behavioral and cognitive developmental delay accompanied by a typical facial gestalt that includes a large mouth with a tented upper lip, a broad and/or beaked nasal bridge, short philtrum, prominent Cupid's bow, deep-set eyes, squared forehead, and overfolded ear helix[3, 5, 13]. Breathing abnormalities and seizures have also been associated with individuals identified with a chromosome 18 deletion (18q-) that encompasses *TCF4*[14].

Identifying the resulting haploinsufficiency of *TCF4* due to heterozygous mutations in the gene has precipitated the growing literature identifying new patients and novel PTHS-linked mutations[1, 3]. Mutations have typically been reported in the bHLH domain where the possibility for disruption of normal E-protein binding function is high[1, 4, 12, 15–17]. Not all mutations result in complete loss of TCF4 protein function, and evidence for dominant-negative effects of mutant protein has been observed[4, 18]. Reported PTHS-linked mutations that are located outside of this bHLH region have not been shown to lead to functional deficiencies in TCF4 protein, and how they result in disease remains an open question [4].

Genotype-phenotype analysis is leading to other novel findings that may play a role in understanding the biological functions of *TCF4*. Missense mutations were identified as increasing the probability that an individual would develop seizures in one study that confirmed *TCF4* deletion in several patients that did not present with seizures[19]. This finding of a clear genotype-phenotype correlation has not been found in other patient populations with truncating mutations, deletions, or missense mutations[5]. The absence of a clear correlation suggests that a general *TCF4* haploinsufficiency is the broad basis to the central phenotypic traits of PTHS. However, variability in phenotype and symptom severity across patients seems to be a characteristic of PTHS, and as new patients are described along with their genetic mutation, linking the specifics of *TCF4* biology to pathophysiology will help address the lack of current therapeutic strategies.

Treatments in PTHS

Current clinical care for PTHS patients mainly comprises treatment aimed at alleviating severe symptoms that are understood and commonly treated outside of the context of this rare disorder. Improving GI dysfunction, treating seizures, and applying ASD therapies to support social and cognitive development are some of the current means for improving the quality of life for PTHS patients. A consensus for care and proven evidence-based interventions are still lacking though, and the limited amount of therapeutic options has been noted in previous PTHS reviews[8]. The small overall patient population inhibits an ability to draw firm conclusions from various case reports, especially with the biological mechanisms of the underlying disorder far from defined.

In the course of addressing epileptic seizures in a PTHS patient, it was observed that administration of valproate also improved the clinical presentations of breathing abnormalities[20]. The correction of breathing abnormalities is also an outcome of one of the other few reports of PTHS treatments[21]. Bouts of hyperventilation resulting in severe apnea were treated in two patients using acetazolamide to create a global tissue metabolic

acidosis that would stimulate breathing through the central chemoreceptor response[21]. The mechanism for this effect is still unclear; the rationale for treatment relies mostly on the previously successful use of acetazolamide to counteract sleep apneas and hyperventilation symptoms seen at altitude[22, 23]. However, novel treatments for the constellation of PTHS deficits and symptoms has been mostly elusive for patients of this rare disorder. In particular, therapies to address the cognitive deficits in this developmental disorder have seemed out of reach due to a lacking description of TCF4 regulation in brain development and function. We will describe recent work that addresses this gap in knowledge with the aim of contributing to a starting point for the conversation about targeted, biology-based therapeutics.

Understanding TCF4 Biology

TCF4 is a basic helix-loop-helix (bHLH) protein further characterized as an E-protein due to its binding of Ephrussi box (E-box) DNA motif (CANNTG) when homodimerized with itself or heterodimerized with other bHLH transcription factors (TF)[24]. There have been at least 18 reported splice variants of *TCF4*, differentiated by N-terminus variations that result from the alternative splicing of 5' exons[25]. The highly conserved bHLH domain motif allows for recognition of a variety of dimerization partners to create E-box binding[26, 27]. Alternative splicing that can alter the affinity for particular dimerization partners leads to diverse opportunities for transcriptional regulation under various physiological contexts[28, 29]. This context dependence has recently become relevant to understanding PTHS biology. The full-length human isoform, TCF4-B, has been described through *in vitro* investigations to activate transcription[12, 15]. However, this variant has also been reported to be a repressor of transcription in various studies[30–32], and knockdown of TCF4 was found to relieve repression of ion channel expression and thus regulate neuronal excitability[33].

