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NK2 homebox 1 gene mutations in a family diagnosed with ataxic dyskinetic cerebral palsy

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Abstract

Benign hereditary chorea caused by mutations in the NK2 homeobox 1 gene (NKX2-1), shares clinical features with ataxic and dyskinetic cerebral palsy (CP), resulting in the possibility of misdiagnosis. A father and his two children were considered to have ataxic CP until a possible diagnosis of benign familial chorea was made in the children in early teenage. The father's neurological condition had not been appreciated prior to examination of the affected son. Whole exome sequencing of blood derived DNA and bioinformatics analysis were performed.

A 7 bp deletion in exon 1 of NKX2-1, resulting in a frame shift and creation of a premature termination codon, was identified in all affected individuals. Screening of 60 unrelated individuals with a diagnosis of dyskinetic or ataxic CP did not identify NKX2-1 mutations. BHC can be confused with ataxic and dyskinetic CP. Occasionally these children have a mutation in NKX2-1.

1. Introduction

Cerebral palsy (CP) describes a group of permanent disorders of the development of movement and posture, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain.[1] The motor dysfunction of CP is divided into three categories: spastic, ataxic and dyskinetic. Spastic CP is characterised by increased muscle tone, ataxic CP is characterised by low muscle tone and cerebellar features including intention tremor, poor balance and poor coordination.[2] Dyskinetic CP is characterised by involuntary movements, either athetosis or dystonia, and mixed muscle tone.[1]

Benign Hereditary Chorea (BHC) is an autosomal dominant movement disorder characterised by non-progressive or very slowly progressive chorea with normal cognitive function. Choreiform movements may involve the limbs, face, neck, trunk and tongue. While chorea is the characteristic movement disorder, atypical features including gait disturbance, dystonia, ataxia, intention tremor, dysarthria and pyramidal signs may accompany BHC. In a minority of affected individuals, the disorder may involve the thyroid presenting as congenital hypothyroidism, and/or lung, with respiratory distress syndrome or recurrent lung infection.[3, 4] Benign Hereditary Chorea results from mutations of the NK2 homeobox 1 gene (*NKX2-1*), located at chromosome 14q13.3, which encodes a transcription factor important for the development of the lung, thyroid and brain.[5] *NKX2-1* consists of three coding exons, which are transcribed into two major *NKX2-1* mRNA isoforms encoding different proteins of 371 and 401 amino acids in length.[4] Deletions, missense mutations and nonsense mutations have been described in *NKX2-1* and result in haploinsufficiency.[6, 7] The gene is highly expressed in the fetal brain and is involved in neuronal migration and development of the basal ganglia.[8] We report a father and his two children who were diagnosed with ataxic CP in early childhood. The father's neurological condition was not appreciated until examination of his son. The clinical diagnosis was revised to BHC, with

identification of a pathogenic mutation in *NKX2-1* by whole-exome sequencing of the family during a research study into the genetic basis of CP. To determine other BHC cases with similar phenotypes, we sequenced *NKX2-1* in 60 unrelated individuals diagnosed with ataxic, dyskinetic and/or athetoid CP.

2. Clinical report

2.1 The Family

The affected family members are a father, his son and his daughter (II-1, III-2 and III-3, in Figure 1) with a previously unreported mutation of the *NKX2.1* gene. The father and his wife are Caucasian, non-consanguineous and have an unaffected son.

2.2 The affected father

He was born at term after a normal pregnancy, labour and delivery but had delayed onset of respiration. There were no problems in the neonatal period. He walked late and is described as having had genu valgum, an awkward gait and a tendency to fall easily; coordination was generally poor through childhood. He was an above average student at school and completed tertiary studies. Neurological examination at the time his son was diagnosed revealed definite though mild ataxia; he was able to stand on one leg for approximately 10 seconds but tandem gait was ataxic. He did not have nystagmus or chorea. When seen at 49 years of age he described mild dysarthria when tired, and mild functional difficulties due to his ataxia. Walking was not usually a problem but he avoided running; when he ran, his legs “locked” and he overbalanced. He avoided carrying things in both hands, as this made him less stable. He found it difficult to use both hands simultaneously to perform separate tasks. Examination revealed an inability to heel-toe walk and minor left intention tremor on finger-nose testing.

