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First *de novo ANK3* nonsense mutation in a boy with intellectual disability, speech impairment and autistic features

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ABSTRACT

Ankyrin-G, encoded by *ANK3*, plays an important role in neurodevelopment and neuronal function. There are multiple isoforms of Ankyrin-G resulting in differential tissue expression and function. Heterozygous missense mutations in *ANK3* have been associated with autism spectrum disorder. Further, in three siblings a homozygous frameshift mutation affecting only the longest isoform and a patient with a balanced translocation disrupting all isoforms were documented. The latter four patients were affected by a variable degree of intellectual disability, attention deficit hyperactivity disorder and autism. Here, we report on a boy with speech impairment, intellectual disability, autistic features, macrocephaly, macrosomia, chronic hunger and an altered sleeping pattern. By trio-whole-exome sequencing, we identified the first *de novo* nonsense mutation affecting all *ANK3* transcripts. Thus, our data expand the phenotype of *ANK3*-associated diseases and suggest an isoform-based, phenotypic continuum between dominant and recessive *ANK3*-associated pathologies.

1. Introduction

ANK3 encodes the Ankyrin-G protein, which is involved in neuronal development and signaling (S. M. Jenkins and Bennett, 2002; Kordeli et al., 1995; Rasband et al., 1999). On the cellular level, Ankyrin-G plays an integral role as a membrane cytoskeleton-linker and scaffolding protein. Similar to other members of the ankyrin family, it consists of an amino-terminal domain containing multiple Ankyrin-repeats, a central spectrin-binding domain, a serine-rich and a carboxy-terminal regulatory domain. The latter is the least conserved and subject to high variation (Kapfhamer et al., 1995). ANK3 was initially identified at the axonal initial segment (AIS) and in the nodes of Ranvier of neurons in the central and peripheral nervous system (S. M. Jenkins and Bennett, 2002; Kapfhamer et al., 1995; Kordeli and Bennett, 1991), regulating the maintenance and targeting of ion channels and cell adhesion molecules (Davis et al., 1996; Zhang and Bennett, 1996). In fact, *ANK3* is subject to tissue-specific splicing resulting in multiple differentially regulated isoforms in various tissues (Peters et al., 1995; Yamankurt et al., 2015). Only in brain samples six distinct isoforms have been identified (Rueckert et al., 2013). However, three are major isoforms in the brain, each showing a tissue-specific distribution pattern and bearing different functions in neuronal circuits (Gasser et al., 2012; P. M. Jenkins et al., 2015; Rueckert et al., 2013). The canonical *ANK3* isoform, sized 190-kDa, is expressed in most tissues specifically regulating dendritic spine morphology and N-methyl-D-aspartate (NMDA) receptor trafficking in the brain (Smith et al., 2014). The other two isoforms, sized 270-kDa and 480-kDa respectively, are localized in the nodes of Ranvier and the initial segments of myelinated axons (Kordeli et al., 1995) and known to fulfill specific functions in the nervous system. The 270-kDa isoform preserves the myelin sheaths of the nodes of Ranvier (Chang et al., 2014) whereas the 480-kDa isoform has a crucial role in the formation of the nodes of Ranvier (P. M. Jenkins et al., 2015) and is involved in the regulation of somatodendritic GABAergic synapses (Tseng et al., 2015). In addition, Ankyrin-G has been detected at paranodes of the central and peripheral nervous system, respectively

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(Rasband et al., 1999).

Previously, heterozygous missense mutations in *ANK3* have been associated with autism spectrum disorders (ASDs) (Bi et al., 2012; Codina-Sola et al., 2015; Epi et al., 2013; Sanders et al., 2012). In addition, genome-wide association studies have implicated ANK3 in the pathogenesis of bipolar disorder and schizophrenia (Ferreira et al., 2008). Moreover, a patient bearing a balanced chromosomal translocation affecting all three *ANK3* isoforms as well as three siblings bearing a homozygous frameshift mutation affecting only the 480-kDa isoform were recently identified. These latter individuals were affected by intellectual disability (ID), attention deficit hyperactivity disorder (ADHD) and ASD (Iqbal et al., 2013). Here we report the first patient harboring a heterozygous *de novo* nonsense mutation that affects all three *ANK3* isoforms.