The regulation of neuronal excitability by TCF4 has intriguing consequences when considered in the context of previously reported Ca^{2+} -dependence of E-protein mediated transcription[34–37]. Ca^{2+} and Ca^{2+} -binding proteins have been shown to influence dimer binding at the bHLH transcription factor domain[38–41]. Evidence that homodimerization binding rates are more sensitive to the inhibitory regulation of these Ca^{2+} -mediated mechanisms suggests that differential control of transcriptional targets occurs with activity depending on the E-protein binding partners involved[38]. Activity is a well-known regulator of neuronal development mediating aspects of cell migration and morphology[42–45]. The finely tuned process of neuronal development unfolds against a fluctuating background of Ca^{2+} signaling from multiple sources including voltage-gated Ca^{2+} -channels and NMDA receptors[46]. Activity-dependent developmental processes reflected in the functional morphology of the brain have been known since the description of ocular dominance column formation[47], and the interaction between Ca^{2+} -mediated mechanisms and activity is well known to influence developmental plasticity in the visual cortex[48]. Many mechanisms of cortical development have been well reviewed including the role of activity[49, 50], the regulation of calcium-dependent processes[51–53], and the role of bHLH transcription factors in corticogenesis [54]. Integrating new findings on TCF4 biology into these broader contexts will be essential to the understanding of the severe neurological deficits in PTHS. If the feedback from Ca^{2+} -mediated mechanisms during nascent activity in

the developing brain plays an important role in shaping TCF4 expression and activity, there may be important implications for circuit formation and its dysregulation under pathophysiological conditions.

Severe developmental outcomes resulting from insufficient *TCF4* were evident in animal models with the first reported descriptions of the *Tcf4* homozygous knockout mice that fail to survive through the first postnatal day[55]. Further studies have shown specific developmental deficits in the pontine nucleus within the hindbrain despite a lack of other gross developmental deficits in these *Tcf4* knockout mice[56]. It has been noted that this hindbrain developmental deficit may be particularly relevant to PTHS biology, as the pontine nucleus plays a role in the hindbrain control of breathing[56, 57]. Confirmation of a corresponding developmental insult in the PTHS patients has yet to be reported, so it remains unclear if patient breathing abnormalities arise from the central pattern generators involved in breathing control in the hindbrain. However, in addition to describing the developmental phenotype of the pontine nucleus, Flora et al. further determined that normal development of this brain region was dependent on specific heterodimerization of *Tcf4* with another bHLH transcription factor, *Atoh1* (*Math1*). The regulation of development for these *Atoh*-dependent regions has been shown to be critical for respiratory rhythmogenesis and oscillatory coordination of the control of breathing[58, 59]. This conclusion, that particular *Tcf4* heterodimers fill a distinct role in the tight control of genetic programs during the development of highly specific brain regions, highlights both a great success of understanding TCF4 biology and the great challenge of complexity when it comes to determining realistic therapeutic strategies for PTHS patients.

A mouse model for *Tcf4* haploinsufficiency has provided insight into the molecular mechanisms that result as a consequence of TCF4 deficiency during development[55]. Originally generated as a model of constitutive germline deletion of one *Tcf4* allele, our recent work has shown that a truncated TCF4 protein is in fact expressed in these mice[18]. Furthermore, we have shown this truncated protein is capable of binding to full length TCF4, confirming other previous reports that this transgenic PTHS mouse model may produce TCF4 haploinsufficiency through a dominant-negative binding mechanism[4, 18]. Although this model does not produce a complete allelic germline deletion, it still represents a powerful model for understanding PTHS biology, as the disruption of normal protein dimerization and DNA binding is a key element of the point mutations or other TCF4 gene disruptions that result in PTHS[4]. A description of behavioral phenotypes in the PTHS mouse was recently reported[60] and was shown to exhibit deficits in line with previously reported mouse models of autism including motor coordination deficits, repetitive behaviors and grooming, preference for isolation over social interaction, reduced vocalizations, and hyperactivity[61–63]. Additionally, the PTHS mouse displays gastrointestinal (GI) deficiencies in the upper GI and distal colon, providing further connection to common human PTHS patient phenotypes including GI disturbances like constipation[64].

