

Homozygous Missense Variant in the N-Terminal Region of ANK3 Gene Is Associated with Developmental Delay, Seizures, Speech Abnormality, and Aggressive Behavior

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Keywords

Autosomal recessive · Homozygous · Missense variant · ANK3 · Intellectual disability · Whole exome sequencing

Abstract

Introduction: Intellectual disability (ID) is a lifelong disability that affects an individual's learning capacity and adaptive behavior. Such individuals depend on their families for day-to-day survival and pose a significant challenge to the healthcare system, especially in developing countries. ID is a heterogeneous condition, and genetic studies are essential to unravel the underlying cellular pathway for brain development and functioning. **Methods:** Here we studied a female index patient, born to a consanguineous Pakistani couple,

showing clinical symptoms of ID, ataxia, hypotonia, developmental delay, seizures, speech abnormality, and aggressive behavior. Whole exome sequencing (WES) coupled with Sanger sequencing was performed for molecular diagnosis. Further, 3D protein modeling was performed to see the effect of variant on protein structure. **Results:** WES identified a novel homozygous missense variant (c.178T>C; p.Tyr60His) in the ANK3 gene. In silico analysis and 3-dimensional (3D) protein modeling supports the deleterious impact of this variant on the encoding protein, which compromises the protein's overall structure and function. **Conclusion:** Our finding supports the clinical and genetic diversity of the ANK3 gene as a plausible candidate gene for ID syndrome.

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Introduction

Intelligence is a complex polygenic human trait, and understanding molecular and biological pathways involved in learning and memory can solve the complex puzzle of how cognition develops. Intellectual disability (ID) is defined as a deficit in an individual's learning and adaptive behavior at an early age of onset [American Psychiatric Association, 2013]. It is one of the major medical, and cognitive disorders with a prevalence of 1–3% in the population worldwide [Leonard and Wen, 2002]. ID often exists with other disabling mental conditions such as autism, attention deficit hyperactivity disorder, epilepsy, schizophrenia, bipolar disorder, or depression. Almost half of the cases appear to have a genetic explanation that ranges from cytogenetically visible abnormalities to monogenic defects [Flint, 2001; Ropers, 2010; Tucker-Drob et al., 2013]. Intellectual disability is a genetically heterogeneous condition, and more than 700 genes have been identified to cause ID alone or as a part of the syndrome. Research in X-linked ID has identified more than 100 disease-causing genes on the X chromosome that play a role in cognition; however, research into autosomal causes of ID is still ongoing [Visser et al., 2016].

Next-generation sequencing (NGS) technology has created a paradigm shift in the genetic diagnosis of common and rare diseases [Black et al., 2015; Hekim et al., 2016]. The application of NGS also led to a dramatic increase in disease gene identification in familial and sporadic ID cases [Grozeva et al., 2015; Hu et al., 2016]. Identifying genes that cause ID is valuable for early diagnosis and genetic counseling. Still, it is noteworthy that an etiological connection has yet to be built for many of the genes identified through high throughput sequencing technologies.

Here, we report a homozygous missense variant in *ANK3* gene that causes syndromic ID in an affected female born to a consanguineous couple.

Materials and Methods

Ethical approval of the study was obtained from the Institutional review board (IRB) of the UOE (UE/S&T/2021/222), as per the guideline of the declaration of Helsinki. Furthermore, written informed consent to publish the molecular data and case report was obtained from the caretaker of the affected individual.

Whole Exome Sequencing and Data Analysis

Peripheral blood samples of the affected and normal individuals were collected in EDTA containing Vacutainer. Genomic DNA was extracted using a GenElute™ Blood Genomic DNA Kit (Sig-

ma-Aldrich, USA), and the extracted DNA was quantified using a Nanodrop-1000 spectrophotometer (Thermal Scientific, Wilmington, USA).