2. Clinical report

The proband is the second child of unaffected non-consanguineous German parents. His older sister is also unaffected. One cousin was said to suffer from speech development delay but attended regular schooling later on in life. Further family history was unremarkable. The pregnancy has been complicated by polyhydramnion and gestational diabetes. At birth, spontaneous at term, the boy presented with macrosomia (+2.63SD) and macrocephaly (+2SD). After one week of feeding difficulties he developed chronic hunger and demanded to be fed every 2 h. Temporary hyperbilirubinaemia needed to be treated with phototherapy for 24 h. Gross motor development was normal (crawling at 10 months, walking at 13 months). In contrast, he presented with delayed language development. Sound and syllables were formed at 12 months, but he did not start using two-word-sentences until the age of 4 years. Dentition was also mildly delayed. Primary macrocephaly (+3,7SD) and macrosomia (+2,4SD) progressed since early childhood. The boy further presented with an unusual sleep rhythm, often sleeping for up to 13 h per day. Seeing and hearing was unremarkable, a regression was not noted. Due to weaknesses in social interaction and communication, lack of empathy as well as specific facial expression a formal ASD assessment was performed at the age of 5 years, revealing an ADOS-2 score of 5. In addition, ADHD was first documented at the age of 5 years. After attending regular kindergarten and first grade, his IQ was tested to be 77 (HAWIK-IV), for which he attended special schooling. Because of his chronic hunger, he was put on a low-carb-diet to prevent an aggravation of adiposity (weight, +2.4SD). Clinical examination at the age of 8 years revealed hypertelorism, full eyebrows, truncal adiposity, short broad hands and digits as well as toes. Extensive laboratory, metabolic and radiological diagnostics yielded unremarkable results (MRI of the brain, lumbar puncture, echocardiogram, echocardiography, EEG, metabolic analysis of blood, cerebrospinal fluid and urine). Previous genetic testing including chromosomal analysis, array-CGH, as well as diagnostics for Sotos- and Fragile-X-syndrome were unremarkable.

3. Materials and methods

3.1. Patient information

All biological samples were obtained following written informed consent from studied individuals or their legal representatives. The Ethical Board of the Medical Faculty of the Hamburg University approved the study. The study was performed in accordance with the Declaration of Helsinki protocols.

3.2. Genetic testing

DNA samples from whole blood were isolated by standard procedures. To elucidate the putative genetic cause in this patient we performed trio whole exome sequencing (trio-WES) with DNA samples of both healthy parents and the index patient, as described before (Hempel et al., 2015). For validation, PCR and Sanger sequencing were performed as described previously (Lessel et al., 2014). Primer pairs are available upon request.

4. Results

4.1. Genetic findings

Trio-WES resulted in approximately 99% of target sequences being covered at least 20-fold with a coverage of at least 175x, in each of the tested DNA samples. Bioinformatic filtering of the obtained data identified no rare candidate variants (minor allele frequency MAF < 0.01) following an autosomal recessive or X-linked mode of inheritance. Further, in line with array-CGH findings no putative CNVs were identified. However, we identified only a single coding non-synonymous heterozygous *de novo* alteration. Namely, a nonsense mutation, c.1990G > T (p.(Gly664*)), in exon 17 of *ANK3* (NM_020987.3). This nonsense mutation affects all three *ANK3* isoforms (Fig. 1) and probably activates the nonsense-mediated mRNA decay (NMD). This alteration was not present in dbSNP139, the ExAC database or the 1000 Genomes data, precluding that it represents a rare polymorphism. This variant was deposited in the

