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ARTICLE

Characterization of the genotypic and phenotypic spectrum of TCF7L2-related neurodevelopmental disorder (TRND)

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ABSTRACT

Purpose: *TCF7L2* (OMIM 602228; HGNC:11641) is a transcription factor and a critical effector of the *Wnt*/ β-Catenin pathway. In 2021, 11 pediatric patients with monoallelic predicted loss-of-function (pLOF) *TCF7L2* variants and syndromic features were observed. Characterization of patients with pLOF *TCF7L2* variants and neurodevelopmental features—herein referred to as *TCF7L2*-related neurodevelopmental disorder—is urgently needed.
Methods: We leveraged multiple methods (eg, GeneMatcher, DECIPHER, literature review, and public/private repositories) to identify an international cohort of 76 patients with pLOF *TCF7L2* variants and neurodevelopmental features and phenotypically characterized them. We also retrospectively searched for an independent cohort of adults with pLOF *TCF7L2* variants ($n = 11$) from more than 60,000 PennMedicine BioBank patients.
Results: Among 76 patients with pLOF *TCF7L2* variants, speech delay (95.3%), craniofacial dysmorphisms (73.3%), ophthalmologic conditions (65.5%), autism (62.1%), and orthopedic abnormalities (52.6%) were the most commonly observed. Phenotypic differences did not cluster by variant type or genomic locus. Among PennMedicine BioBank patients, an association of nominal significance with type 2 diabetes with renal manifestations (odds ratio = 5.8; $P = .03$) was detected, warranting further investigation.
Conclusion: This study represents the most comprehensive characterization of *TCF7L2*-related neurodevelopmental disorder to date, a novel neurodevelopmental disorder, defining its genotypic and phenotypic spectra. We opened a Simons Searchlight natural history study that is now available for patient enrollment to enhance the understanding of this condition.
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Introduction

TCF7L2 (OMIM 602228; HGNC:11641)—formerly TCF4-transcription factor 4—is a key mediator of canonical *Wnt*/β-catenin (CTNNB1) signaling across human biology and disease.[1,2] In fact, genome-wide association studies have linked *TCF7L2* single nucleotide variants with an increased risk for type 2 diabetes mellitus (T2D), colon cancer, bipolar disorder, schizophrenia, and autism spectrum disorder (ASD).[2-4] *TCF7L2* is located on chromosome 10q25.2, and the encoded protein is a member of the T-cell factor/lymphoid enhancer-binding factor family of proteins, which complex with CTNNB1 to bind DNA via a highly conserved high-mobility group (HMG Box) domain.[1,5] Transcriptional regulation of *Wnt*-responsive genes is vital for fetal development and adult tissue homeostasis.[1,2] *TCF7L2* specifically has a complex splicing pattern that generates distinct protein isoforms—each with agonistic and antagonistic activities on the transcriptional regulation of canonical *Wnt*/CTNNB1 signaling pathways.[2,6]

TCF7L2 is especially critical for central nervous system development. In fact, *TCF7L2* homozygous knockout in mice results in neural tube duplications and severe truncations of the caudal endoderm incompatible with life.[7] Single-cell RNA sequencing of human neuronal tissue has demonstrated *TCF7L2* expression in several central nervous system cell populations throughout development,[8] and *TCF7L2* has been implicated in astrocyte maturation,[8,9] thalamic and midbrain development,[10,11] and premyelination and maturation of oligodendrocytes.[12,13] In humans, *TCF7L2* has a high intolerance to loss-of-function variation (probability of loss-of-function intolerance = 1). Several large-scale studies over the past decade have identified occasional patients with intellectual disability (ID) and ASD with variants in *TCF7L2*.[14-19]

Recently, Dias et al[20] reported a small case series of 11 pediatric patients with de novo heterozygous nonsense, splice-site, and missense variants in *TCF7L2* who had neurodevelopmental syndromic features. This small cohort all had developmental delays in addition to a range of ophthalmologic, orthopedic, and neuropsychiatric comorbidities, such as ASD and attention-deficit/hyperactivity disorder (ADHD). All variants were in highly conserved regions expected to disrupt *TCF7L2* function. Functional mouse models with 2 of these variants demonstrated reduced *TCF7L2* HMG Box binding, anxiety-like phenotypes, and impaired vocalization consistent with previous murine ASD models.[21] These findings collectively suggest that haploinsufficiency of *TCF7L2* contributes to the risk for a novel neurodevelopmental disorder, herein referred to as *TCF7L2*-related neurodevelopmental disorder (TRND).

Given the small sample size and limited research to date, many questions remain, including those regarding TRND's natural history and phenotypic and genotypic spectrum. In this study, we sought to establish this comprehensively for future investigation.

Materials and Methods

During this retrospective cohort study, we identified patients with predicted loss-of-function (pLOF) variants in

TCF7L2 from June 2022 to October 2024 (Figure 1). We also opened a Simons Searchlight natural history study (https://www.simonssearchlight.org/research/what-we-study/tcf7l2/), which is currently available for prospective patient enrollment.

Identifying and classifying patients with pLOF *TCF7L2* variants

Orthogonal identification strategies were implemented, including: (1) patient identification with GeneMatcher[22] through the Matchmaker Exchange Network, (2) initiating contact with clinicians of cases published in DECIPHER,[23] (3) collating patient data from public/private repositories (Simons Simplex Collection (SSC),[24] Simons Foundation Autism Research Initiative (SFARI),[24] Simons Foundation Powering Autism Research for Knowledge (SPARK), the Clinical Variant database,[25] and Ambry Genetics), and (4) outreach to authors/clinicians of cases yielded from systematic literature reviews. A literature review was conducted using the keyword search term "TCF7L2" on PubMed, and corresponding authors were contacted regarding potential cases. Following initial identification ($n = 125$), 37 cases were excluded because they were either (1) identified as duplicates under different source-specific identifiers and were removed to ensure that patients were uniquely represented or (2) outside the coding sequence (eg, intron variants).

