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Upregulation versus loss of function of *NTRK2* in 44 affected individuals leads to 2 distinct neurodevelopmental disorders



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ABSTRACT

Purpose: Heterozygous pathogenic variants in *NTRK2* (HGNC: 8032) have been associated with global developmental delay. However, only scattered cases have been described in small or general studies. The aim of our work was to consolidate our understanding of *NTRK2*-related disorders and to delineate the clinical presentation.

Methods: We reported an extended cohort of 44 affected individuals, of whom 19 are from the literature and 25 were previously unreported.

Results: Our analysis led to splitting the cohort into 2 entities.

Conclusion: One group had variants in the cholesterol-binding motif of the transmembrane domain, with most of these being the recurrent variant c.1301A>G p.(Tyr434Cys). These variants probably lead to upregulation of tropomyosin receptor kinase B activity and to a severe phenotype of developmental delay/intellectual disability, muscular hypotonia, therapy-refractory epilepsy, visual impairment and blindness, and feeding difficulties. The second group had truncating variants or variants that presumably disturb the 3D structure of the protein leading to loss of function. These individuals had a remarkably milder phenotype of developmental delay, obesity, and hyperphagia.

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Introduction

NTRK2 (HGNC: 8032) is associated with 2 different phenotypes in OMIM. This is based on the publications of Hamdan et al¹ for the “Developmental and epileptic encephalopathy 58, DEE58” (OMIM 617830) and Yeo et al,² Miller et al,³ and Hamdan et al¹ for the “Obesity, hyperphagia, and developmental delay, OBHD” (OMIM 613886). Because this correlation is based on a few cases, we sought to recapitulate the literature, collect further individuals, and delineate the phenotype. *NTRK2* encodes tropomyosin receptor kinase B (TRKB), which binds to brain-derived neurotrophic factor (BDNF) and neurotrophin 4.^{4,5} TRKB is mainly expressed in the central nervous system. It is known to regulate the growth and survival of neurons and neuronal plasticity.^{6,7} Learning and hippocampal activity are also influenced by changes in this receptor.⁸

There have been several studies that functionally characterized previously published variants.^{2,9–11} One of these studies, conducted by Sonoyama et al, functionally analyzed variants of affected individuals with OBHD with neurons and PC12 cells. They found that of the 10 analyzed variants, 5 were located in the kinase domain and lead to loss of function of TrkB in different cell lines, causing obesity and impaired hippocampal synaptogenesis.¹¹ This hypothesis that OBHD is caused by loss-of-function (LoF) variants was already proposed by the mouse model of Xu et al, who found that mutant mice with a quarter of the wild-type expression of TrkB develop hyperphagic obesity.¹² Long et al analyzed 2 variants located in 2 different domains. They supported the findings of Sonoyama et al showing that variants in the kinase domain result in loss of function, whereas the recurrent variant c.1301A>G p.(Tyr434Cys), located in the transmembrane domain, upregulates the TRKB pathways. The increase of TRKB signaling has previously been linked to seizures. A study by Almoguera et al proposes that it causes a drug-resistant form of epilepsy.¹³

Although there has been a substantial amount of literature on *NTRK2* and the encoded protein TRKB, a comprehensive summary describing the phenotype in larger cohorts is lacking. Our search of the literature identified 19 affected individuals with clinically relevant variants (variants of uncertain significance, likely pathogenic, and pathogenic) in *NTRK2*^{1–3,10,11,14–16} with OBHD, DEE58, or with an unspecified neurodevelopmental delay (NDD) phenotype. We further added 25 patients that were not yet published. Our cohort of 44 affected individuals and a thorough review of the literature combined with molecular modeling allowed us to describe the correlation of variants in different domains to a phenotypic spectrum of the *NTRK2*-related disorder.

Materials and Methods

Literature review

To identify reports of patients with *NTRK2* variants, we searched PubMed for articles that included “*NTRK2*” or “TRKB” in the title or abstract. We reviewed the main text of the studies that seemed promising, ie, whether the studies mentioned individuals with an *NTRK2* variant. If the articles included references to studies with additional individuals, we rechecked whether we had already included these articles. Furthermore, we checked the variant databases Human Gene Mutation Database and ClinVar for possible patients.