DNA sample of the affected female (II-2) was subjected to WES by using HiSeq 2500 systems (Illumina, San Diego, CA, USA) as described previously [Hayat et al., 2020; Umair et al., 2021]. The exomic sequences were enriched by using SureSelect XT Human All Exon 50 Mb kit version 5 (Agilent Technologies, Santa Clara, CA, USA). The sequence reads were aligned with the human genome assembly hg19 (GRCh37) using Burrows-Wheeler Aligner (BWA v 0.7.5), and variants were called by using tools such as PINDEL (v 0.2.4 t), SAM tools (v 0.1.18), and Exome Depth (v1.0.0). The Bam file was visualized with Golden Helix Genome Browser to see coverage of candidate ID genes in WES data. Variants obtained were analyzed using BaseSpace (Illumina; <https://basespace.illumina.com/>) using standard methods.

Variant Pathogenicity

To evaluate the pathogenicity of the variants, various online protein prediction tools were used including MutationTaster, EIGEN, PROVEAN, MetaRNN, MutPred, and FATHMM-MKL, etc. The frequency of the identified candidate variant was further cross-checked with in-house 2000+ exomes, Exome Aggregation Consortium (ExAC), 1000 Genomes, and genome aggregation database (gnomAD). Conservation of the mutated amino acid was checked across different species using NCBI-Homologene (<https://www.ncbi.nlm.nih.gov/homologene>).

Sanger Sequencing

Co-segregation analysis of the prioritized variants was performed by using the standard Sanger sequencing method [Waqas et al., 2022; Nayab et al., 2021]. DNA sequences flanking the prioritized variants were downloaded from Ensembl genome browser (<https://m.ensembl.org>), and PCR primers were designed using primer 3 software (<http://primer3.ut.ee>). Bi-directional Sanger sequencing was performed by using DTCS sequencing kit (Beckman Coulter, USA), and the data were analyzed by using BioEdit-7.2 by aligning sanger sequenced ABI files to the reference sequence.

3D Protein Modeling

Protein structure prediction was performed for the novel homozygous missense variant (c.178T>C; p.Tyr60His) in the *ANK3* gene. The partial amino acid sequence of Ankyrin-3 (ANK3) encoding protein was retrieved from the UniProt protein sequence accessible database with accession number Q12955 in FASTA format [Venter et al., 2001]. Due to the non-availability of the solved structure as a limitation of *ANK3* experimentally, its amino acid sequence was submitted to the I-TASSER server for the prediction of structure [Roy et al., 2010; Yang et al., 2015]. Both the predicted structures were aligned and displayed using PyMol (Schrödinger, LLC) program.

Results

Clinical Report

The proband (II-2) is born to a consanguineous Pakistani couple with two unaffected siblings and had no history of disease trait in the extended family (Fig. 1a). The