Patients with *TCF7L2* variants were classified as pLOF and "likely TRND" (Supplemental Table 1) if: (1) any neurodevelopmental phenotype was reported, (2) the patient had no other variants known to contribute to a neurodevelopmental phenotype (per report using patient-specific identification methods listed in Supplemental Table 1), and (3) the following inclusion criteria for each variant class were met:

- Nonsense variants were located ≥50 nucleotides upstream of the final exon-exon junction, and, thus, expected to cause nonsense-mediated decay of the transcript[20,26];
- Splice-site variants scoring highly on splicing prediction tools (MaxEntScan[27] |delta score| ≥8 or SpliceAI[28] score ≥0.8);
- Out-of-frame indel variants expected to introduce a stop codon ≥50 nucleotides upstream of the final exon-exon junction[20,26];
- Missense variants with <0.01% total allele frequency in the Genome Aggregation Database version 4.1.0[29] and with scores supportive of pathogenicity in at least 2/4 predictive tools (AlphaMissense[30] 0.56-1.0, ClinPred[31] ≥0.5, PolyPhen[32] "possibly" or "probably damaging" ≥0.446, and/or PHRED-like CADD[33] ≥25);
- Copy number variant (CNV) deletion variants expected to disrupt *TCF7L2* not otherwise spanning genes known to explain the presenting phenotype; or
- In-frame indel variants that are not present in the Genome Aggregation Database[29] in a highly conserved region.

Pathogenicity scores generated using prediction algorithms for missense and splice-site variants are summarized in Supplemental Table 2. Some identified patients had suspicious *TCF7L2* variants and no other clear genetic contributor for their neurodevelopmental phenotype but did not meet the aforementioned criteria. These cases were separately classified as "suspected TRND," with case-specific reasons for exclusion in Supplemental Table 3.

Genetic variants were primarily identified using exome sequencing and/or Sanger sequencing ($n = 49$). Remaining cases were identified using non-Sanger genome sequencing ($n = 7$), other non-Sanger assays ($n = 6$; molecular inversion probe assay, expanded gene panel, microarray, and/or Fluorescence in situ hybridization), or were otherwise unable to be confirmed due to case-specific limitations ($n = 14$). All reported variants were converted to the MANE Select transcript (NM_001367943.1) using the Ensembl Variant Effect Predictor tool.[34] An overview of patient identification and classification is summarized in Figure 1. Composite genotypic and phenotypic information for "likely TRND" and "suspected TRND" cases is available in Supplemental Tables 1 and 3, respectively.

Phenotypic characterization of TRND patients

Following patient identification, clinicians were provided with a tabular survey (Supplemental Table 4) to complete with de-identifiable data regarding their case, including free-text response questions regarding genotypic, phenotypic, and historical details. Of the 76 "likely TRND" and 12 "suspected TRND" patients, 2 patients (P20 and P7), could not be further characterized through the tabular survey by the referring clinician/site/author, and only publicly available data were used in these cases. Written responses were clarified during follow-up, and phenotypic information was standardized using the Human Phenotype Ontology (HPO)[35] during analysis. Translated phenotypic details were corroborated with clinicians and updated at the study's conclusion. To characterize the spectrum of features shared across "likely TRND" patients, we extracted HPO-standardized terms that cooccurred in at least 2 patient cases, comprising 81 features (Figure 1; Supplemental Table 1). Ten broad categories of non-neurodevelopmental phenotypic features were additionally included in the analysis (Supplemental Table 1).

Interrogation of adult biobank and electronic health record (EHR) data for individuals with pLOF *TCF7L2* variants

We also conducted a retrospective screen of the PennMedicine Biobank (PMBB) at the University of Pennsylvania—a