Study participants

Through GeneMatcher and other collaborative efforts,^{17,18} we gathered 25 previously unreported affected individuals with disease-associated variants in *NTRK2*. All families or responsible individuals provided written consent. The study was led by the Institute of Human Genetics at the University of Leipzig Medical Center in cooperation with universities, research institutes, and private health providers worldwide. The clinical data were compiled into a standardized table (Table 1) from the participating institutes. We also incorporated information from 19 affected individuals with previously published heterozygous missense variants in *NTRK2* into this table, which we gathered from our literature review. We provided no information on ethnicity as this is irrelevant considering that most variants are de novo and ultrarare. Furthermore, we unfortunately had a heterogeneous depth and professionalism in the description of clinical information.

Genetic analyses

All variants were detected via massive parallel sequencing techniques, such as exome or genome analysis. For de novo variants, the analysis was a trio, or the de novo status was confirmed via Sanger sequencing. The impact of the splice variant was confirmed with RNA analysis. To obtain a comprehensive assessment of the in silico predicted pathogenicity of the variants, all were analyzed using REVEL,¹⁹ CADD,²⁰ and SpliceAI²¹ (Table 2; see the paragraph below on molecular modeling for further details). We used gnomAD v4.0.0 to distinguish whether our variants of interest had been reported in the presumed healthy population. We mapped the variants to the Matched Annotation from the NCBI and EMBL-EBI transcript of *NTRK2* (NM_006180.6) GRCh37.²² All variants were classified according to the

guidelines of the American College of Medical Genetics and Genomics.²³ Unless stated otherwise, frequencies of clinical features were described as the number of affected versus assessed individuals. Cases from the literature with weak evidence to support clinical relevance, such as inherited variants and/or in silico evidence strongly suggesting the variant was not pathogenic, were not included in this overview.

Facial analysis

We conducted facial analysis on only 5 affected individuals who consented to the usage of their photos by Gestalt-Matcher.^{24,25} We used 7459 images of 449 disorders in the GestaltMatcher Database²⁶ as the control cohort and measured the degree of similarity of the facial features of these 5 affected individuals in comparison with the control cohort.

Molecular modeling

Structure-based predictions of pathogenicity were performed using AlphaMissense²⁷ for all missense variants. Detailed modeling of variants was done with Variant Interpretation and Prediction Using Rosetta²⁸ using a TRKB model generated with ColabFold.²⁹ Structural analysis of the interaction with neurotrophin was based on the complex crystal structure of neurotrophin-4/5 bound to the neurotrophin binding domain of TRKB³⁰ (PDB: 1HCF).

Results

Cohort overview

We established a cohort of 44 affected individuals (individual 1 to individual 44). Of these, 19 have been previously reported in the literature,^{1-3,10,11,14-16} and 25 were new. Eighteen had the recurrent variant NM_006180.6:c.1301A>G p.(Tyr434Cys). In 22 individuals, the identified variant was de novo (including 9 with c.1301A>G p.(Tyr434Cys)), whereas in 17 individuals, the inheritance was unknown, and in 1 case, the variant was inherited from an affected father, who was not part of our cohort. Ages of the individuals ranged from 6 months to 45 years (median was 7 years). Individual 31 and individual 32 were affected siblings.

In total, we described 25 different variants. Of these, 20 were missense variants, 3 were splice variants, and 2 were nonsense variants. In individual 2, with a splice variant at +3, RNA analysis confirmed in-frame (72 bp) skipping of exon 5 (second coding exon).

Based on the American College of Medical Genetics and Genomics variant classification criteria,²³ 18 were classified

as variants of uncertain significance, 6 were likely pathogenic variants, and 1 was a pathogenic variant (c.1301A>G p.(Tyr434Cys)) (Table 1). All variants were absent from gnomAD 4.0.0.

Clinical overview

Because not all clinical information was available for all affected individuals, we presented varying absolute numbers depending on the individuals for whom we had information on the respective clinical features.