2.3 The affected son

Following a normal pregnancy, he was born at 39 weeks gestation by caesarean delivery after failure of labour to progress with Apgar scores of 9 and 9 at one and five minutes, birth weight 2970 g (10th-50th percentile), birth length 51.5 cm (50th-90th percentile) and birth head circumference 34.0 cm (10th-50th percentile). There was no respiratory distress or abnormalities on newborn screening. A paediatrician documented developmental delay with ataxia at 16 months of age and a diagnosis of ataxic CP was made. He sat at 18 months, walked and had about 40 words at 20 months and used phrases by three years. When seen by a neurologist at 20 months he had an unsteady gait, fell frequently and there were some choreiform movements. At 24 months he had choreoathetoid movements and ataxia persisted. He had speech therapy between two and three years. A diagnosis of BHC was considered at 12 years of age. At 15 years he had choreiform movements and no ataxia; the chorea was exacerbated by emotion. Handwriting was poor and he ran awkwardly, fell frequently and had had a number of fractures. Intelligence was above average, his speech was clear and he was a gifted swimmer in spite of his neurological disorder.

2.4 The affected daughter

Following a normal pregnancy, she was born at 40 weeks gestation by elective caesarean delivery with Apgar scores of 6 and 9 at one and five minutes, birth weight 3440 g (50th percentile), birth length 53 cm (90th percentile) and birth head circumference 34.0 cm (10th-50th percentile). There was mild respiratory distress attributed to meconium aspiration. Newborn screening was normal. Motor milestones were delayed: she sat at 6-8 months, crawled at 12 months and walked at 17 months, with frequent falls but without the jerky movements seen in her brother. Speech development was normal. A neurologist assessed her at 20 months, at which time she was considered to have an ataxic gait; upper limb

movement was normal. At 3.5 years, speech and cognitive function were normal and there were mild choreoathetoid movements and an ataxic gait. At 11 years, there was chorea affecting her face, trunk, arms and legs; there was no ataxia. She was a gifted student, with good handwriting.

2.5 Other cases of dyskinetic or ataxic CP

We selected 60 unrelated Caucasian individuals with a diagnosis of dyskinetic or ataxic CP from the Australian CP Research Study[9] – 29 (48%) had ataxic CP, 16 (27%) had dyskinetic athetoid CP, 10 (17%) had dyskinetic dystonic CP and 5 (8%) had dyskinetic CP without known subtype. There were no reported family histories of CP.

Research ethics approval was obtained from Women's and Children's Health Network Human Research Ethics Committee (Approval No. REC 1946/4/10) and signed parental consent was obtained from the family and all participants involved in the mutational screening.

3. Methods

3.1 DNA isolation

For each individual in the three generation pedigree DNA was isolated from whole blood using a QIamp DNA Midi kit (Qiagen, Stanford, CA) following the manufacturer's instructions. DNA had previously been extracted from buccal swabs obtained from a convenience cohort of volunteer cerebral palsy cases selected for follow up mutational screening.[10]

3.2 Whole-exome sequencing and analysis

Initially whole-exome sequencing was performed for the three affected individuals (II-1, III-2 and III-3) and the two unaffected individuals in the second and third generation (II-2 and III-1). Following Nimblegen Exome sequence capture, enriched genomic DNA was massively parallel sequenced on the Illumina HiSeq 2000 platform (Macrogen), which returned on average 48.3 million 100 bp pair end reads per individual. These reads were quality trimmed using the FASTX toolkit. Quality reads were mapped to the hg19 build of the human genome by using Burrows-Wheeler Alignment tool. Samtools were used to generate BAM files. Sequence variants were realigned, recalibrated and reported with Genome Analysis ToolKit and categorised with Annovar. We filtered the variants based on dbSNP132 and 1000 genomes, exonic/splice sites, nonsynonymous and then further filtered for inheritance models including, autosomal dominant, autosomal recessive both homozygous and compound heterozygous and *de novo* variants.