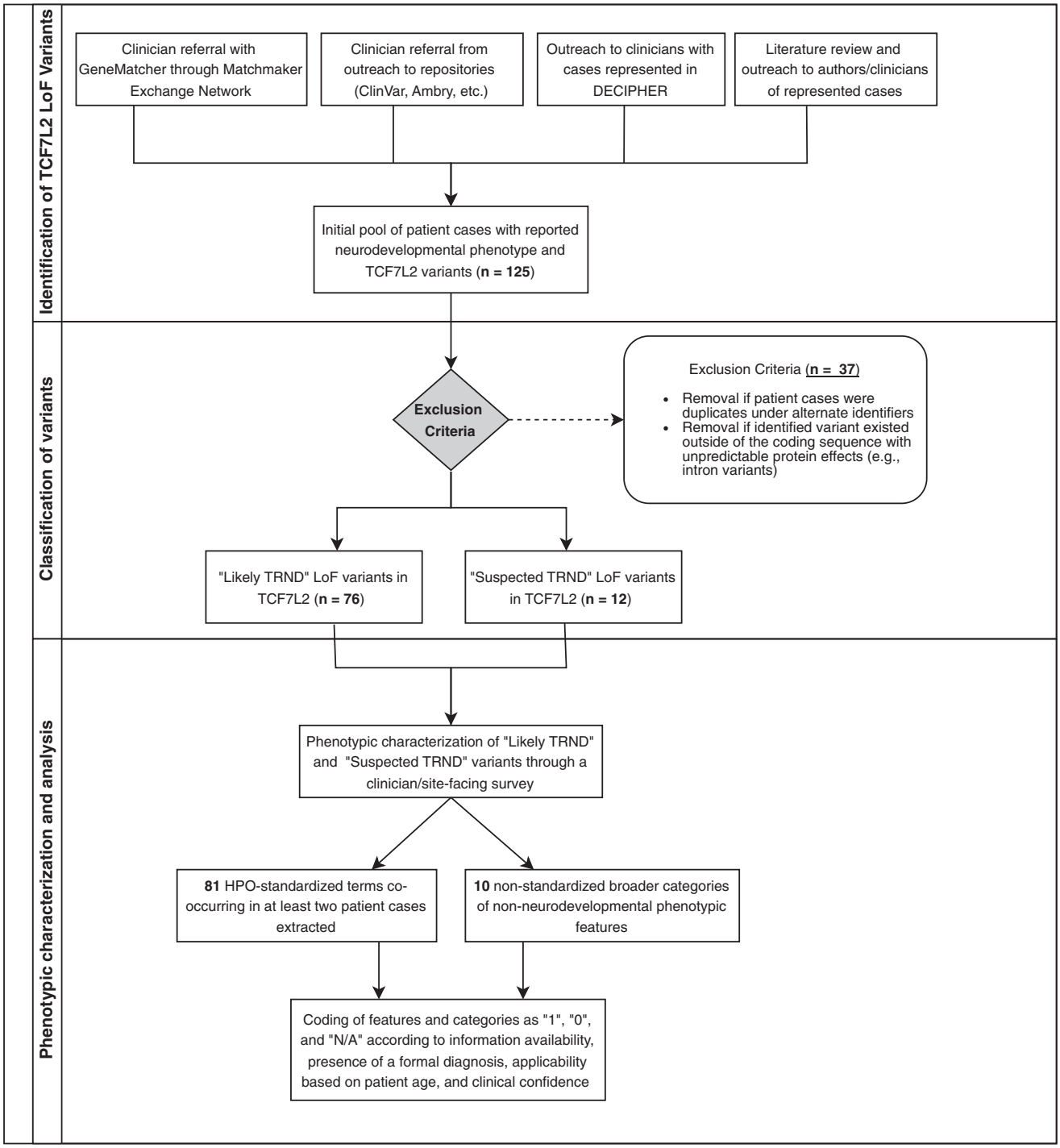


Figure 1 Search, identification, and characterization of *TCF7L2*-related neurodevelopmental disorder (TRND) patient cases. Orthogonal approaches were adopted to identify and characterize patient cases with predicted loss of function *TCF7L2* variants. A total of 76 variants were classified as likely loss-of-function *TCF7L2* variants resulting in TRND (Supplemental Table 1). Twelve patient cases were suspected to have TRND but did not otherwise meet criteria (Supplemental Table 3). We phenotypically characterized "likely TRND" and "suspected TRND" cases using a clinician/site-facing tabular survey with free-response questions. Of the 76 "likely TRND" and 12 "Suspected TRND" patients, 2 patients (P20 and P7), could not be further characterized with additional information through the tabular survey by the referring clinician/site/author. For analytical purposes, we extracted Human Phenotype Ontology (HPO)-standardized terms that cooccurred in at least 2 patient cases, comprising 81 features, and 10 categories of non-HPO broader categories of phenotypic features. For the purposes of aggregated counts, patients were assigned a "1" for a phenotypic feature if a qualified health care professional reported its presence in the survey on clinical assessment or through patient report. Similarly, "0" was designated in the absence of the phenotypic feature on clinical assessment or through report, and "N/A" was assigned if phenotypic information was unavailable or could not otherwise be definitively determined or clinically assessed, altering the denominator per phenotypic feature or category. ClinVar, Clinical variant database.

repository linked to the PennMedicine EHR system—to identify adults with pLOF variants in *TCF7L2*. The biobank includes approximately 60,000 adult patient volunteers who have had a genome-wide single-nucleotide polymorphism array performed; a subset of these volunteers also received exome sequencing. pLOF variants in *TCF7L2* were defined as frameshift, splicing, or stop-gain variants, as annotated by ANNOVAR,[36] and were filtered for pathogenicity using REVEL[37] scores of ≥ 0.5. Following enforcement of this criterion, 12 different heterozygous pLOF variants in *TCF7L2* were detected among an initial pool of 22 PMBB patients. Eleven patients had nonsense or out-of-frame indel variants that did not meet the $\geq$50 nucleotides before the last exon-exon junction threshold criterion and were excluded, resulting in a final sample size of $n = 11$. Age- and sex-matched controls were identified by selecting patients with common variants in *TCF7L2* (allele frequency $\geq$ 10%). Principal component analysis-based matching on Euclidean distance was used to randomly select approximately 10 controls per case from the $n = 22$ unfiltered dataset ($n = 198$ controls). All diagnoses in the EHR for each patient were recorded, as well as any International Classification of Diseases, 9th revision codes. Median age and body mass index (BMI) were reported relative to the date of the last encounter. PMBB "phecodes"–groups of International Classification of Diseases, 9th revision codes depicting clinically meaningful phenotypes–were compared between control and pLOF cohorts. Statistical significance was evaluated using Fisher exact tests with Benjamini-Hochberg (BH) correction for categorical variables, and Mann-Whitney U tests were used for quantitative variables.

Results

pLOF *TCF7L2* variants in highly conserved protein domains are present in TRND

Seventy-six patients were identified as "likely TRND," with nonsense ($n = 10$), out-of-frame indel ($n = 18$), missense ($n = 33$), splice-site ($n = 10$), in-frame indel ($n = 1$), and CNV ($n = 4$) variants in *TCF7L2*. The median patient age at the most recent recorded clinical assessment was 8.17 years (interquartile range [IQR] = 3.94-13.25), and 55.71% were male. Most patients for whom reported ethnicity was available were identified by clinicians as White Non-Hispanic (39.47%). An additional 12 patients were identified as "suspected TRND," with insufficient evidence to meet the criteria. Characteristics of both cohorts are included in Table 1.

Most variants ($n = 72$) were predicted to impact the coding sequence of (1) the full-length isoforms of *TCF7L2* (FL-*TCF7L2*) and (2) the short isoforms of *TCF7L2*, which lack the N-terminal CTNNB1 binding domain and act as a dominant-negative antagonist of canonical *Wnt* signaling (DN-*TCF7L2*). However, 4 cases (P11, P17, P18, and P21) included variants before exon 4, predicted to impact only FL-*TCF7L2* and long non-coding RNA (lncRNA), excluding DN-*TCF7L2* isoforms (NM_001349870.2, NM_001349871.1).

Of the 76 identified variants, 7 were shared by 2 patients each (14 patients total). The 7 shared variants among "likely TRND" cases are as follows:

(1) NM_001367943.1:c.1268A>G, NP_001354872.1:p.(Tyr423Cys), NG_012631.1:g.207190A>G, and NC_000010.11:g.113152439A>G.
(2) NM_001367943.1:c.1001+1G>A, NP_001354872.1:p.?, NG_012631.1:g.205875G>A, and NC_000010.11:g.113151124G>A.
(3) NM_001367943.1:c.634_635dup, NP_001354872.1:p.(Pro213ArgfsTer13), NG_012631.1:g.196016_196017dup, and NC_000010.11:g.113141265_113141266dup.
(4) NM_001367943.1:c.162del, NP_001354872.1:p.(Asn55IlefsTer53), NG_012631.1:g.5669del, and NC_000010.11:g.112950918del.
(5) NM_001367943.1:c.686-1G>A, NP_001354872.1:p.?, NG_012631.1:g.198673G>A, and NC_000010.11:g.113143922G>A.
(6) NM_001367943.1:c.1172T>C, NP_001354872.1:p.(Leu391Pro), NG_012631.1:g.207094T>C, and NC_000010.11:g.113152343T>C
(7) NM_001367943.1:c.1604C>T, NP_001354872.1:p.(Ser535Leu), NG_012631.1:g.220467C>T, and NC_000010.11:g.113165716C>T.