We have recently reported on the role of TCF4 to regulate neuronal excitability in the medial prefrontal cortex of both the PTHS mouse and another in vivo model of PTHS that replicates TCF4 haploinsufficiency prior to neurogenesis[33]. Abnormal neuronal function in these rodent models may correspond to a possible mechanism of the cognitive deficits associated

with the corresponding prefrontal association cortex in humans with psychiatric disorders[65]. Our study identified multiple ion channels that are capable of being regulated by TCF4 and alter neuronal spiking under conditions of TCF4 dysfunction. One channel gene in particular, *Scn10a*, is interestingly ectopically overexpressed due to TCF4 haploinsufficiency[33]. Indeed, neuronal firing was normalized through both pharmacological and genetic rescue experiments aimed at blocking the function and expression of SCN10a. This peripherally expressed ion channel has not previously been reported to play a role in neuronal function within the CNS[66, 67], and thus presents a possible novel target for therapeutics aimed at correcting circuit function and cognitive deficits in PTHS.

The pattern of TCF4 expression through the lifespan that is conserved across species, peaking prior during late prenatal life, is clearly suggestive of an important role in development[33]. Although it declines from its developmental peak, TCF4 expression is maintained throughout life[33]. Recent experiments have shown that this expression past development has functional consequences for the establishment of synaptic connections in the drosophila neuromuscular junction[68]. In this preparation, TCF4 was shown to restrict neurite branching and synapse number[68]. Interestingly, TCF4 was found to repress Neurexin expression in these postmitotic neurons[68], establishing a link to the synaptically-associated gene that is linked to multiple psychiatric disorders and specific cases of a PTHS-like disorder in patients that do not appear to have a TCF4 mutation[69]. This work highlights the developing description of ASD-linked genes across multiple independently identified disorders. It also points to a challenge of developing strategies to treat PTHS symptoms, namely, that TCF4 may play an important function not only in neurodevelopment, but also in the maintenance of neuronal mechanisms that might contribute to cognitive deficits.

Recent work has also identified the dysregulation of transcription and DNA methylation in genes important to learning and memory in the PTHS mouse[60]. Both a non-specific HDAC inhibitor, the small molecule suberoylanilide hydroxamic acid (SAHA), and a subtype specific *Hdac2* knockdown by antisense oligonucleotides (ASOs) were able to normalize the expression of some learning and memory related genes that are dysregulated in this PTHS model[60]. Both HDAC inhibition treatments were also shown to improve deficiencies exhibited by the PTHS mice in certain learning and memory tasks[60]. This is a promising avenue for potentially reversing cognitive deficits in PTHS during a post-development therapeutic window.

TCF4 Biology in other psychiatric disorders

TCF4 is a clinically pleiotropic gene and its role in the pathology of other psychiatric disorders may be very relevant to understanding the biological implications of haploinsufficiency in PTHS. Genome wide association studies have identified common single nucleotide polymorphisms within TCF4 introns that are associated with a diagnosis of schizophrenia[70–72]. Furthermore some common variants are associated with schizophrenia-linked cognitive endophenotypes in both patients and control carriers[73–75]. However, the mechanisms for these associations are not known. The risk for psychiatric

disease and dysregulated cortical development seems to be a shared outcome whether TCF4 expression is disrupted, as in PTHS, or overexpressed. Duplication of TCF4 is known to result in developmental delays across neurodevelopmental diagnoses[76]. A partial duplication of TCF4 was found through analysis of copy number variations in DNA from the brain of major depressive disorder patients[77]. Possibly suggestive of common mechanisms of ASD pathophysiology, the upregulation of TCF4 expression has been shown in another disorder on the autism spectrum, 2q23.1 deletion syndrome[78]. Indeed, the complexity of phenotype outcome due to variable expression of TCF4 isoforms is emerging from reports describing a range of cases of TCF4-linked ID. An inherited familial translocation that results in both disrupted and overexpressed transcripts depending on the specific TCF4 isoform has been associated with mild ID[79]. Forms of developmental delays with ID that do not exhibit other characteristics of PTHS have been found to result when TCF4 mutations lead to reduced expression of particular isoforms[80, 81]. Exome sequencing has also identified de novo TCF4 mutations in children with severe non-syndromic forms of sporadic ID[82, 83]. The range of TCF4-associated disorders is representative of the myriad of potential interactions the protein encounters during regulation of expression at multiple target genes. While the range of described cellular and molecular phenotypes is diverse and growing for psychiatric disorders and ASD in particular, these insights are contributing hope that there are underlying pathways and common systems to be uncovered[84, 85].