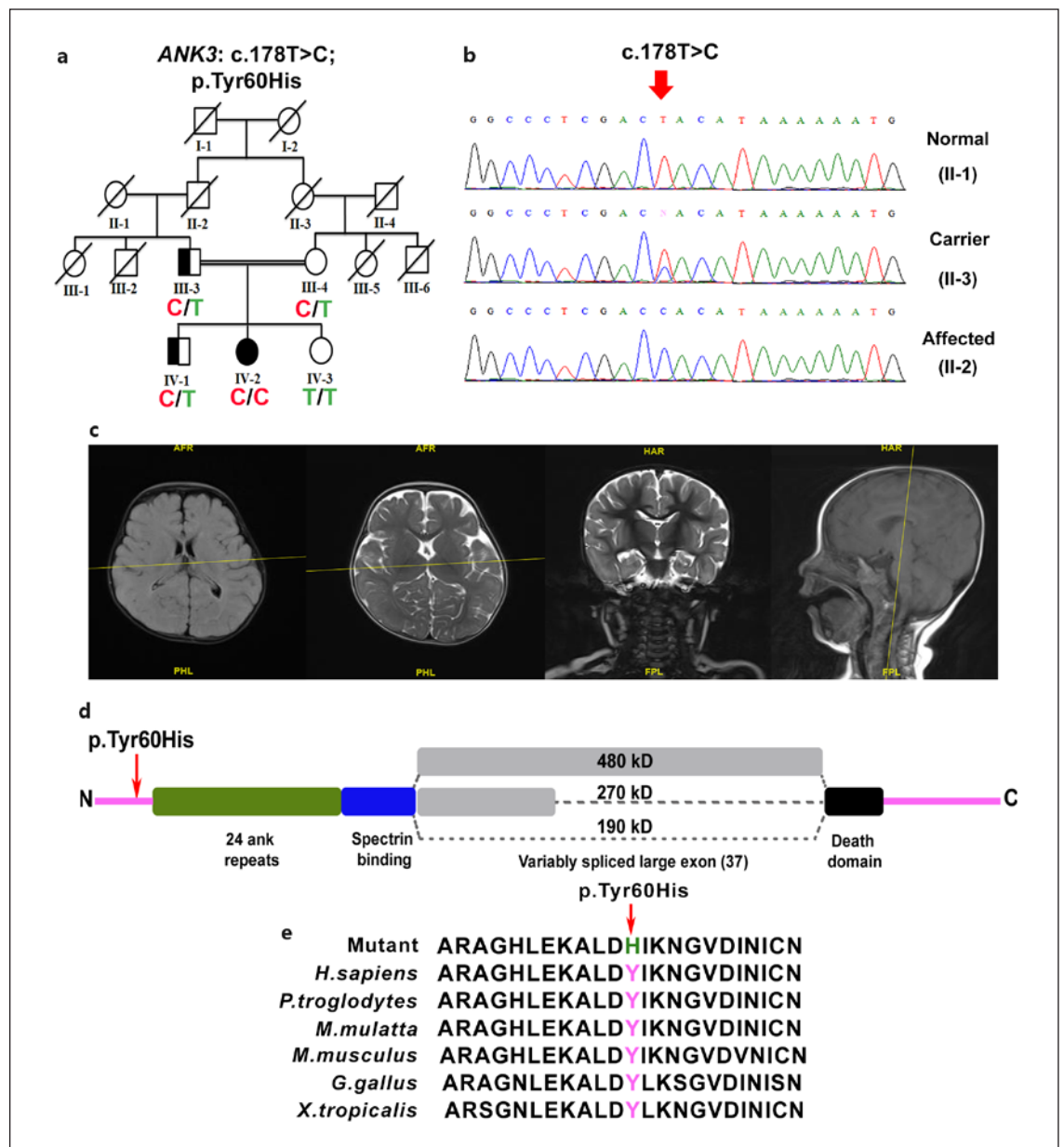


Fig. 1. Pedigree and haplotype of the family. **a** Haplotype of the c.178T>C variant in the family where parents (III-3, III-4) and brother (IV-1) are heterozygous carriers and affected individual (IV-2) is homozygous affected, and sister (IV-3) is homozygous wild type. Pedigree depicts autosomal inheritance pattern with parents having a consanguineous marriage. **b** Sanger sequencing data of normal, carrier, and affected individual. **c** T2 weighted and

flair MRI scan of an affected individual showing a mild bilateral increase in periventricular signal, ex vacuo enlargement of the ventricular atrium, splenium atrophy, and mild volume loss of the anterior body of corpus callosum. **d** Different domains present in the three isoforms of ANK3 protein. **e** Amino acid conservation of Tyr60 across different species representation.

proband was a product of normal spontaneous vaginal delivery (NSVD) with a birth weight of 3.8 kg. At the age of 1.9 years, she exhibited the following growth parameters: height 86 cm (10–25 percentile), weight 10.5 kg (<5th percentile), and head circumference of 44 cm (<5th

percentile), while she exhibited 111 cm height (10–25 percentile), 16.5 kg weight (<5th percentile), and 48.3 cm head circumference (<5th percentile) at the age of 6 years. The body mass index (BMI) was calculated at the age of 2 and 3 years, and she showed normal growth with BMI

Table 1. Patient and cyst characteristics

Variable	Total
Age, median (IQR), years	67 (56–73)
Gender, n (%)	
Female	25 (62.5)
Male	15 (37.5)
Presentation	
Incidental	29 (72.5)
Abdominal pain	6 (15.0)
Pancreatitis	4 (10.0)
Weight loss	1 (2.5)
Indication for EUS-FNA, n (%)	
Mucinous with WF	36 (90.0)
Indeterminate cyst type	4 (10.0)
Cyst location, n (%)	
Head	22 (55.0)
Body	7 (17.5)
Tail	11 (27.5)
Cyst size, median (IQR), mm	30.0 (15–75)
Cyst CEA levels, median (IQR), ng/mL*	63 (7.8–1,271.8)
Cyst glucose levels, median (IQR), mg/dL [#]	10 (10.0–47.5)
Cyst amylase levels, median (IQR), U/L [§]	566 (40.0–18,540.0)
Final diagnosis, n (%)	
IPMN	26 (65.0)
SCA	6 (15.0)
MCN	3 (7.5)
PC	3 (7.5)
SPN	2 (5.0)
Follow-up, median (IQR), months	12.0 (4.0–18.8)