All other variants were found in unique patients. The spectrum of *TCF7L2* variants was mapped to the MANE select transcript, NM_001367943.1 (Figure 2). Patient cases with "likely TRND" clustered at the C-terminal ends of the CTNNB1 binding domains and HMG Box domain. Variants were also clustered at a highly conserved region between both domains.

TRND patients most frequently presented with developmental, ophthalmologic, and orthopedic abnormalities

Of the 76 "likely TRND" patients, they were marginally heavier and of relatively average height compared with reference populations—with median weight and height Z-scores of 0.85 (IQR = −0.25 to 1.60) and 0.14 (−0.95 to 0.66), respectively. 81 HPO-standardized ontology terms were described by clinicians in at least 2 TRND patients, as well as 10 broader categories of non-HPO, non-neurodevelopmental features. HPO codes associated with each term are listed in Supplemental Table 1. Among "likely TRND" cases (Figure 3A and B, Supplemental Figure 1), nearly all presented with speech delay (61/64, 95.31%). Other frequently reported neurodevelopmental phenotypes included ASD (36/58, 62.07%; OMIM 209850), gross motor delay (29/58, 50.0%), ID (27/56,

Table 1 Features of *TCF7L2*-related neurodevelopmental disorder patient cohorts

Cohort Features	Patient Cohort with "Likely TRND" ($n = 76$)	Patient Cohort with "Suspected TRND" ($n = 12$)
Median age, y (1st quartile, 3rd quartile)[a]	8.17 (3.94, 13.25)	13 (6.5, 22.5)
Sex (% female)[b]	31/70 (44.29%)	3/9 (33.33%)
Ethnicity distribution[c]		
White Non-Hispanic	30 (39.47%)	4 (33.33%)
Asian Non-Hispanic	2 (2.63%)	2 (16.67%)
Black or African American Non-Hispanic	2 (2.63%)	0 (0.0%)
Hispanic	1 (1.32%)	0 (0.0%)
Not known or prefer not to disclose	41 (53.95%)	6 (50.0%)
Median height z-score (1st quartile, 3rd quartile)[d]	0.14 (–0.95, 0.66)	–0.45 (–0.66, 0.06)
Median weight z-score (1st quartile, 3rd quartile)[e]	0.85 (–0.25, 1.60)	0.23 (–0.14, 0.79)
Variant class		
Nonsense (stop-gain)	10 (13.16%)	4 (33.33%)
Out-of-frame indel	18 (23.68%)	1 (8.33%)
Missense	33 (43.42%)	6 (50.0%)
Splice site donor/acceptor variant	10 (13.16%)	1 (8.33%)
In-frame indel	1 (1.32%)	N/A
CNV deletion	4 (5.26%)	N/A
Cases with shared: unique variants	14:62	4:8
Inheritance pattern		
De novo[f]	58 (76.32%)	4 (33.33%)
Inherited[g]	7 (9.21%)	4 (33.33%)
Unknown	11 (14.47%)	4 (33.33%)
Missense variant location		
HMG Box domain	12 (36.36%)	N/A
CTNNB1 binding domain	8 (24.24%)	2 (33.33%)
Neither	13 (39.39%)	4 (66.67%)

Patients with predicted loss of function *TCF7L2* variants were collected using an orthogonal approach, including GeneMatcher, DECIPHER, individual outreach to referred clinicians, and literature review prior to phenotypic characterization. Seventy-six patient cases were identified with a median age of 8.17 years, and 44.29% of whom were female. Patients who were suspected to have *TCF7L2*-related neurodevelopmental disorder but did not meet criteria were classified as "suspected TRND."

CTNNB1, β-catenin; *CNV*, copy number variant; HMG, high-mobility group; *N/A*, not available *TRND*, *TCF7L2*-related neurodevelopmental disorder.

[a]Median and quartiles found relative to number of patients with available information ($n = 64$ for "likely TRND" and $n = 6$ for "suspected TRND").

[b]Reflective of the number of patients with available information about sex ($n = 70$ for "Likely TRND" and $n = 9$ for "Suspected TRND").

[c]Ethnicities based on categories defined by the Center for Disease Control. There were no patients of American Indian and Alaskan Native descent or Native Hawaiian or Other Pacific Islanders presented in this cohort.

[d]Median and quartiles found relative to number of patients with available information ($n = 48$ for "likely TRND" and $n = 4$ for "suspected TRND").

[e]Median and quartiles found relative to number of patients with available information ($n = 49$ for "likely TRND" and $n = 4$ for "suspected TRND").

[f]De novo or likely de novo ($n = 4$ could not confirm absence in both parents).

[g]Most inherited variants had unknown parental phenotypes ($n = 4$). Others had a parent with mosaicism with school difficulties but no reported neurodevelopmental delays ($n = 2$). One patient had a homozygous inherited variant in *TCF7L2* with no neurodevelopmental delays in either parent.

48.21%), ADHD/attention-deficit disorder (20/47, 42.55%; OMIM 143465), learning disability (9/32, 28.13%), and fine motor delay (12/54, 22.22%). When the degree of ID was clinician-reported, or intelligence quotient scores were provided, most patients with ID were in the mild-to-moderate range (11/16, 68.75%); a minority (5/16, 31.25%) were classified as "severe" or overlapping with the "severe" range.

Among patients for whom information was available, the median age at patients' first words ($n = 30$; 7 non-verbal; 39 unknown) was markedly delayed, to 30 months (IQR = 18.75-36), and their first steps/ability to walk ($n = 28$; 48 unknown) was mildly delayed, to 15 months (IQR = 13.13-18.13). Among those with reported behavioral abnormalities, aggression (7/21, 33.33%) was among those most frequently reported, aside from ASD-like and ADHD-like features.