Global and social development

All assessed individuals had NDD and speech delay (36/36). Almost all had a motor delay (31/33). Although 18 of 30 assessed individuals were nonverbal (most of them were heterozygote for c.1279G>T p.(Gly427Cys) or c.1301A>G p.(Tyr434Cys)), some had poor speech because of limited vocabulary (6/30) or were verbal but had difficulty with pronunciation for example, because of jaw malformation (2/30). Four individuals were able to speak normally at their most recent examination (4/30). Information on intellectual disability was available for 31 individuals. All but 1 had an intellectual disability (30/31), ranging from moderate learning difficulties (11/31) to mild or moderate (6/31), or severe or profound intellectual disability (13/31, predominated by individuals with c.1279G>T p.(Gly427Cys) or c.1301A>G p.(Tyr434Cys)). Regarding behavioral abnormalities, all but 3 were affected (30/33). More than a third of the assessed individuals were formally diagnosed with autism spectrum disorder (13/33), whereas another third presented with autistic features without a formal diagnosis (10/33). Difficulties controlling emotions were reported in 16 of 26 individuals, leading to aggressive outbursts or self-mutilation in 7 and 2 individuals, respectively. However, the opposite was also described because 2 individuals (individual 25 and individual 41) were excessively placid.

Epilepsy

Twenty-five individuals were diagnosed with epilepsy (25/36). The median age of onset was 5.5 months. Of all the individuals with epilepsy, 15 had seizures that were refractory to treatment (15/25, 13 of them had c.1279G>T p.(Gly427Cys) or c.1301A>G p.(Tyr434Cys)).

Neuromotor features

Thirteen, ie, almost half of the individuals, were ambulatory, 1 could not yet walk but was slowly reaching motor milestones at 1.6 years old (14/29), whereas 15 remained non-ambulatory. Twenty-four individuals presented with neuromuscular hypotonia (24/33). Another 5 individuals had a choreiform movement disorder (5/33), 3 presented with cerebral palsy (3/33), and 2 individuals had a mild resting tremor (2/33).

Weight, growth, and dysmorphic features

Nine individuals were at a healthy weight and had a body-mass index (BMI) in the 5th to 85th percentiles for children (9/31), 3 were underweight (3/31), 3 were overweight with a BMI in the 85th to 95th percentile (3/31), and 16 were obese with a BMI $\geq$ 95th percentile (16/31). None of the individuals who were overweight or obese had the variants c.1279G>T p.(Gly427Cys) or c.1301A>G p.(Tyr434Cys). Within the overweight/obese group, 8 presented with hyperphagia. All underweight individuals required enteral feeding. Regarding height, most were in the normal range, (16/25), 3 were short (<5th percentile, 3/25), and 6 were tall (>95th percentile, 6/25). Eight had microcephaly (8/23), 6 had macrocephaly (6/23), and 1 had an obstructive hydrocephalus. Based on clinical descriptions, there was no consistent facial dysmorphism; facial features were unremarkable, or no information on facial features was provided. We were able to perform facial analysis with GestaltMatcher on 5 individuals for whom photographs were provided. Of the 5 individuals, we found that 2 pairs exhibited similarity in facial gestalt (individuals 3 and 29, and individuals 4 and 44). However, this was not the case for individuals 29 and 44, although they otherwise shared similar clinical features and variants in the tyrosine kinase domain (Supplemental Figure 1).

Ophthalmological findings

Information on vision was available for 30 individuals. In about three quarters (23/30), vision was impaired. More than half were blind (17/30). Blindness was attributed to optic nerve hypoplasia/pallor in 11 individuals, 7 individuals had cortical visual impairment. Six individuals (6/30) had nystagmus or astigmatism.

Additional findings

In several cases, additional features were reported. However, information on other affected individuals was often lacking for these specific findings. Impaired short-term memory was reported in 4 individuals, high tolerance to pain stimuli was noted in 6 individuals, 11 individuals had feeding difficulties, whereas 6 required enteral feeding at ages ranging from 2.9 to 20 years (median of 5.5 years). Eight individuals had various respiratory issues, including 5 with obstructive sleep apnea (5/9), and 2 who were tracheostomy-dependent (2/9). The obstructive sleep apnea was highly likely a consequence of obesity in the affected individuals. See details in Supplemental Table 1 and in the supplemental case reports.

Structural analysis

TRKB is a transmembrane protein with a single transmembrane segment. The extracellular part contains several globular domains, namely leucine-rich repeats (residues 32-195) and 2 Ig-fold domains (residues 196-285 and 286-380).

The intracellular part harbors the globular kinase domain (residues 547-830).