EUS-FNA, endoscopic ultrasound fine needle aspiration; WF, worrisome features; CEA, carcinoembryonic antigen; IPMN, intraductal papillary mucinous neoplasm; SCA, serous cystadenoma; MCN, mucinous cystic neoplasm; SPN, solid pseudopapillary neoplasm; PC, pseudocyst; IQR, interquartile range. *Data available for 30 lesions. [#]Data available for 29 lesions. [§]Data available for 25 lesions.

values of 15.8 (32nd percentile) and 14.4 (12th percentile), respectively. The affected female showed clinical symptoms of ID, growth retardation, hypotonia, ataxia, speech impairment, seizures, and aggressive behavior.

The first concern arose at the age of 4–5 months, when parents observed developmental delay and febrile seizure, which were resolved spontaneously with no medications. The proband also has two other healthy siblings (II-1, II-3). Her developmental milestones were delayed as she started to sit at 9 months, crawled at 13 months. She also suffers from a speech delay and speaks one- to two-word sentences with a non-fluent pattern. Features such as hearing impairment and eye anomaly were not observed (Table 1).

The biochemical analysis of serum, acylcarnitine, ammonia, lactic acid, creatine kinase, coagulation profile, lipid profile, total homocysteine was normal. The estimation of urine amino acids, organic acids, creatine, guanidinoacetate, and purine/pyrimidine showed unremarkable findings. The brain MRI revealed a mild bilateral increase in periventricular signal with some ex vacuo enlargement of the ventricular atrium. Normal morphology and signal intensity of the supra- and infratentorial structures and no restriction diffusion was observed. The ventricular system appears normal in size and configuration. Atrophy of the splenium and mild volume loss of the anterior body of the corpus callosum was also observed (Fig. 1c). EEG examination was performed at a 21-channel sleep EEG recording with international 10/20 electrode placement. No significant slow or fast wave activity was observed. Photoc stimulation with frequencies of 1–30 Hz produced no changes to the background activity. Nerve conduction assay and auditory brainstem responses (ABS) were unremarkable.

Molecular Diagnosis and Pathogenicity

The whole exome sequencing (WES) was performed using the DNA of an affected member (II-2) to identify recessive homozygous and compound heterozygous variants as described previously [Younus et al., 2018]. Variants in genes already known to cause syndromic or non-syndromic ID were shortlisted for downstream co-segregation analysis with Sanger sequencing standard protocols as described earlier [Umair et al., 2017]. Similarly, we also screened for digenic inheritance or dual diagnosis where variants in two genes cause overlapping phenotypes [Posey et al., 2017].

Based on this step-by-step filtering process for screening criteria, the homozygous missense variant (c.178T>C; p.Tyr60His) was identified in the *ANK3* gene located on chromosome 10q21.2 (Fig. 1d). The identified missense variant (c.178T>C; p.Tyr60His) co-segregated with the disease phenotype in the family (Fig. 1b). A list of homozygous variants obtained after WES filtration is presented in online supplementary Table 1 (available online at www.karger.com/doi/10.1159/000526381) and genes for syndromic or non-syndromic ID were obtained from OMIM (<https://omim.org/>). The Sanger sequencing revealed that the carrier (II-1) was heterozygous for the identified *ANK3* variant (c.178T>C), while one of the normal siblings (II-3) was homozygous wild type and the proband (II-2) was homozygous affected.