A few patients experienced regression (8/55, 14.55%), primarily of expressive language. Psychiatric diagnoses were less commonly reported (7/53, 13.21%), with anxiety being the most frequent (5/54, 9.26%; OMIM 607834). Other neurological complications included sleep

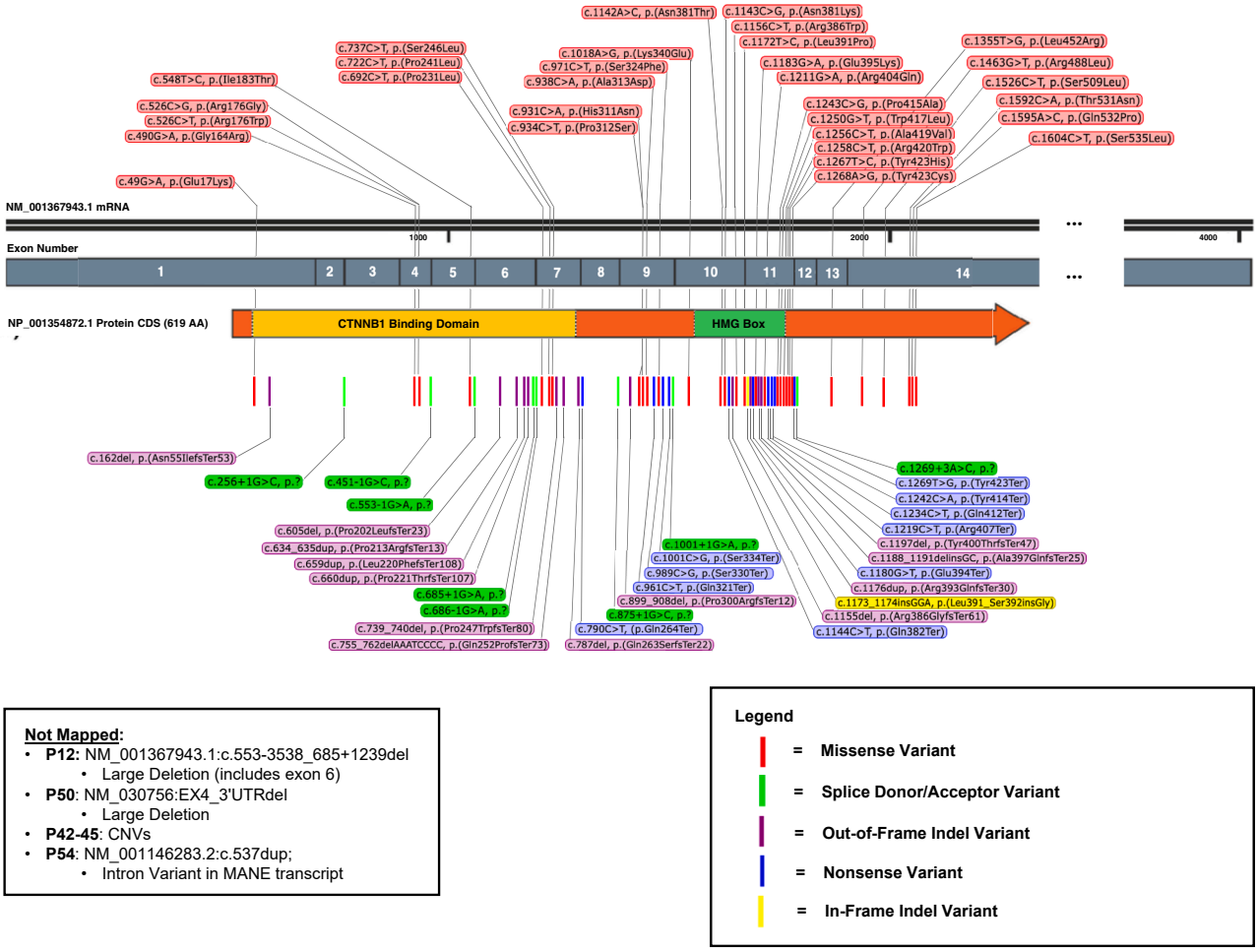


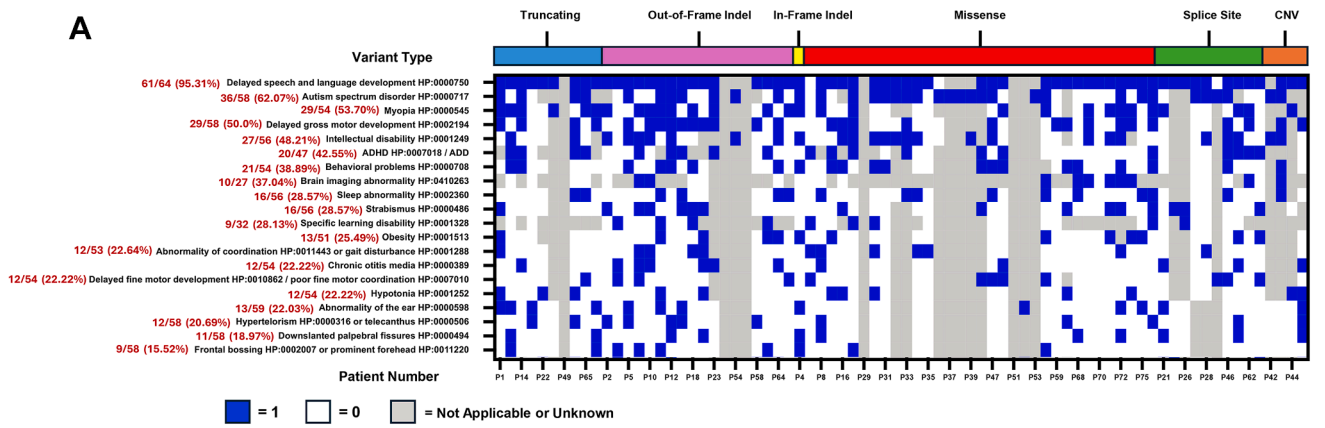
Figure 2 Gene map of patients with likely *TCF7L2*-related neurodevelopmental disorder (TRND). The spectrum of genetic variants represented in "likely TRND" patient cases are mapped to the *TCF7L2* MANE select transcript (NM_001367943.1) and its associated protein product (NP_001354872.1). Variants are distributed across highly conserved regions of the *TCF7L2* genes, including foci within the CTNNB1 Binding, the High-Mobility Group (HMG) Box domains, as well as in a region between them. AA, amino acid; mRNA, messenger RNA.

abnormalities (16/56, 28.57%), coordination abnormalities or gait disturbances (12/53, 22.64%), hypotonia (12/54, 22.22%), seizures (8/56, 14.29%), and toe walking (6/53, 11.32%). Most seizures manifested as generalized (7/8, 87.5%); 4 were uncomplicated febrile seizures, and all had an onset at 7 years of age, or age was otherwise not specified.