Our analyses predicted that the deletion of the amino acids 71 to 96, because of the splice variant c.287+3G>C, disrupts the structure of the leucine-rich repeats. Additionally, c.302C>A p.(Ser101Tyr) was predicted to destabilize this structure. The c.986A>G p.(Tyr329Cys) substitution replaces Tyr329, which forms interactions with neurotrophin, by a smaller cysteine residue, and was predicted to decrease neurotrophin binding affinity. Both missense variants were also classified as likely pathogenic by AlphaMissense (Supplemental Table 2).

AlphaMissense classified all variants of the intracellular kinase domain (residues 547-830) as pathogenic, except for c.2000C>G p.(Ser667Trp) (Supplementary Table 2). But we considered c.2000C>G p.(Ser667Trp) pathogenic by specific modeling with VIPUR. Notably, some variants in the kinase domain were predicted not only to destabilize the 3D structure but also to affect the 2 conserved tyrosines 722 and 723 in the activation segment of the kinase, which becomes phosphorylated upon kinase activation.³¹

Individual 25 heterozygote for c.1438G>A p.(Gly480Ser), which is located in the nonglobular intracellular region and present only in some *NTRK2* isoforms with weaker expression. This variant was classified as likely benign by AlphaMissense, and specific modeling with VIPUR did not detect any unfavorable structural effects for this variant. In line with this observation, the CADD and the REVEL values of this variant were relatively weak. The phenotype of the affected individual was not fully aligned with other affected individuals. Reanalysis of the data did not reveal other variants of relevance. Thus, we were not certain whether this is the potentially causative variant in the affected individual.

Most interestingly, 2 of the variants with severe phenotypes (c.1279G>T p.(Tyr427Cys) and c.1301A>G p.(Tyr434Cys)) are in a unique location within the transmembrane domain of TRKB, which contains a cholesterol-binding motif³² (residues Arg428-Val438). The c.1301A>G p.(Tyr434Cys) and c.1279G>T p.(Tyr427Cys) exchanges resulted in rather low AlphaMissense scores (Supplemental Table 2), which was in line with the results from manual modeling, suggesting that both exchanges do not critically disturb the overall TRKB structure. However, previous studies have shown that cholesterol interaction was lost in the single p.(Tyr433Phe) mutant of murine *Ntrk2* (corresponding to c.1301A>G p.(Tyr434Cys) in human TRKB), indicating the central role of the tyrosine in this interaction with cholesterol.³³ In addition to compromised cholesterol sensing, the p.(Tyr433Phe) variant in mice showed an altered response to BDNF in terms of *Ntrk2*-dimerization and *Ntrk2*-phosphorylation, and changes in the translocation to lipid rafts.³³ Based on these findings, it appeared most likely that in humans, c.1301A>G p.(Tyr434Cys) and c.1279G>T p.(Gly427Cys) do not disrupt TRKB structure, but instead induce altered

functional properties (see the Discussion section for further details).

Splitting phenotypes by variant types

Literature, clinical information, and structural analyses clearly showed 2 distinct variant and phenotype groups.

The 18 affected individuals with the recurrent variant c.1301A>G p.(Tyr434Cys) in the transmembrane domain had a profound or severe intellectual disability and severe motor delay with global/central hypotonia. Remarkably, none exhibited hyperphagia and all had normal weight or were even underweight because of feeding difficulties that partially required enteral feeding. All except 1 individual had seizures, and the individual without seizures had an abnormal electroencephalogram with focal epileptiform discharges in the left temporo-occipital region (individual 16). Nine individuals, with a median age of 3 years, had hypsarrhythmia. It was unclear whether hypsarrhythmia persisted at older ages. Seventeen of 18 individuals had significant visual impairment, with the majority being blind. It appeared that neurological structures, particularly the optic nerve, were implicated in the cases of blindness. Also, individual 5 with the variant c.1279G>T p.(Gly427Cys) seemed to be part of this group.

On the other hand, 17 individuals (individuals 28–44) had a missense variant in the tyrosine kinase domain (starting at AA551; Figure 1). All these individuals were overweight or obese. They had global developmental delay, but the language development in this group was remarkably better and the intellectual disability was mild to moderate. Assessed individuals achieved walking at a median age of 25.5 months. Moreover, most individuals demonstrated catch-up in motor development. Seizures were reported in only 4 individuals, of which 2 had absence seizures, whereas specific information was lacking for the other 2. Visual impairments were also less severe in this group; only 2 individuals had strabismus, and 1 was reported with cortical visual impairment. None was blind. Impaired short-term memory was noted in 4 individuals, impaired nociception in 4 individuals, and 3 obese individuals had obstructive sleep apnea requiring continuous positive airway pressure, which was probably a consequence of their obesity (Figure 2).