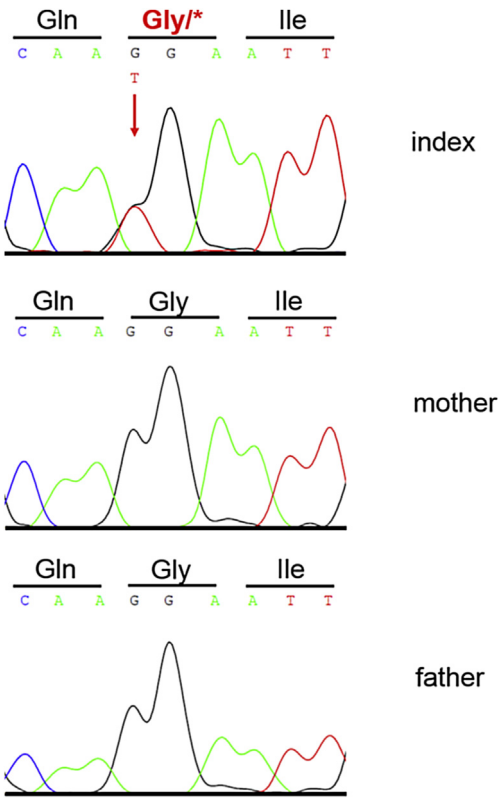

Fig. 1. Sanger sequence chromatograms of parts of *ANK3* after PCR amplification of genomic DNA of the affected individual and his parents. The amino acid translation is shown in the three-letter code above the chromatograms. The red arrow indicates the heterozygous mutation at positions c.1990G > T, (p.(Gly664*)), present only in DNA sample of the affected individual. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Leiden Open Variation Database accessible at https://databases.lovd.nl/shared/genes/ANK3 (Variant ID: 0000170837). Further, the probability of loss-of-function intolerance (pLI), for *ANK3* is 1.00, suggesting strong intolerance. Sanger sequencing confirmed the *de novo* nature of the mutation (Fig. 1).

5. Discussion

Heterozygous missense mutations in *ANK3* have been identified in individuals affected by autism spectrum disorder (Bi et al., 2012; Codina-Sola et al., 2015; Epi et al., 2013; Sanders et al., 2012). Moreover, a heterozygous disruption of all three *ANK3* isoforms, due to a balanced chromosomal translocation, was described in a single patient to result in a more severe phenotype including autism spectrum disorder, mild ID, speech delay, muscular hypotonia, spasticity and behavioral difficulties including ASD, ADHD and an altered sleeping pattern (Iqbal et al., 2013). An even more severe phenotype was described in three siblings of a consanguineous family carrying a homozygous truncating mutation in only the largest brain-specific isoform of *ANK3*. These three

Table 1
Clinical characteristics of patients with *ANK3* mutations.

Patients	PKMR14 family			Patient with translocation	ASD cohort	Our patient
Reference	Iqbal et al., 2013			Iqbal et al., 2013	Bi et al., 2012 Sanders et al., 2012 Epi et al., 2013 Codina-Sola et al.,2015	This study
Sex	female	female	male	male	female/male	male
Genotype	homozygous nonsense mutation	homozygous nonsense mutation	homozygous nonsense mutation	translocation	heterozygous missense mutations	heterozygous nonsense mutation
Age at last examination (years)	25	22	18	14	various	9
Development						
Intellectual disability	moderate	moderate	moderate	mild	n/a	mild
Speech delay	+	+	+	+	n/a	+
Musc. hypotonia	+	+	+	+	n/a	+
Other						
Spasticity	+	+	+	−	n/a	−
Hyperactivity	+	+	+	+	n/a	+
Autistic features	n/a	n/a	n/a	+	+	+
Epilepsy	−	−	+	−	n/a	−
Aggressiveness	+	+	+	+	n/a	+
Sleeping disorder	+	+	+	+	n/a	+
Chronic hunger	n/a	n/a	n/a	n/a	n/a	+
Dysmorphic features	−	−	−	+	n/a	−
Macrocephaly	n/a	n/a	n/a	n/a	n/a	+
Macrosomia	n/a	n/a	n/a	n/a	n/a	+
cCT/brain MRI	n/a	normal	normal	n/a	n/a	normal
***ANK3* mutation**	p.Thr3666fs*/ p.Thr3666fs*	p.Thr3666fs*/ p.Thr3666fs*	p.Thr3666fs*/ p.Thr3666fs*	Translocation with breakpoint in intron 30 of *ANK3*	various (s. Fig. 1)	p.Gly664fs*/ wt

+, present; -, absent, n/a not available.