Conclusion

The severe and systemic developmental phenotypes of PTHS present a formidable researchers and clinicians interested in developing effective therapeutics to enhance the quality of life for these patients. Evidence that the expression of TCF4 is maintained in the brain past an early peak of expression during development[33] suggests that treatment of PTHS cognitive deficits may be possible if TCF4 plays a significant role in the maintenance of neuronal function through adulthood. This is especially important for a rare disease like PTHS that is most commonly acquired through random spontaneous mutation, where identification of the genetic abnormality and developmental deficits occur well past birth.

Multiple recent studies have begun to highlight potential therapeutic routes that may provide hope for PTHS patients and their families. Regulators of neuronal excitability have been shown to play a direct role in neuronal dysfunction related to TCF4 haploinsufficiency, specifically an ectopically expressed ion channel SCN10a may be a promising target for normalizing neuronal function in PTHS[33]. Evidence that TCF4 functions postnatally in post-mitotic neurons to regulate synapse formation[68], and results that HDAC inhibition can improve cognitive behaviors of a PTHS mouse model[60] both also present an emerging picture that amelioration of the widespread developmental phenotypes of PTHS may be a future possibility.

Acknowledgements

This work was supported by NIH grant (R56MH104593) and Pitt-Hopkins Research Foundation Award to B.J.M.

Brady J. Maher reports grants from the NIH/NIMH during the conduct of study. He reports employment at the Lieber Institute for Brain Development and grants from NIMH outside of the submitted work.

References

1. Amiel J, Rio M, de Pontual L, et al. (2007) Mutations in TCF4, encoding a class I basic helix-loop-helix transcription factor, are responsible for Pitt-Hopkins syndrome, a severe epileptic encephalopathy associated with autonomic dysfunction. *Am J Hum Genet* 80:988–993. [PubMed: 17436254]
2. Brockschmidt A, Filippi A, Charbel Issa P, et al. (2011) Neurologic and ocular phenotype in Pitt-Hopkins syndrome and a zebrafish model. *Hum Genet* 130:645–655. [PubMed: 21544580]
3. Zweier C, Sticht H, Bijlsma EK, et al. (2008) Further delineation of Pitt-Hopkins syndrome: phenotypic and genotypic description of 16 novel patients. *J Med Genet* 45:738–744. [PubMed: 18728071]
4. Sepp M, Pruunsild P, Timmusk T (2012) Pitt-Hopkins syndrome-associated mutations in TCF4 lead to variable impairment of the transcription factor function ranging from hypomorphic to dominant-negative effects. *Hum Mol Genet* 21:2873–2888. [PubMed: 22460224] • Description of PTHS-related mutations in relation to resulting TCF4 function. Evidence of a range of mutation impact from subtle deficiencies to dominant-negative effects.