Most of the remaining 8 individuals seemed to be part of the latter, mild group. Please refer to the Discussion.

Discussion

General symptoms of pathogenic variants in *NTRK2*

All herein-described variants in *NTRK2* led to a neurodevelopmental disorder. This was unsurprising because of the

known functions of the encoded protein TRKB. For example, BDNF, the neurotransmitter binding to TRKB, was known to be essential for a broad spectrum of neurological phenotypes.³⁴ Also, mouse models showed that *Ntrk2* is crucial in hippocampal long-term potential and learning.^{35,36}

Variants in the transmembrane cholesterol-binding sequence motif

The recurrent variant NM_006180.6:c.1301A>G p.(Tyr434Cys), had been previously reported in 8 individuals,^{1,16} and we reported 10 additional individuals. A potential explanation for the recurrence of this variant was that it had been associated with NDD for several years, making its identification easier. It seemed more likely that position 434 may be prone to mutagenesis or that this variant may lead to a selective clonal expansion in the gonads. Explaining this was beyond the scope of this study. The common finding of seizures in this group was striking because Torres et al found a statistically significant association with temporal lobe epilepsy or epilepsy in general and variants in *NTRK2*.³⁷ Among those with the recurrent variant with seizures, 13 had seizures that were refractory to treatment, whereas others required multiple anticonvulsants to achieve seizure control. This finding supported the hypothesis by Almoguera et al, who proposed that variants in *NTRK2* increasing TRKB signaling might lead to drug-resistant epilepsy.¹³

Besides the recurrent variant, c.1279G>T p.(Gly427Cys) was another variant in this domain. The single individual reported with this variant, individual 5, had similar clinical features to those with the recurrent variant. She had severe intellectual disability, was nonverbal and nonambulatory, and had drug-resistant epilepsy. She was severely underweight, and tracheostomy and gastrostomy tube dependent.

The c.1301A>G p.(Tyr434Cys) and c.1279G>T p.(Gly427Cys) missense variants are both located in the nonglobular transmembrane domain of TRKB and affect a cholesterol-binding sequence motif. This segment of the gene is not conserved (MetaDome); however, findings suggested that the location of the variants in the cholesterol-binding sequence in the transmembrane domain and the cysteine residues are prerequisites for the development of the observed severe phenotype. The cysteine residues may confer a special structural property, such as facilitating homodimer formation via intermolecular disulfide bonds, thereby enhancing TRKB activity. Further experimental studies are required to gain a complete structural and mechanistic understanding of the function of these cysteine variants in the transmembrane domain. Considering the similarities in phenotype and the shared pathomechanism, we believe that lumping these individuals into 1 group for the severe phenotype of epileptic encephalopathy and neurodevelopmental delay is plausible.