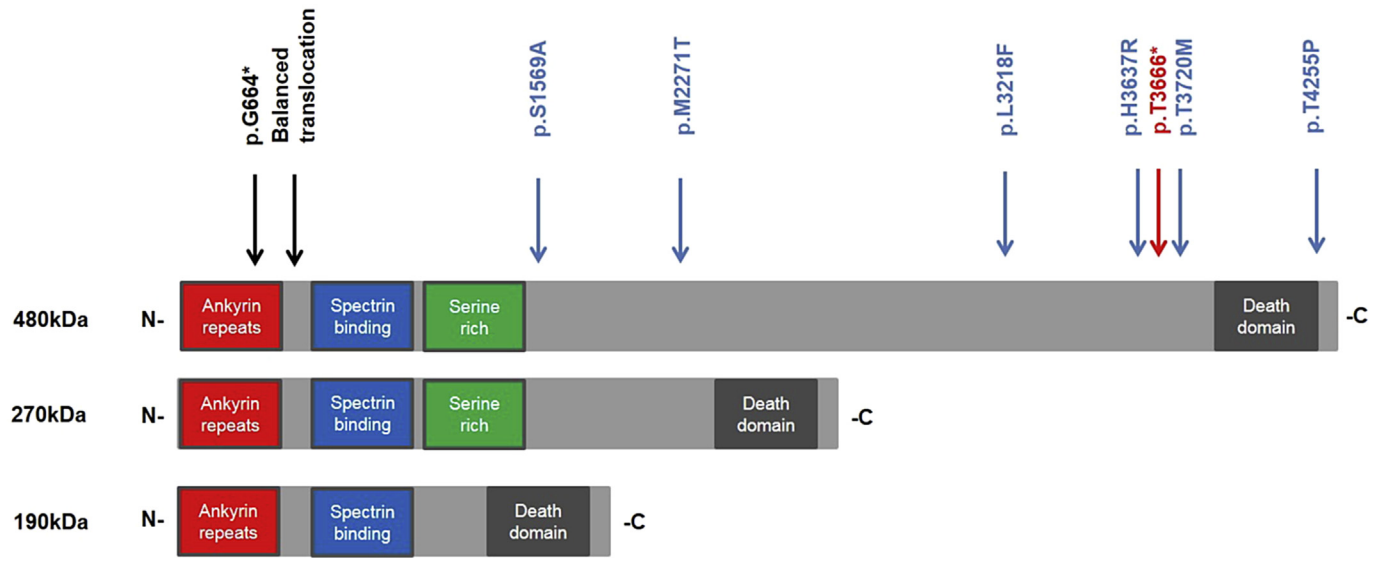

Fig. 2. Protein structure of the three ANK3 isoforms with indicated positions of identified mutations. Black arrows indicate position of heterozygous truncating mutation/translocation; red arrow indicates position of the homozygous truncating mutation; blue arrows indicate heterozygous missense mutations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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children, born to unaffected parents, presented with moderate ID, speech delay and behavioral difficulties including ADHD and sleep disturbances (Iqbal et al., 2013) (Table 1). In addition to the previously published *ANK3*-phenotypic spectrum, our patient presented with chronic hunger, macrosomia and macrocephaly. Re-examination of our patients' family revealed that both his father and parental grandfather showed macrocephaly, though both were of normal intelligence and unremarkable character. Indeed, macrocephaly is a trait often segregating in families and known to be inherited independently (Williams et al., 2008). In contrast, chronic hunger and macrosomia were not observed in any of the family members, thus possibly expanding the phenotypic *ANK3*-spectrum.

Notably, there is a high phenotypic similarity between the patient presented here and the previously published patient bearing a heterozygous disruption due to the chromosomal translocation at the similar position. Both alterations are located within the amino-terminal domain consisting of multiple Ankyrin repeats or rather shortly behind (Fig. 2). Both alterations likely activate the NMD and result in haploinsufficiency. However, even if they result in a stable protein it would lack the central spectrin-binding domain, the serine-rich and the carboxy-terminal regulatory domain. The homozygous truncating variant is located within the very C-terminus of the largest brain-specific isoform (480-kDa), not affecting the other two brain specific isoforms. Based on these findings we suggest that alterations in *ANK3* result in a genotypic-phenotypic continuum. Heterozygous missense mutations predispose to ASD with mild developmental delay. Heterozygous disruption of all three isoforms results in a more severe neurological phenotype including mild ID, speech delay, hypotonia, behavioral difficulties such as ASD, ADHD and an altered sleeping pattern. Last but not least, homozygous truncating mutations affecting only the largest brain-specific isoform (480-kDa) of *ANK3* cause an even more severe neurodevelopmental phenotype. These data may suggest that fetuses bearing homozygous truncating variants affecting all isoforms of *ANK3* may not be viable, similar to the situation in mice (P. M. Jenkins et al., 2013). However, it remains somewhat unclear why despite its broad role in multiple tissues none of the so far described individuals bearing alterations in *ANK3* developed any non-neurological symptoms.

Taken together, we present here the first heterozygous *de novo* nonsense mutation affecting all *ANK3* isoforms. Thus, our data, above expanding the phenotype, suggest an isoform-based phenotypic continuum between dominant and recessive *ANK3*-associated pathologies.

Conflict of interest

The Authors have no conflict of interest to report.

Internet resources

1000 Genomes, http://browser.1000genomes.org.
dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/
ExAC Browser, http://exac.broadinstitute.org/

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