5. Whalen S, Héron D, Gaillon T, et al. (2012) Novel comprehensive diagnostic strategy in Pitt-Hopkins syndrome: clinical score and further delineation of the TCF4 mutational spectrum. *Hum Mutat* 33:64–72. [PubMed: 22045651]
6. Grant SFA, Thorleifsson G, Reynisdottir I, et al. (2006) Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 38:320–323. [PubMed: 16415884]
7. Forrest MP, Hill MJ, Quantock AJ, et al. (2014) The emerging roles of TCF4 in disease and development. *Trends Mol Med* 20:322–331. [PubMed: 24594265]
8. Sweatt JD (2013) Pitt-Hopkins Syndrome: intellectual disability due to loss of TCF4-regulated gene transcription. *Exp Mol Med* 45:e21. [PubMed: 23640545]
9. Pitt D, Hopkins I (1978) A syndrome of mental retardation, wide mouth and intermittent overbreathing. *Aust Paediatr J* 14:182–184. [PubMed: 728011]
10. Marangi G, Ricciardi S, Orteschi D, et al. (2012) Proposal of a clinical score for the molecular test for Pitt-Hopkins syndrome. *Am J Med Genet A* 158A:1604–1611. [PubMed: 22678594]
11. Van Balkom IDC, Vuijk PJ, Franssens M, et al. (2012) Development, cognition, and behaviour in Pitt-Hopkins syndrome. *Dev Med Child Neurol* 54:925–931. [PubMed: 22712893]
12. de Pontual L, Mathieu Y, Golzio C, et al. (2009) Mutational, functional, and expression studies of the TCF4 gene in Pitt-Hopkins syndrome. *Hum Mutat* 30:669–676. [PubMed: 19235238]
13. Marangi G, Ricciardi S, Orteschi D, et al. (2011) The Pitt-Hopkins syndrome: report of 16 new patients and clinical diagnostic criteria. *Am J Med Genet A* 155A:1536–1545. [PubMed: 21671391]
14. Soileau B, Hasi M, Sebold C, et al. (2015) Adults with Chromosome 18 Abnormalities. *J Genet Couns* 24:663–674. [PubMed: 25403900]
15. Forrest M, Chapman RM, Doyle AM, et al. (2012) Functional analysis of TCF4 missense mutations that cause Pitt-Hopkins syndrome. *Hum Mutat* 33:1676–1686. [PubMed: 22777675]
16. Zweier C, Peippo MM, Hoyer J, et al. (2007) Haploinsufficiency of TCF4 causes syndromal mental retardation with intermittent hyperventilation (Pitt-Hopkins syndrome). *Am J Hum Genet* 80:994–1001. [PubMed: 17436255]
17. Brockschmidt A, Todt U, Ryu S, et al. (2007) Severe mental retardation with breathing abnormalities (Pitt-Hopkins syndrome) is caused by haploinsufficiency of the neuronal bHLH transcription factor TCF4. *Hum Mol Genet* 16:1488–1494. [PubMed: 17478476]
18. Rannals MD, Page SC, Campbell MN, et al. (2016) Neurodevelopmental models of transcription factor 4 deficiency converge on a common ion channel as a potential therapeutic target for Pitt Hopkins syndrome. *Rare Diseases* 4:e1220468. [PubMed: 28032012]
19. Rosenfeld JA, Leppig K, Ballif BC, et al. (2009) Genotype–phenotype analysis of TCF4 mutations causing Pitt-Hopkins syndrome shows increased seizure activity with missense mutations. *Genet Med* 11:797–805. [PubMed: 19938247]