The conservation of the amino acid (Tyr60) in the different *ANK3* orthologs was searched using homologue

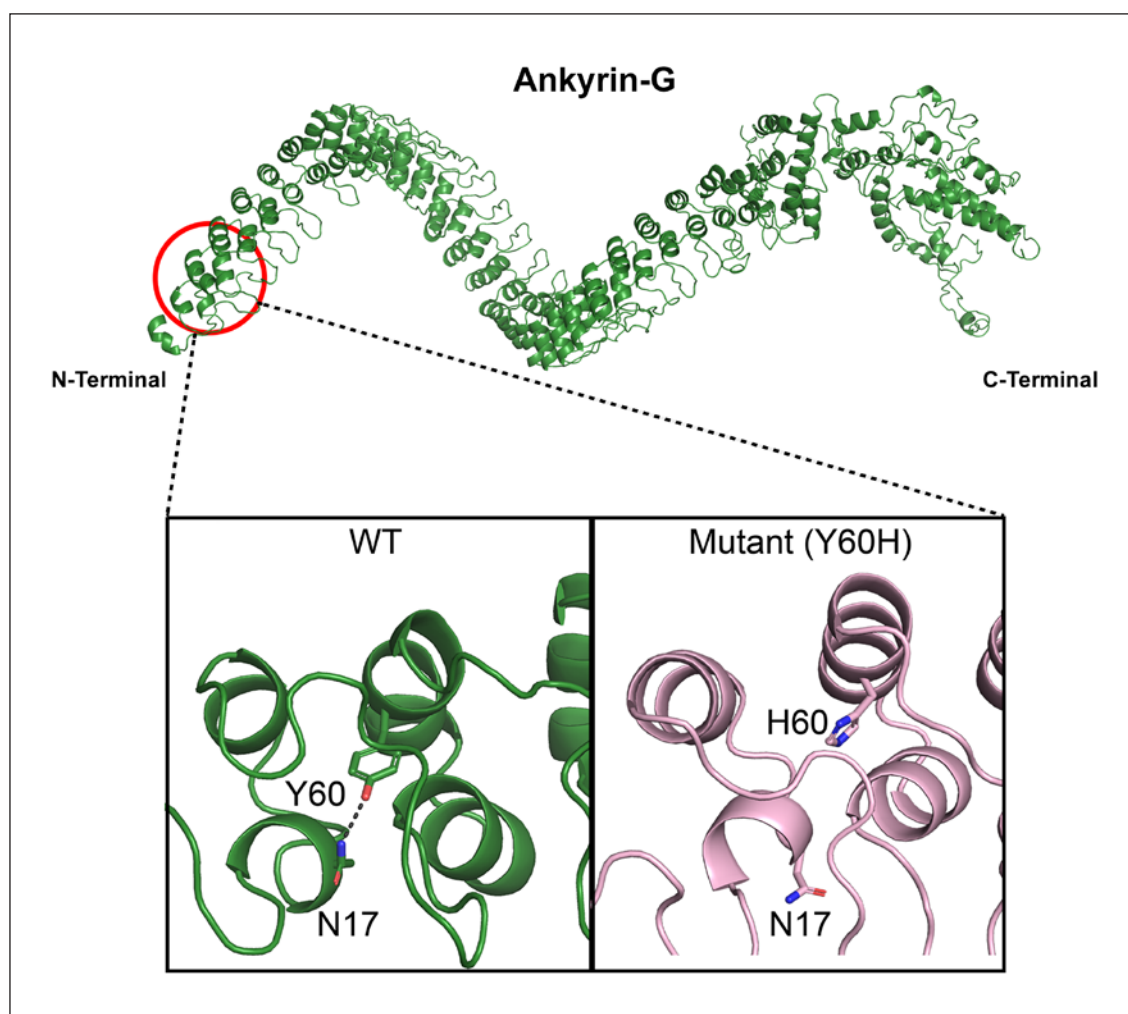


Fig. 2. ANK3 protein structure and in silico 3D modeling of the mutant proteins. In silico predicted structural representation of ankyrin-G wild type protein. The red circle shows Y60 (tyrosine at 60 position) and is depicted in green color representing the wild type, while H60 (histidine at 60 position) shows a change in tinted color representing the mutated position.