3.3 Sanger sequencing

To confirm the mutation in the family unique primers incorporating the 7 bp deletion were designed using Primer3 (v.0.4.0), (forward 5'-CTGTTCCCTCATGGTGTCTGGT-3', reverse 5'-GAATCATGTCGATGAGT CCAAAG-3'). For the 60 unrelated individuals diagnosed with dyskinetic or ataxic CP, the entire protein coding region of the *NKX2-1* gene was amplified in four fragments. Four primer pairs were designed using Primer3 (v.0.4.0). Primer set 1: (forward: 5'-CAGTCGATCCCCTACTCAGC-3', reverse: 5'GTAACAGAGGAGGAGAGATGGT TG-3'), primer set 2: (forward: 5'-AATGCTTTGGGTCTCGTCTCT-3', reverse: 5'-CACT TTCTTGTAGCTTTCCTCCAG-3'), primer set 3: (forward: 5'-TACGTGTACATCCAA CAAGATCG-3', reverse: 5'-TACCAAGTCCCTGTTCTTGGAC-3'), primer set 4: (forward: 5'-TCGAAAGAGGGAACTGAGACTGAG-3', reverse: 5'-

GCCAGGTTGTTAAGAAAA GTCGA -3'). Sanger sequencing was performed using BigDye terminator chemistry 3.1 (ABI) and sequenced using an ABI prism 3700 genetic analyser (Applied Biosystems, Foster City, CA, USA). Sequencing data was analysed using DNASTAR Lasergene 10 Seqman Pro (DNASTAR, Inc. Madison, WI, USA).

4. Results

Analysis of whole-exome sequencing data for all individuals in generations II and III identified a 7 bp deletion within exon 1 of *NKX2-1* in all three affected individuals. Both unaffected individuals do not carry this deletion (Figures 1). Results were confirmed by Sanger sequencing (Figure 2A). Neither of the father's unaffected parents had the deletion, suggesting that it occurred *de novo* in the father (II-1) and was transmitted in an autosomal dominant manner to his affected children (III-2 and III-3). The deletion in *NKX2-1* (NM_003317: exon1: c.84_90del) is predicted to result in a premature termination codon (PTC) and a truncated NKX2-1 protein (p.M59fs*39) (Figure 2B). It is plausible that the PTC containing *NKX2-1* mRNA is degraded by non-sense mediated mRNA decay (NMD) resulting in the absence of one copy of the NKX2-1 protein. The effect of NMD on the c.84_90del *NKX2-1* mRNA could not be tested as *NKX2-1* is not expressed in routinely available tissue (i.e. blood, skin or saliva). The c.84_90del mutation is predicted to affect both *NKX2-1* mRNA isoforms.

Sanger sequencing of 60 unrelated participants from the Australian CP Research Study who had been diagnosed with dyskinetic or ataxic CP harboured no *NKX2-1* mutations.

5. Discussion

We describe two siblings who were considered to have ataxic CP in early childhood. Their father had a mild cerebellar syndrome, not appreciated at the time of their presentation. Diagnosis was based on the presence of delayed motor milestones and a non-progressive movement disorder, ataxic in nature, associated with gait disturbance and frequent falls. As the children grew older, choreoathetosis became the predominant neurological feature, although ataxia persisted through the first decade. It was recognised that the diagnosis was BHC when reviewed by a neurologists in their early teenage years. Coincidentally, a mutation was identified by whole-exome sequencing in *NKX2-1* at around the same time because the family was participating, in the Australian CP Research Study. The affected individuals in this family shared a 7 bp deletion of exon 1 of *NKX2-1* resulting in a frame shift, with creation of a premature termination codon. This is predicted to cause haploinsufficiency, either as a result of a non-functional, truncated NKX2-1 protein or 50% reduction of the protein levels due to NMD degradation of the premature termination codon containing *NKX2-1* mRNA allele.[6, 7]

While BHC, with its characteristic choreiform movements, would not commonly be confused with ataxic CP, similar cases have been reported[11, 12]. Doyle et al.¹² reported siblings with an *NKX2-1* mutation who had congenital hypothyroidism, global developmental delay and later ataxia, choreoathetosis and dysarthria. Their mother had been diagnosed with CP during childhood; she was described as having ataxia as an adult. Carre et al[12] described a child with an *NKX2-1* mutation who had congenital hypothyroidism and respiratory distress syndrome and in whom ataxic movements and psychomotor delay presented in the first year.

BHC may be difficult to diagnose in early childhood before the characteristic choreiform movements are apparent, especially in the absence of a family history of the

disorder and when the child has an atypical movement disorder at the time of first diagnostic evaluation. It is possible that there are a small number of BHC cases among patients diagnosed to have dyskinetic or ataxic CP. However, we did not find any individuals with an *NKX2-1* mutation among 60 unrelated cases of dyskinetic or ataxic CP.