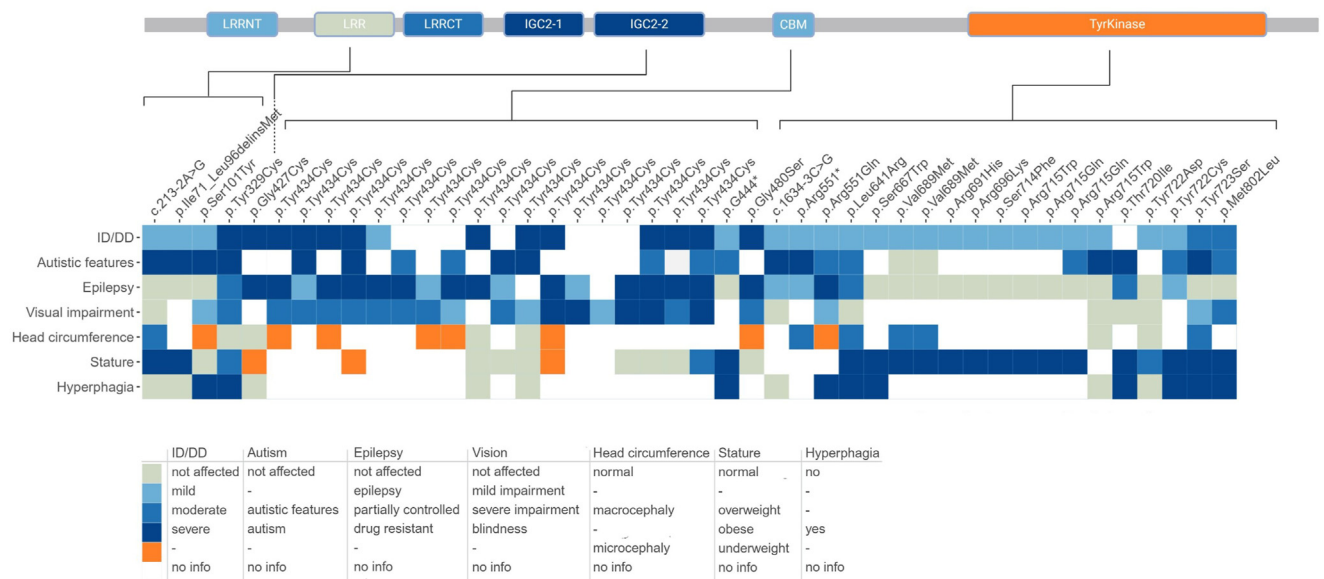


Figure 1 Distribution of symptoms within the cohort in regard to the variant's localization. The variants are aligned vertically according to the amino acid position, whereas the main symptoms are presented below. The severity of symptoms is represented by a color gradient. Intellectual disability or developmental delay is shown in the order of light to dark blue as mild, moderate, or severe. For epilepsy, the gradient reflects responsiveness to treatment, partial control, or drug resistance. Vision ranges from minor impairments to severe impairment and blindness. Autism is divided into autistic features or a diagnosis of autism. Body mass is shown as overweight in blue, obese in dark blue, and underweight in orange. Hyperphagia is dark blue. Macrocephaly in dark blue, microcephaly in orange. Above is the schematic structure of TRKB with its domains, indicating the domains in which variants are located. The domains from left to right are leucine-rich repeats N-terminal (LRRNT), leucine-rich repeats (LRR), tyrosine kinase receptor C2 Ig-like domain (LRRCT), immunoglobulin I-set domain 1 (IGC2-1), immunoglobulin I-set domain 2 (IGC2-2), cholesterol-binding motif in the transmembrane domain (CBM), and tyrosine kinase domain (TyrKinase).

Variants with loss of function

Seventeen individuals (individuals 28-44) had a missense variant in the tyrosine kinase domain (starting at AA551; Figure 1). Affected individuals had mild to moderate developmental delay; however, they caught up regarding motor developmental delay. All individuals were overweight or obese. Of note, Xu et al found that mice with a hypomorphic variant were overweight with increased food intake as well, suggesting that TrkB was an important element in a signaling cascade responsible for the regulation of energy balance and feeding behavior.¹² Our molecular modeling showed that the variants of this domain lead to a destabilization of the 3D structure, which would support that they are LoF variants.

Five individuals (individuals 1, 2, 24, 26, and 27) had a splice or stop variant that likely results in nonsense-mediated decay. Because molecular modeling clearly predicted that the missense variant in individual 3, c.302C>A p.(Ser101Tyr), disrupts the 3D structure of the globular extracellular domain, we summed the symptoms of the individuals 1, 2, 3, 24, 26, and 27 together as probable LoF variants. All but 1 (individual 2) had mild or moderate intellectual disability. All but individual 3 were morbidly obese. However, individual 3 had hyperphagia. Only individuals 26 and 27 had epilepsy. Because our structural analysis predicted the disruption of the 3D structure for all

variants in the tyrosine kinase domain, we suggested pooling the variants of this domain with the LoF variants above into 1 group, which, contrary to the variants in the transmembrane cholesterol-binding sequence motif, leads to loss-of-function and a milder phenotype of obesity and hyperphagia (Figure 2).

Unclear cases

Individual 25 was included in this cohort as she had a de novo missense variant in *NTRK2* and NDD. Clinically, she was similar to individuals with variants in the transmembrane domain because she had a profound intellectual disability, treatment-refractory epilepsy, functional blindness because of profound cortical visual impairment, dependence on enteral feeds, and was underweight. However, as noted in the results, molecular modeling and in silico predictions did not predict a significant structural effect of the variant that was located in a low-expressed transcript. Additionally, the affected individual had features that were unique or rarely noted in other individuals, such as progressive brain atrophy, hypothermia, and bradycardia. Therefore, it was unclear whether the variant in this position was (solely) responsible for her presentation or whether other unidentified genetic or nongenetic factors played a role.