20. Maini I, Cantalupo G, Turco EC, et al. (2012) Clinical and polygraphic improvement of breathing abnormalities after valproate in a case of Pitt-Hopkins syndrome. *J Child Neurol* 27:1585–1588. [PubMed: 22378662]
21. Verhulst SL, De Dooy J, Ramet J, et al. (2012) Acetazolamide for severe apnea in Pitt-Hopkins syndrome. *Am J Med Genet A* 158A:932–934. [PubMed: 22407847]
22. Swenson ER, Leatham KL, Roach RC, et al. (1991) Renal carbonic anhydrase inhibition reduces high altitude sleep periodic breathing. *Respir Physiol* 86:333–343. [PubMed: 1788493]
23. De Backer WA (1995) Central sleep apnoea, pathogenesis and treatment: an overview and perspective. *Eur Respir J* 8:1372–1383. [PubMed: 7489805]
24. Henthorn P, Kiledjian M, Kadesch T (1990) Two distinct transcription factors that bind the immunoglobulin enhancer uE5/ke2 motif. *Science*
25. Sepp M, Kannike K, Eesmaa A, et al. (2011) Functional diversity of human basic helix-loop-helix transcription factor TCF4 isoforms generated by alternative 5' exon usage and splicing. *PLoS One* 6:e22138. [PubMed: 21789225]
26. Blake DJ, Forrest M, Chapman RM, et al. (2010) TCF4, schizophrenia, and Pitt-Hopkins Syndrome. *Schizophr Bull* 36:443–447. [PubMed: 20421335]
27. Goldfarb AN, Lewandowska K, Pennell CA (1998) Identification of a highly conserved module in E proteins required for in vivo helix-loop-helix dimerization. *J Biol Chem* 273:2866–2873. [PubMed: 9446597]
28. Massari ME, Grant PA, Pray-Grant MG, et al. (1999) A conserved motif present in a class of helix-loop-helix proteins activates transcription by direct recruitment of the SAGA complex. *Mol Cell* 4:63–73. [PubMed: 10445028]
29. Powell LM, Jarman AP (2008) Context dependence of proneural bHLH proteins. *Curr Opin Genet Dev* 18:411–417. [PubMed: 18722526]
30. Petropoulos H, Skerjanc IS (2000) Analysis of the inhibition of MyoD activity by ITF-2B and full-length E12/E47. *J Biol Chem* 275:25095–25101. [PubMed: 10833525]
31. Furumura M (2001) Involvement of ITF2 in the Transcriptional Regulation of Melanogenic Genes. *J Biol Chem* 276:28147–28154. [PubMed: 11382753]
32. Lu Y, Sheng D-Q, Mo Z-C, et al. (2005) A negative regulatory element-dependent inhibitory role of ITF2B on IL-2 receptor α gene. *Biochem Biophys Res Commun* 336:142–149. [PubMed: 16126178]
33. Rannals MD, Hamersky GR, Page SC, et al. (2016) Psychiatric Risk Gene Transcription Factor 4 Regulates Intrinsic Excitability of Prefrontal Neurons via Repression of SCN10a and KCNQ1. *Neuron* 90:43–55. [PubMed: 26971948] • First report of TCF4 directly regulating neuronal excitability. Haploinsufficiency of TCF4 results in the loss of repression of the ion channel gene SCN10a which becomes ectopically expressed and represents a potential therapeutic target.
34. Hauser J, Sveshnikova N, Wallenius A, et al. (2008) B-cell receptor activation inhibits AID expression through calmodulin inhibition of E-proteins. *Proc Natl Acad Sci U S A* 105:1267–1272. [PubMed: 18203819]
35. Hauser J, Saarikettu J, Grundström T (2008) Calcium regulation of myogenesis by differential calmodulin inhibition of basic helix-loop-helix transcription factors. *Mol Biol Cell* 19:2509–2519. [PubMed: 18353974]
36. Hauser J, Wallenius A, Sveshnikova N, et al. (2010) Calmodulin inhibition of E2A stops expression of surrogate light chains of the pre-B-cell receptor and CD19. *Mol Immunol* 47:1031–1038. [PubMed: 20022378]
37. Hermann S, Saarikettu J, Onions J, et al. (1998) Calcium regulation of basic helix-loop-helix transcription factors. *Cell Calcium* 23:135–142. [PubMed: 9601609]
38. Corneliusen B, Holm M, Waltersson Y, et al. (1994) Calcium/calmodulin inhibition of basic-helix-loop-helix transcription factor domains. *Nature* 368:760–764. [PubMed: 8152489]
39. Onions J, Hermann S, Grundström T (1997) Basic helix-loop-helix protein sequences determining differential inhibition by calmodulin and S-100 proteins. *J Biol Chem* 272:23930–23937. [PubMed: 9295343]