The most frequently observed nondevelopmental features included ophthalmological conditions (38/58, 65.52%) such as myopia (29/54, 53.70%), strabismus (16/56, 28.57%, OMIM 185100), and astigmatism (6/52, 11.54%, OMIM 603047), and musculoskeletal/orthopedic abnormalities (30/57, 52.63%) such as abnormal thorax morphology (8/53, 15.09%), clinodactyly (5/54, 9.26%), Achilles tendon contractures (4/53, 7.55%), bilateral club feet (4/54, 7.41%), and pes planus (3/53, 5.66%). Three cases were described with insulin resistance/pre-diabetes ($n = 1$) or diabetes (type 1, $n = 1$, OMIM 222100; T2D, $n = 1$, OMIM 125853). Other notable conditions included respiratory/ear, nose, and throat abnormalities (18/55, 32.73%), including chronic otitis media infections (12/54, 22.22%, OMIM 166760). Hearing deficits were described in some cases (8/52, 15.38%), many of which were conductive or due to a history of chronic infections.

Abnormal brain imaging was reported in 10 of 27 patients (37.04%) with available magnetic resonance imaging. A subset of 4 patients demonstrated periventricular abnormalities on magnetic resonance imaging, including periventricular hyperintensities, white matter volume loss, and/or leukomalacia. Two of these cases were described as having signs of upper motor neuron dysfunction and spastic paraplegia. Although 2 had unremarkable pregnancies and deliveries, 1 was born prematurely, and 1 was complicated by perinatal asphyxia.

Dysmorphic craniofacial features were frequent (44/60, 73.33%); however, specific features varied and were not uniformly observed. The most frequently reported features included abnormalities of ear morphology (13/59, 22.03%), hypertelorism and/or telecanthus (12/58, 20.69%; OMIM 145400), down-slanted palpebral fissures (11/58, 18.97%),



	Frequency
Craniofacial Dysmorphic Features	44/60 (73.33%)
Vision/Ophthalmologic Abnormalities	38/58 (65.52%)
Orthopedics/MSK Abnormalities	30/57 (52.63%)
Respiratory/ENT Abnormalities	18/55 (32.73%)
Skin/Hair/Nails Abnormalities	12/55 (21.82%)
Gastrointestinal Abnormalities	10/54 (18.52%)
Cardiac Abnormalities	9/56 (16.07%)
Endocrine Abnormalities	7/50 (14.0%)
Psychiatric Diagnoses	7/53 (13.21%)
Urogenital Abnormalities	4/54 (7.41%)

Figure 3 Top 25% highest frequency phenotypic attributes occurring in *TCF7L2*-related neurodevelopmental disorder (TRND) patient cases. Eighty-one HPO-standardized phenotypic terms cooccurring in 2 or more "likely TRND" patients were extracted as well as 10 nonstandardized broader categories of nonneurodevelopmental features. A. The top 25% highest frequency HPO-standardized phenotypes among "likely TRND" patients are grouped by variant type. Although the top 25% highest frequency phenotypic attributes are included, the full distribution is included in Supplemental Figure 1. The highest frequency HPO terms among "likely TRND" patient cases included speech delay HP:0000750 (95.31%), autism spectrum disorder HP:0000717 (62.07%), myopia HP:0000545 (53.70%), delayed gross motor development HP:0002194 (50.0%), and intellectual disability HP:0001249 (48.21%). B. The highest frequency broader categories of nonneurodevelopmental phenotypic features included craniofacial dysmorphic features (73.33%), vision/ophthalmologic abnormalities (65.52%), and orthopedics/musculoskeletal (MSK) abnormalities (52.63%). For the top 25% highest frequency phenotypic attributes (in A), differences in phenotypic frequency were evaluated across (C) variant type, (D) in comparison with the study by Dias et al[20] (11 patients from the primary cohort and 1 identified from the Dias et al[6] supplement; $n = 12$), and (E) across isoform types predicted to be disrupted. Comparisons of the full distributions are included in Supplemental Figures 2 to 4. CNV, copy number variant; ENT, ear, nose, and throat; HPO, Human Phenotype Ontology; WT, wild type.

and frontal bossing and/or prominent forehead (9/58, 15.52%).

Patients with shared genetic variants ($n = 7$ variants among 14 patients) had similar neurodevelopmental presentations; however, some features, such as craniofacial dysmorphic features and orthopedic abnormalities, were variably present, suggesting potential differences in the penetrance of certain phenotypes and/or variable expression. For example, unrelated patients P11 and P18 (NM_001367943.1:c.162del, NP_001354872.1:p.(Asn55IlefsTer53), NG_012631.1:g.5669del, NC_000010.11:g.112950918del) both presented with speech delay, gross motor delay, concentration deficiency (no formal diagnosis of attention-deficit disorder/ADHD), behavioral issues, and myopia. However, P11 additionally presented with craniofacial dysmorphic features, orthopedic abnormalities (including abnormal thorax morphology), hypotonia, and an absence of facial/axillary hair discordant with age. P18, by

contrast, presented with a growth disorder with delayed bone age, primary enuresis, strabismus, no craniofacial dysmorphic features, and no orthopedic abnormalities.

Other patients with shared variants, P8 and P48 (NM_001367943.1:c.1172T>C, NP_001354872.1:p.(Leu391Pro), NG_012631.1:g.207094T>C, NC_000010.11:g.113152343T>C), had speech delay, concentration deficiency, or diagnosed ADHD, normal initial motor development, myopia, orthopedics abnormalities (lumbar hyperlordosis vs brachydactyly and syndactyly), and recurring ear infections and chronic otitis media. However, P48 had no neurologic or craniofacial dysmorphic features, whereas P8 additionally presented with ASD, limb hypertonia, and cranial nerve IV paresis, as well as craniofacial dysmorphic features (hypertelorism and down-slanted palpebral fissures, among others).

Compared with patients first identified in Dias et al[20] ($n = 12$), the remaining cases in this cohort ($n = 64$) had no statistically significant differences across all HPO-standardized terms and categories of phenotypic features. There was a nominally increased frequency of ASD in our cohort (69.57% vs 33.33% Dias; nominal $P = .042$) and a decreased frequency of hypertrichosis (0% vs 22.2% Dias; nominal $P = .029$). However, these differences did not survive correction for multiple hypothesis testing (Figure 3C; Supplemental Figure 2).