Table 2. Depicting pathogenicity of the identified homozygous mutant (c.178T>C; [p.Tyr60His]) by using different online software

S. No.	Tool used	Prediction score	Prediction
1	BayesDel addAF	0.1791	Damaging
2	EIGEN	0.5734	Pathogenic
3	FATHMM-MKL	0.9889	Damaging
4	FATHMM-XF	0.9273	Damaging
5	LIST-S2	0.9326, 0.933	Damaging
6	MetaRNN	0.8146	Damaging
7	MutationTaster	0.9999	Disease causing
8	MutPred	0.741	Pathogenic
9	PrimateAI	0.87	Damaging
10	PROVEAN	-4.51, -4.03, -3.31	Damaging
11	GERP++ score	4.15	Conserved

Table 3. List of mutations/variants already reported until now in *ANK3* gene (HGMD) for intellectual disability syndrome

S. No.	cDNA change	Protein change	Mutation type	Reported phenotype
1	c.1A>G	(p.Met1Val)	Start loss	Atrioventricular canal defects with extracardiac anomalies and neurodevelopmental disorder
2	c.58G>T	(p.Glu20Term)	Nonsense	Autism spectrum disorder
3	c.647A>G	(p.Lys216Arg)	Missense	Lennox-Gastaut syndrome
4	c.1448G>A	(p.Arg483Gln)	Missense	Autism spectrum disorder
5	c.1621C>T	(p.Arg541Term)	Nonsense	Tourette syndrome
6	c.1990G>T	(p.Gly664Term)	Nonsense	Intellectual disability, speech impairment, and autistic features
7	c.2327C>A	(p.Thr776Lys)	Missense	Cardiomyopathy, arrhythmogenic
8	c.2516C>T	(p.Thr839Met)	Missense	Brugada syndrome
9	c.3662C>T	(p.Pro1221Leu)	Missense	Autism spectrum disorder
10	c.4085G>C	(p.Gly1362Ala)	Missense	Brugada syndrome
11	c.4465C>T	(p.Pro1489Ser)	Missense	Autism spectrum disorder
12	c.4705T>G	(p.Ser1569Ala)	Missense	Autism spectrum disorder
13	c.4826C>T	(p.Thr1609Met)	Missense	Brugada syndrome
14	c.5309C>G	(p.Thr1770Arg)	Missense	Brugada syndrome
15	c.5582C>T	(p.Thr1861Met)	Missense	Neurodevelopmental disorder
16	c.6812T>C	(p.Met2271Thr)	Missense	Autism
17	c.6916A>G	(p.Lys2306Glu)	Missense	Brugada syndrome
18	c.7096T>C	(p.Ser2366Pro)	Missense	Autism spectrum disorder
19	c.7267C>T	(p.Arg2423Cys)	Missense	Autism spectrum disorder
20	c.7469C>T	(p.Pro2490Leu)	Missense	Neurodevelopmental disorder
21	c.7928A>G	(p.Asn2643Ser)	Missense	Bipolar disorder, association with
22	c.8592G>T	(p.Lys2864Asn)	Missense	Neurodevelopmental disorder
23	c.8921A>T	(p.Asn2974Ile)	Missense	Brugada syndrome
24	c.9652C>T	(p.Leu3218Phe)	Missense	Intellectual disability, cerebral and cerebellar atrophy, and delayed myelination
25	c.9971A>G	(p.Tyr3324Cys)	Missense	Intellectual disability and behavioral problems
26	c.10152G>C	(p.Gln3384His)	Missense	Increased nuchal translucency
27	c.10910A>G	(p.His3637Arg)	Missense	Autism spectrum disorder
28	c.11090C>G	(p.Ser3697Cys)	Missense	Brugada syndrome
29	c.11159C>T	(p.Thr3720Met)	Missense	Autism spectrum disorder
30	c.12500T>C	(p.Val4167Ala)	Missense	Brugada syndrome
31	c.2614+1G>T	IVS23 ds G-T +1	Splice site	Pulmonary arterial hypertension
32	c.-344520C>T	CCTT A TCA T A TGAGCTCCT AGAAACAAGGA(C-T)GCTCAG AGGTCCCCTGCTCCCCA TTCTGTT, -344520 relative to initiation codon	Regulatory region	Autism spectrum disorder with intellectual disability
33	c.10981_10982delCA	p.(Gln3661Valfs*22)	Small Deletions	Cerebellar vermis hypoplasia and microcephaly

Table 3 (continued)

S. No.	cDNA change	Protein change	Mutation type	Reported phenotype
34	c.10995delC	p.(Thr3666Leufs*2)	Small Deletions	Intellectual disability, ADHD-like syndrome and behavioral problems
35	c.11033delC	p.(Pro3678Leufs*45)	Small Deletions	Intellectual disability
36	chr10: 61994446–62029987	–	Gross deletion	Congenital heart defect
37	Balanced translocation –	–	Complex rearrangements	Attention deficit hyperactivity disorder, autism, and sleeping problems

(<https://www.ncbi.nlm.nih.gov/homologene>), and the results revealed that Tyr60 is highly conserved across different species (Fig. 1e).