40. Larsson G, Schleucher J, Onions J, Hermann S (2001) A novel target recognition revealed by calmodulin in complex with the basic helix-loop-helix transcription factor SEF2- 1/E2- 2. *Proteins*
41. Larsson G, Schleucher J, Onions J, et al. (2005) Backbone dynamics of a symmetric calmodulin dimer in complex with the calmodulin-binding domain of the basic-helix-loop-helix transcription factor SEF2-1/E2-2: a highly dynamic complex. *Biophys J* 89:1214–1226. [PubMed: 15894636]
42. Zhang LI, Poo MM (2001) Electrical activity and development of neural circuits. *Nat Neurosci* 4 Suppl:1207–1214.
43. Spitzer NC (2006) Electrical activity in early neuronal development. *Nature* 444:707–712. [PubMed: 17151658]
44. Cancedda L, Fiumelli H, Chen K, Poo M-M (2007) Excitatory GABA action is essential for morphological maturation of cortical neurons in vivo. *J Neurosci* 27:5224–5235. [PubMed: 17494709]
45. Luhmann HJ, Fukuda A, Kilb W (2015) Control of cortical neuronal migration by glutamate and GABA. *Front Cell Neurosci* 9:4. [PubMed: 25688185]
46. Komuro H, Rakic P (1998) Orchestration of neuronal migration by activity of ion channels, neurotransmitter receptors, and intracellular Ca²⁺ fluctuations. *J Neurobiol* 37:110–130. [PubMed: 9777736]
47. Hubel DH, Wiesel TN, LeVay S (1977) Plasticity of ocular dominance columns in monkey striate cortex. *Philos Trans R Soc Lond B Biol Sci* 278:377–409. [PubMed: 19791]
48. Kleinschmidt A, Bear MF, Singer W (1987) Blockade of “NMDA” receptors disrupts experience-dependent plasticity of kitten striate cortex. *Science* 238:355–358. [PubMed: 2443978]
49. Shatz CJ (1990) Impulse activity and the patterning of connections during CNS development. *Neuron* 5:745–756. [PubMed: 2148486]
50. Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. *Science* 274:1133–1138. [PubMed: 8895456]
51. Konur S, Ghosh A (2005) Calcium signaling and the control of dendritic development. *Neuron* 46:401–405. [PubMed: 15882639]
52. Rosenberg SS, Spitzer NC (2011) Calcium signaling in neuronal development. *Cold Spring Harb Perspect Biol* 3:a004259. [PubMed: 21730044]
53. Uhlén P, Fritz N, Smedler E, et al. (2015) Calcium signaling in neocortical development. *Dev Neurobiol* 75:360–368. [PubMed: 25652687]
54. Ross SE, Greenberg ME, Stiles CD (2003) Basic helix-loop-helix factors in cortical development. *Neuron* 39:13–25. [PubMed: 12848929]
55. Zhuang Y, Cheng P, Weintraub H (1996) B-lymphocyte development is regulated by the combined dosage of three basic helix-loop-helix genes, E2A, E2-2, and HEB. *Mol Cell Biol* 16:2898–2905. [PubMed: 8649400]
56. Flora A, Garcia JJ, Thaller C, Zoghbi HY (2007) The E-protein Tcf4 interacts with Math1 to regulate differentiation of a specific subset of neuronal progenitors. *Proc Natl Acad Sci U S A* 104:15382–15387. [PubMed: 17878293]
57. Huang W-H, Tupal S, Huang T-W, et al. (2012) Atoh1 governs the migration of postmitotic neurons that shape respiratory effectiveness at birth and chemoresponsiveness in adulthood. *Neuron* 75:799–809. [PubMed: 22958821]
58. Rose MF, Ren J, Ahmad KA, et al. (2009) Math1 is essential for the development of hindbrain neurons critical for perinatal breathing. *Neuron* 64:341–354. [PubMed: 19914183]
59. Tupal S, Huang W-H, Picardo MCD, et al. (2014) Atoh1-dependent rhombic lip neurons are required for temporal delay between independent respiratory oscillators in embryonic mice. *Elife* 3:e02265. [PubMed: 24842997]
60. Kennedy AJ, Rahn EJ, Paulukaitis BS, et al. Tcf4 Regulates Synaptic Plasticity, DNA Methylation, and Memory Function. *CellReports*. doi: 10.1016/j.celrep.2016.08.004• Evidence that HDAC inhibitors can alleviate learning and memory deficits in a PTHS mouse model. Also provides a thorough description of behavioral phenotypes that mirror other ASD mouse models.
61. Crawley JN (2004) Designing mouse behavioral tasks relevant to autistic- like behaviors. *Ment. Retard. Dev. Disabil. Res. Rev*