TRND patient phenotypes do not grossly cluster by genomic locus or transcript domain

Next, we sought to investigate whether variant type and genomic locus were associated with phenotypic differences among patients. A comparison between variant types is displayed in Figure 3D and Supplemental Figure 3. When compared with non-CNV variant groups, patients with CNV variants exhibited higher frequencies of cardiac abnormalities than patients with missense (100.0% vs 9.09% missense; BH-adjusted $P = .029$) and out-of-frame indel variants (100.0% vs 7.14% out-of-frame indel; BH-adjusted $P = .029$). This difference was nominally significant when compared with other variant types (eg, splice-site and nonsense variants) but did not survive multiple hypothesis testing. Other inter-group differences were not significant following BH adjustment; differences of nominal significance are summarized in Supplemental Figure 3.

Despite the limited sample size, we investigated whether phenotypic differences existed among variants predicted to disrupt all 17 RefSeq isoforms of both FL- and DN-*TCF7L2* ($n = 72$) vs FL-*TCF7L2* only (15 isoforms; $n = 4$) (Figure 3E). Variants predicted to affect the protein sequence of only FL-*TCF7L2* isoforms were more frequently associated with short palpebral fissures (50.0% vs 3.70%; nominal $P = .021$) and skin/hair/nail abnormalities (75.0% vs 17.65%; nominal $P = .029$), including sparse lateral eyebrows (50.0% vs 0%; nominal $P = .004$) and slow-growing hair (50.0% vs 0%; nominal $P = .004$). However, these differences did not survive multiple hypothesis correction. Although not statistically significant, 2 of 4 patients with variants predicted to impact only FL-*TCF7L2* transcripts had notable endocrine abnormalities (50.0% vs 10.9%; nominal $P = .09$), including a growth disorder with delayed bone age and absent axillary and facial hair in adolescence. Differences between the 2 isoform groups, across all HPO-standardized phenotypes, are summarized in Supplemental Figure 4.

Next, we sought to evaluate whether clinical presentation varied based on variant location within the *TCF7L2* gene (Supplemental Figure 5). Generally, specific phenotypes across patients were well-distributed in type and location across the *TCF7L2* transcript, but a few trends were noted. Many of the sub-phenotypes of orthopedic/musculoskeletal abnormalities were concentrated beyond exon 6. Notably, severe phenotypes, including seizures, did not cluster grossly with location on the *TCF7L2* transcript but were often sequestered between exons 5 and 11 (Supplemental Figure 5). A subset of patients with variants clustering at the terminal end of the HMG Box domain presented with cooccurring craniofacial features, including frontal bossing, down-slanted palpebral fissures, and ear morphology abnormalities.

Small retrospective adult cohort with pLOF *TCF7L2* variants nominally associated with increased risk of T2D

To explore phenotypes in adults with pLOF *TCF7L2* variants, we conducted a retrospective analysis of PMBB adults. Following enforcement of inclusion and exclusion criteria (Methods), 11 patients were identified, of whom 2 had nonsense, 7 had splice-site, and 2 had out-of-frame indel variants introducing a premature stop codon (Supplemental Table 5). All had 2 or more EHR clinician encounters documented. Notably, however, none of the variants impacted all *TCF7L2* isoforms. In fact, among 9 of 11 patients, only 1 FL-*TCF7L2* isoform was impacted. A neurodevelopmental phenotype could not be confirmed in any PMBB patients in this cohort ($n = 11$), potentially because of a lack of neurodevelopmental phenotypes or limitations of this methodology, and these patients represent a distinct group compared with "likely TRND" patients. The median age of this cohort was 55.06 years (IQR = 54.0-74.5); 7 of the 11 patients (63.6%) were male, and the median BMI was 31.75 (IQR = 26.78-35.44). Comparatively, the median age of the control cohort ($n = 198$) was 60.11 years ($P = .3944$); 119/198 (60.1%) were male ($P = 1$), and the median BMI was 28.60 (IQR = 24.85-32.61; $P = .1539$). The lack of neurodevelopmental phecodes and the inability to confirm a neurodevelopmental phenotype limit the ability to make clear inferences from this cohort to TRND patients. PMBB patients did trend toward a marginally increased risk of some features identified from the "likely TRND" cohort, including congenital deformities

of the limbs/feet (odds ratio [OR] = 9.8; nominal $P = .15$) and myopia (OR = 1.1375; nominal $P = \sim 1$) in the absence of an increased risk of other congenital anomalies; however, these associations were not statistically significant.

Across all 1748 distributed phecodes, T2D with renal manifestations (OR = 5.81; 3/11 vs 12/198; nominal $P = .03$) and chronic kidney disease (OR = 8.91; 3/11 vs 8/198; nominal $P = .01$) were both represented at increased frequency among PMBB patients meeting pLOF *TCF7L2* variant criteria. *TCF7L2* has been historically associated with metabolic disorders; metabolic syndromes, including dysmetabolic syndrome X, were trending towards an association with patients in the PMBB cohort (OR = 6.5; nominal $P = .196$), but the difference was not statistically significant. Some nonmetabolic pathologies were nominally associated with the PMBB patient cohort compared with controls, including unspecified diseases of hair and hair follicles (OR = 8.58; nominal $P = .046$). However, no differences survived multiple hypothesis testing. Neurodevelopmental and other phenotypic comparisons between the "likely TRND" and PMBB pLOF TCF7L2 variant cohorts were not performed because of a lack of overlapping phecodes, including age-relevant diagnoses. A composite summary of ORs and *P*s for the full list of phecodes is provided in Supplemental Table 6.

Discussion

This study characterizes the largest cohort of patients to date with *TCF7L2* pLOF variants constituting TRND. There are several key findings that arise from this study.