To investigate whether c.178T>C; p.Tyr60His is rare and disease causing, we screened this *ANK3* variant in different online databases, such as, gnomAD (<http://gnomad.broadinstitute.org/>), ExAC (<http://exac.broadinstitute.org>), dbSNP and in the 1000 Genomes Project (<http://www.internationalgenome.org/>), or in-house 2000 exome database (<https://www.ncbi.nlm.nih.gov/projects/SNP/>) (Table 2). The variant was not present in public databases and was predicted to be disease-causing by mutation tester, provean (score –4.03), and polyphen-2 (score 1). As the variant is present at the N-terminal region, it affects all three isoforms of the *ANK3*.

3D Protein Modeling

The predicted structural topology of *ANK3* protein is shown in Figure 2. The mutational position (60 amino acid position) was falling in the N-terminal helix, so to investigate whether c.178T>C; p.Tyr60His alters the protein structure, we performed 3D modeling. The results showed that tyrosine (60th) makes a hydrogen bond with asparagine at 17th residue in wild-type protein. However, this tyrosin-asparagine bond is disrupted after the substitution of tyrosine with histidine (Fig. 2).

Discussion

Mutations and single nucleotide polymorphisms in *ANK3* have been linked to neurodevelopmental and psychiatric disorders. Here, we report a proband (II-2) exhibiting features such as ID, GDD, seizures, poor speech development, and hypotonia that can be broadly characterized as syndromic ID. Using WES, we identified a

novel homozygous missense variant (c.178T>C; p.Tyr60His) *ANK3* gene previously associated with autosomal recessive mental retardation type 37 (OMIM 615493). The variant results in the substitution of T nucleotides, which results in the change of tyrosine at amino acid position 60 into histidine amino acid (Fig. 1b). The total number of *ANK3* mutations in HGMD are 40, out of which 33 are missense/nonsense, 1 splice site mutation, 1 mutation in regulatory region, 3 small and 1 gross deletion, and 1 complex mutation (Table 3). The variant is very rare and predicted to be disease causing using various online tools.

The features reported in the present study were also identified in a previous finding of heterozygous missense variants; c.4705T>G, c.11159C>T, and c.12763A>C; to be associated with autism susceptibility to neuropsychiatric disorders [Bi et al., 2012]. A homozygous frameshift mutation, c.10995delC (p.Thr3666LeufsX2), was identified in patients with moderate ID, hypotonia, speech delay, sleeping disorder, and hyperactivity [Iqbal et al., 2013]. Another study reported a heterozygous non-sense de novo mutation, c.1990G>T (p.Gly664*), in *ANK3* to cause mild ID, hypotonia, speech impairment, autistic features, hyperactivity, aggressive behavior, sleeping disorder, macrocephaly, macrosomia, and chronic hunger [Kloth et al., 2017]. Heterozygous frameshift and non-sense mutations have been reported in patients with variable neurodevelopmental phenotypes [Kloth et al., 2021]. A balanced chromosomal translocation in all three isoforms of *ANK3* was reported to cause more severe phenotypes including ASD, mild ID, speech delay, muscular hypotonia, ADHD, altered sleeping patterns, and spasticity [Iqbal et al., 2013]. Our patient harbored a missense variant in *ANK3* and shared symptoms of ID, hypotonia, aggressive behavior, and speech delay with previously reported patients with *ANK3* mutation. The additional

clinical symptoms of our patient included seizures and ataxia, which further adds to the phenotypic expansion of the *ANK3* mutations.