62. Roulet FI, Crawley JN (2011) Mouse Models of Autism: Testing Hypotheses About Molecular Mechanisms In: Hagan JJ (ed) *Molecular and Functional Models in Neuropsychiatry*. Springer Berlin Heidelberg, pp 187–212
63. Crawley JN (2012) Translational animal models of autism and neurodevelopmental disorders. *Dialogues Clin Neurosci* 14:293–305. [PubMed: 23226954]
64. Grubišić V, Kennedy AJ, Sweatt JD, Pargura V (2015) Pitt-Hopkins Mouse Model has Altered Particular Gastrointestinal Transits In Vivo. *Autism Res* 8:629–633. [PubMed: 25728630]
65. Goldberg TE, Weinberger DR (1988) Probing prefrontal function in schizophrenia with neuropsychological paradigms. *Schizophr Bull* 14:179–183. [PubMed: 3059467]
66. Vijayaragavan K, O’Leary ME (2001) Gating properties of Nav1. 7 and Nav1. 8 peripheral nerve sodium channels. *Journal of Neuroscience*
67. Blair NT, Bean BP (2003) Role of tetrodotoxin-resistant Na⁺ current slow inactivation in adaptation of action potential firing in small-diameter dorsal root ganglion neurons. *J Neurosci* 23:10338–10350. [PubMed: 14614093]
68. D’Rozario M, Zhang T, Waddell EA, et al. (2016) Type I bHLH Proteins Daughterless and Tcf4 Restrict Neurite Branching and Synapse Formation by Repressing Neurexin in Postmitotic Neurons. *Cell Rep* 15:386–397. [PubMed: 27050508] • Description of TCF4 playing a role in post-mitotic neuronal function. Functional expression of TCF4 in post-mitotic neurons restricts neurite branching and regulates synapse number.
69. Zweier C, de Jong EK, Zweier M, et al. (2009) CNTNAP2 and NRXN1 Are Mutated in Autosomal-Recessive Pitt-Hopkins-like Mental Retardation and Determine the Level of a Common Synaptic Protein in Drosophila. *Am J Hum Genet* 85:655–666. [PubMed: 19896112]
70. Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium (2011) Genome-wide association study identifies five new schizophrenia loci. *Nat Genet* 43:969–976. [PubMed: 21926974]
71. Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014) Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 511:421–427. [PubMed: 25056061]
72. Ripke S, O’Dushlaine C, Chambert K, et al. (2013) Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nat Genet* 45:1150–1159. [PubMed: 23974872]
73. Quednow BB, Ettinger U, Mössner R, et al. (2011) The schizophrenia risk allele C of the TCF4 rs9960767 polymorphism disrupts sensorimotor gating in schizophrenia spectrum and healthy volunteers. *J Neurosci* 31:6684–6691. [PubMed: 21543597]
74. Zhu X, Gu H, Liu Z, et al. (2013) Associations between TCF4 gene polymorphism and cognitive functions in schizophrenia patients and healthy controls. *Neuropsychopharmacology* 38:683–689. [PubMed: 23249814]
75. Hui L, Rao W-W, Yu Q, et al. (2015) TCF4 gene polymorphism is associated with cognition in patients with schizophrenia and healthy controls. *J Psychiatr Res* 69:95–101. [PubMed: 26343600]
76. Talkowski ME, Rosenfeld JA, Blumenthal I, et al. (2012) Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. *Cell* 149:525–537. [PubMed: 22521361]
77. Ye T, Lipska BK, Tao R, et al. (2012) Analysis of copy number variations in brain DNA from patients with schizophrenia and other psychiatric disorders. *Biol Psychiatry* 72:651–654. [PubMed: 22795968]
78. Mullegama SV, Alaimo JT, Chen L, Elsea SH (2015) Phenotypic and molecular convergence of 2q23.1 deletion syndrome with other neurodevelopmental syndromes associated with autism spectrum disorder. *Int J Mol Sci* 16:7627–7643. [PubMed: 25853262]
79. Maduro V, Pusey BN, Cherukuri PF, et al. (2016) Complex translocation disrupting TCF4 and altering TCF4 isoform expression segregates as mild autosomal dominant intellectual disability. *Orphanet J Rare Dis* 11:62. [PubMed: 27179618]
80. Kalscheuer VM, Feenstra I, Van Ravenswaaij-Arts CMA, et al. (2008) Disruption of the TCF4 gene in a girl with mental retardation but without the classical Pitt–Hopkins syndrome. *Am J Med Genet* 146A:2053–2059. [PubMed: 18627065]

81. Kharbanda M, Kannike K, Lampe A, et al. (2016) Partial deletion of TCF4 in three generation family with non-syndromic intellectual disability, without features of Pitt-Hopkins syndrome. *Eur J Med Genet* 59:310–314. [PubMed: 27132474]
82. Hamdan FF, Daoud H, Patry L (2013) Parent–child exome sequencing identifies a de novo truncating mutation in TCF4 in non-syndromic intellectual disability. *Clinical*
83. Rauch A, Wiczorek D, Graf E, et al. (2012) Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet* 380:1674–1682. [PubMed: 23020937]
84. de la Torre-Ubieta L, Won H, Stein JL, Geschwind DH (2016) Advancing the understanding of autism disease mechanisms through genetics. *Nat Med* 22:345–361. [PubMed: 27050589]
85. Mullins C, Fishell G, Tsien RW (2016) Unifying Views of Autism Spectrum Disorders: A Consideration of Autoregulatory Feedback Loops. *Neuron* 89:1131–1156. [PubMed: 26985722]