The family reported here exemplifies the problem that clinicians face in distinguishing between CP and genetic neurological disorders that include the motor components of CP among their features, especially when the diagnostic evaluation is being done early in life. Other examples are disorders caused by mutations in *GAD1*, [13, 14] *KANK1*, [15] and the adaptor protein complex-4 (*AP4E1*, *AP4M1*, *AP4B1*, and *AP4S1*). [16-19]

This study highlights the importance of genetic investigation of individuals with CP, because a proportion, yet to be defined, will have an underlying genetic disorder, with clinical features that meet the currently accepted criteria for diagnosis of CP.

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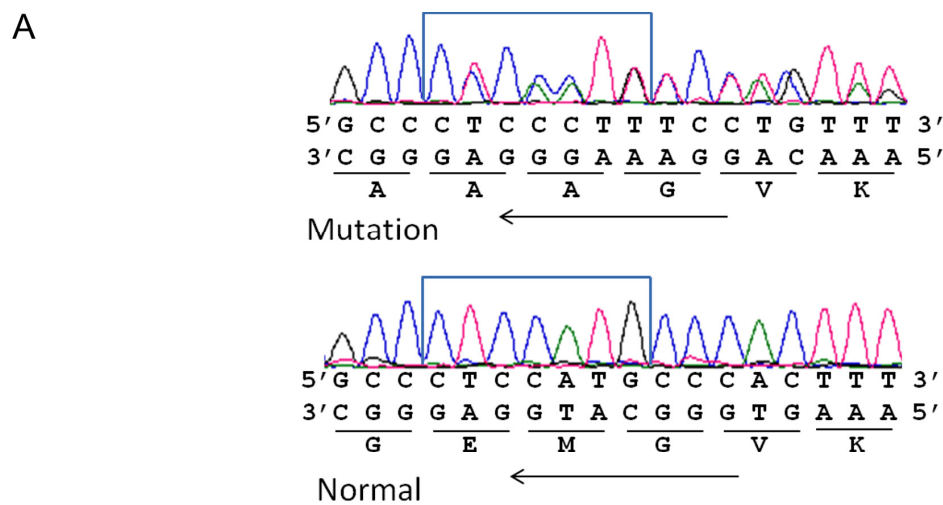
Conflicts of interest

Authors have no conflict of interest.

Figure legends

Figure 1: Three generation pedigree with *de novo* mutation in II-1 and subsequent autosomal dominant transmission in III-2 and III-3. All family individuals were sequenced with Sanger sequencing. *Represents individuals carrying the mutation, remaining family members were unaffected. See Figure 2 for Sanger sequencing trace.

Figure 2: (A) Fragments of sequence chromatograms from an affected individual (heterozygous mutation) and an unaffected individual (normal homozygous) from 5' – 3' and corresponding amino acid sequences. (B) Comparison of amino acid sequences of wildtype and mutant NKX2-1 proteins. The change was at position 59 introducing a premature termination codon at position 98 resulting in a truncated protein. ↓ represents exon/exon boundaries.



B

	1	11	21	31	41	51	
wt	MWSGGSGKAR	GWEAAAGGRS	SPGRLSRRRI	MSMSPKHTTP	FSVSDILSPL	EESYKKVGME	60
mut	MWSGGSGKAR	GWEAAAGGRS	SPGRLSRRRI	MSMSPKHTTP	FSVSDILSPL	EESYKKVGAA	60
wt	GGGLGAPLAA	YRQGQAAPPT	AAMQQHAVGH	HGAVTAAYHM	TAAGVPQLSH	SAVGGYCNGN	120
mut	ASGLRWRRTG	RARRHRQQRP	CSSTPWGTTA	PSPBPTT*			120
	LGNMSELPPY	QDTMRNSASG	PGWYGANPDF	RFPAISRFMG	PASGMNMSGM	GGLGSLGDVS	180
	KNMAPLPSAP	RRKRRVLF SQ	AQVYELERRF	KQQKYL SAPE	REHLASMIHL	TPTQVKIWFQ	240
	NHRYKMKRQA	KDKAAQQQLQ	QDSGGGGGGG	GTGCPQQQQA	QQQSPRRVAV	PVLVKDGKPC	300
	QAGAPAPGAA	SLOGHAQQQA	QHQAQAAQAA	AAAI SVGSGG	AGLGAHPGHQ	PGSAGQSPDL	360
	AHHAASPAAL	QGQVSSLSHL	NSSGSDYGTM	SCSTLLYGRT	W		420

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