ANK3 gene encodes for an ankyrin-G protein, which contains a globular head domain comprised of membrane binding repeats, a spectrin cytoskeleton binding domain, a serine-rich domain, and a C-terminal regulatory domain [Smith and Penzes, 2018]. The N-terminal ankyrin repeat domains (ANKRD) play a significant role in protein-protein interactions through variations in adaptive surface residues [Mosavi et al., 2004]. Ankyrin-G is mainly located in nodes of Ranvier and the axon initial segment (AIS) in the central and peripheral nervous system [Jenkins and Bennett, 2002; Kordeli and Bennett, 1991; Kosaka et al., 2008]. *ANK3* produces multiple tissue-specific isoforms, and six distinct isoforms have been identified in brain samples [Rueckert et al., 2013]. The 190-kDa isoform regulates dendritic spine morphology and NMDA receptor trafficking in the brain [Smith et al., 2014]. The 270 kDa isoform preserves the myelin sheath of the nodes of Ranvier [Chang et al., 2014], whereas the 480 kDa isoform plays a significant role in nodes of Ranvier formation and regulation of GABAergic synapses [Jenkins et al., 2015; Tseng et al., 2015].

ANK3 modulates the Wnt signaling pathway and cell proliferation [Durak et al., 2015]. Besides this, the involvement of *ANK3* gene has been deciphered in regulating sodium channels to promote sodium retention [Edinger et al., 2014]. At the nodes on Ranvier, ankyrin-G is recruited along with voltage-dependent Na⁺ channels where ankyrin-G ensures proper action potential propagation by stabilizing Na⁺ channels [Jenkins et al., 2015]. It was also demonstrated that ankyrin-G plays an essential role in the axo-dendritic cell polarity [Sobotzik et al., 2009]. *Ank3* deficient mice exhibit loss of neuronal excitatory process and depletion of neurogenesis [Bennett and Lambert, 1999; Paez-Gonzalez et al., 2011]. *Ank3* W1989R mice showed a reduction of GABAergic synapses in the forebrain resulting in pyramidal cell hyperexcitability and disruption in network synchronization [Nelson et al., 2018]. Mice model with *Ank3* exon 1b deletion showed marked reduction of ankyrin-G expression at the AIS and PV+ interneurons [Lopez et al., 2017].

There is an emerging theme of shared molecular pathways for neurodevelopmental and neuropsychiatric disorders. Autism spectrum disorder is co-morbid with intellectual disability in almost 50% of the cases [Anagnostou et al., 2014]. Similarly, the prevalence of epilepsy with ID is 22.2% [Robertson et al., 2015; Nøstvik et al., 2021]. Genomic data analysis has shown the clustering of genes in various mental

and neurodevelopmental disorders [Moreno-De-Luca et al., 2013; Khan et al., 2022]. The Association of *ANK3* mutations with ASD, schizophrenia, ID, and bipolar disorder is additional evidence to this theme. The variation can occur due to the presence of a genetic modifier and the exact interaction of mutation with genetic background is an area to be explored. Similarly, affected individuals having severe NDDs can be early-diagnosed using parenteral diagnosis. Prenatal genetic testing for monogenetic disorders (PGT-M) can be performed, which might help parents wishing to have future pregnancies [Alyafe et al., 2021a, b]. Although there is no specific management in these cases, patients are treated with supportive treatment.

In conclusion, we provide additional evidence that homozygous variants in the *ANK3* gene cause syndromic ID in humans. This study also expands the mutation spectrum of ID neurological disorder; it could also be useful for the early parental diagnosis and genetic counseling of affected families.

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Statement of Ethics

The research committee of respective institute UOE approved the study (UE/S&T/2021/222). Written informed consent to perform genetic testing and use the clinical data for academic purposes was taken from patient guardians prior to collecting the blood samples with appropriate counseling.

Conflict of Interest Statement

All authors declare that they have no conflict of interest to disclose.

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Author Contributions

Muhammad Younus, Memoona Rasheed, Muhammad Umair drafted the manuscript. Amjad Khan, Ahmed Waqas collected samples, clinical data, analyzed the data, and performed experiments. Zhaohan Lin, Saeed A. Asiri, Ibrahim A. Almazni per-

formed the in silico analysis. Sarfraz Shafiq, Imran Iqbal, Hanif Ullah, Amjad Khan analyzed the genomic data and revised the manuscript. Muhammad Umair, Ahmed Waqas designed the research study.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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