First, the spectrum of *TCF7L2* genetic variants implicated in TRND includes missense, nonsense, indel, and splice region variants. CNV variants did not differ phenotypically, except for cardiovascular abnormalities. Consistent with Dias et al,[20] several missense variants localized to the C-terminal ends of the HMG Box and CTNNB1 domains, though de novo variants were also identified between the CTNNB1 and HMG Box domains and upstream of Exon 4. This study identifies *TCF7L2* variants before Exon 4 and could yield insight into functional differences between FL- and DN-*TCF7L2* transcripts. Variants without predicted effects on the coding sequence of DN-*TCF7L2* transcripts were associated with differences in vision/ophthalmological, endocrine, and skin/hair/nail manifestations, though the differences were not statistically significant, and caution should be used in interpreting this result. Additional cases are warranted, and if these differences are re-observed with a greater sample size, one hypothesis is that impacting DN-*TCF7L2* transcripts contributes to these differences. DN-*TCF7L2* has unique patterns of selective tissue expression (eg, DN-*TCF7L2* expression has been detected in murine brain but not in murine liver or pancreas), which can be postulated to contribute to these phenotypic differences.[38,39]

Second, across this expanded cohort, neurodevelopmental, visual, and orthopedic/musculoskeletal abnormalities were most prevalent and likely formed the defining syndromic features of TRND (Figure 3A; Supplemental Figure 1). Craniofacial dysmorphism was observed in a majority of patients (44/60, 73.33%), with heterogeneous features, most commonly abnormal ear morphology, hypertelorism, down-slanted palpebral fissures, as well as frontal bossing and/or a prominent forehead. These findings align with Dias et al,[20] though a nominally higher frequency of ASD and underrepresentation of hypertrichosis was observed; however, the Dias cohort was small ($n = 12$), and this did not survive multiple hypothesis correction. Increased ASD representation may be attributed to selection bias, as ASD repositories and articles were searched to find patients in this study. Importantly, this study expands on and establishes a spectrum of sub-phenotypes across phenotypic categories. Abnormal thorax morphology, Achilles tendon contractures, and clinodactyly were the most prevalent orthopedic/musculoskeletal abnormalities, whereas myopia, strabismus, and astigmatism were the most frequently represented ophthalmologic abnormalities. Phenotypic differences by isoform or variant type of nominal significance did not survive multiple hypothesis testing and should be interpreted with caution. Phenotypic differences also did not cluster by genomic locus. Interestingly, some patients, such as SP1, had a de novo nonsense variant but no reported neurodevelopmental phenotype. Additionally, some patients (eg, P11/18) shared variants but differed in key phenotypic traits. There are several potential explanations for this, including variable expressivity and/or differences in penetrance, additive contributions of other unknown variants, epigenetic differences, differences because of mode of inheritance (mosaicism and differences in mutational burden for germ-line vs post-zygotic variants), and/or limitations in data collection (eg, inter-observer differences) or timing of symptom manifestation. Functional studies on biospecimens from these patients may clarify mechanisms contributing to variable feature manifestation.

Third, this study includes a biobank-based search of thousands of adults for pLOF variants in *TCF7L2*. In a retrospective case-control analysis of PMBB patients, carriers of pLOF *TCF7L2* variants demonstrated a nominally increased risk of T2D with renal manifestations ($P = .03$), but this did not survive multiple hypothesis testing, and there was a trend towards increased BMI between the cohorts. Expanded searches across additional biobanks could clarify the validity of this association. Because of phecode restraints and the retrospective design, pediatric neurodevelopmental phenotypes (and other indirect phenotypic markers of the "likely TRND" group) in these adult PMBB

patients could not be assessed. In addition to the ambiguity added by the absence of a reported neurodevelopmental phenotype, interpretation of these findings is further limited by nominal significance and the absence of functional evidence supporting loss-of-function. Thus, direct comparisons with the pediatric group and inference of relative risk in TRND are not possible. Neurodevelopmental follow-up of adult PMBB patients and validation of functionally reduced *TCF7L2* expression in patients' banked samples are required to: (1) correlate with TRNDs natural history; and (2) estimate TRND population incidence.

Since *TCF7L2*s discovery as a locus strongly associated with T2D,[4] there has been controversial evidence regarding how intronic risk alleles put patients at increased risk for T2D, as a result of agonistic or antagonistic effects on *TCF7L2* expression or non-*TCF7L2*-mediated mechanisms. In the primary "Likely TRND" cohort, only 2 cases of diabetes were identified, one of which was an adult with T2D, potentially reflecting the patient cohort's young median age. Future functional investigation of this relationship is needed through patient-derived sources (eg, induced pluripotent stem cells).[40]

This study has several limitations. First, uniform characterization of patients was challenging because of heterogeneous identification methods, leading to potential data gaps and increased susceptibility to ascertainment bias. Furthermore, as previously noted, searching ASD repositories may introduce selection bias, increasing representation of ASD. We sought to mitigate both of these risks by implementing HPO-standardized ontology terms across cases and differentiating the absence ("0") of phenotypic features vs lack of clinical information ("N/A") to accommodate differences in confidence in age-specific clinical assessments. Making this distinction mitigated the dilution of features that would otherwise be underrepresented by the inclusion of patients from these repositories. Importantly, no other features were grossly enriched among these patients that would likely also be overrepresented with ASD. Secondly, retrospective EHR data analysis has inherent limitations, including incomplete or inaccurate documentation of clinical features, a lack of longitudinal data predating tertiary care at PennMedicine, and potential ascertainment/referral bias. The adult cohort further lacks neurodevelopmental phenotyping, limiting direct comparisons with the pediatric TRND group. Thirdly, by the nature of this investigation, a lack of functional studies limits the validation of some identified variants, and, toward that end, in vitro and *in vivo* validation experiments replicating these variants would further corroborate these findings.

In conclusion, this study establishes an expanded genotypic and phenotypic spectrum for TRND that requires further investigation and functional validation. We have launched a natural history study with Simons Searchlight (https://www.simonssearchlight.org/research/what-we-study/tcf7l2/) that is open for enrollment to further characterize patients with TRND.

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Approved researchers can obtain the Simons Searchlight population as well as the Simons Simplex Collection and SPARK population datasets described in this study by applying at https://base.sfari.org.

This study makes use of data generated by the DECIPHER community. A full list of centers who contributed to the generation of data is available from https://deciphergenomics.org/about/stats and via email from contact@deciphergenomics.org.

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Data Availability

All de-identified genetic and phenotypic data generated from survey collection and during analysis are included in this published article and its supplemental content.

Ethics Declaration

This study received ethics approval from the Institutional Review Board at the University of Pennsylvania (#851470). For data not already available in repositories, clinician and author participants obtained informed consent from patients' families to be represented in this study.

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Conflict of Interest

Amy Crunk is an employee of and may own stock in GeneDx, LCC. Meghan Towne is an employee of and may own stock in Ambry Genetics Corporation. Evan E. Eichler is a scientific advisory board member of Variant Bio, Inc. Caroline Dias and Wendy K. Chung are unpaid advisors for the TRND Network. Sally Nijim, Mimi Kim, Melissa Denish, Michael V. Gonzalez, and David C. Fajgenbaum are unpaid members of the TRND Network. All other authors declare no conflicts of interest.

Additional Information

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