Figure 2 Radar chart of symptoms comparing upregulation and loss of function of TRKB. Illustration of symptoms comparing upregulation (missense variants in the cholesterol-binding motif) and loss of function aiding to delineate the frequency of symptoms. The symptoms are shown in respective corners of the chart. The concentric circles demonstrate the lines for 0%, 50%, and 100%.

Also still not fully clarified was individual 4, who had a c.986A>G p.(Tyr329Cys) variant in the globular extracellular domain (immunoglobulin I-set domain 2; [Figure 1](#)). He had severe intellectual disability, was not hypotonic, and was able to walk, although he had difficulty with balance. He had hypoplastic optic nerves and significant visual impairment. He was overweight and exhibited hyperphagia. The c.986A>G p.(Tyr329Cys) variant was predicted to reduce binding affinity for neurotrophin, resulting in a loss of function. However, the affected individual showed symptoms of both phenotypes of *NTRK2*. Further analysis is needed to clarify whether individuals 4 and 25 have other genetic variants that may explain the symptoms or whether the splitting of the 2 phenotypes of *NTRK2* is not yet perfect, as it seemed to be in other cases.

Conclusion

Our findings corroborated existing literature, showing that an increase or a decrease in TRKB signaling results in 2 distinct clinical presentations. We, therefore, supported distinctly splitting individuals with pathogenic variants in *NTRK2* into 2 groups based on phenotype-genotype

correlation: pathogenic variants in the small transmembrane segment of the cholesterol-binding sequence leading to upregulation of TRKB are associated with severe developmental delay and epileptic encephalopathy, blindness, microcephaly, and other features, whereas variants resulting in absent protein or altered protein function were associated with milder developmental delay, obesity, hyperphagia, and behavioral disorders.

The ClinGen clinical validity framework for the evaluation of the gene-disease relationship was applied, including the clinical/genetic data from the newly described patients. A strong level of evidence was achieved for the association of *NTRK2* to autosomal dominant Developmental and epileptic encephalopathy 58 (#MIM 617830), and to Obesity, hyperphagia, and developmental delay (#MIM 613886), using both genetic and functional evidence.

Data Availability

All variants were uploaded to ClinVar either in the context of submitting this manuscript or because the coauthors have done this in advance. All of the clinical information that has

been gathered is included in either [Supplemental Table 1](#) or in the [Supplemental Case Reports](#).

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Author Contributions

Conceptualization: E.B., R.A.J.; Data Curation: E.B., J.D.R., E.Z., L.W., A.W., J.W., A.T., A.T.-W., B.S., R.R., J.P., J.O., O.M., A.M.-F., M.L., J.L., D.L., N.J.L., I.K., M.K., E.K., G.J., V.J., B.I., D.H., A.M.H., T.B.H., N.B.G., D.D., M.D., W.D., K.A.D.L.R.P., M.D., J.C., S.C., B.C., A.B., L.B., B.G.B., M.A., S.S.A., M.N.A., D.A., A.A., A.B.-A.; Investigation: E.B., H.S.; Supervision: R.A.J.; Visualization: R.T.J., E.B.; Writing-original draft: E.B., R.A.J., H.S.; Writing-review and editing: E.B., R.A.J., J.D.R., L.B., J.L., E.K., M.D., B.C., N.L., A.B.-A., J.V., J.C., H.S.

Ethics Declaration

All probands were clinically examined by experienced pediatricians and/or human geneticists and were enrolled and sampled according to standard local practice in approved human subjects' protocols as part of routine clinical care at the respective institutes. The project was approved by the ethics committee of the University of Leipzig, Germany (224/16-ek and 402/16-ek) and was conducted in concordance with the declaration of Helsinki. Written informed consent of all examined probands or their legal representatives was obtained after advice and information about the risks and benefits of the study.

Conflict of Interest

The authors declare no conflict of interest. A.B.-A. and E.Z. are employees of CENTOGENE GmbH. A.T. is an employee of and may own stock in GeneDx, LLC.

Additional Information

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