De novo Loss of function Mutations in *KIAA2022* Are Associated with Epilepsy and Neurodevelopmental Delay in Females

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Conflicts of Interest
Megan Cho, Kyle Retterer, Francisca Millan, Aurora Pujol, Amber Begtrup, and Dianalee McKnight are employees of GeneDx. Wendy Chung is a consultant to BioReference Laboratories. The other authors declare that they have no conflict of interest.

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Graphical Abstract

Current age (years)	8	11	11	6	7
Developmental delay/ID	Yes	Yes	Yes	Yes	Yes
Current speech abilities	Limited language; 2 word phrases, unable to name common objects	Limited language; 2 word phrases	Babbling, some specific sounds	Short 3-word phrases	Can speak in sentences; language on the CELF is at ~ 4 year level
ADD/ADHD	Hyperactive and inattentive	Yes	Hyperactive	Attention difficulties	Not hyperactive, but distracted in classroom environment
Seizures	Yes – onset at 22 mos	Yes – onset at 2 years	Yes	Yes – first at 4 mos	Yes
Seizure type / EEG results	Generalized epilepsy; absence seizures (intractable)	Generalized, tonic clonic seizures (intractable)	Myoclonic, atonic drop seizures, absence, tonic, tonic-clonic (intractable)	Myoclonic epilepsy - diagnosed at age 1.5 yo; myoclonic astatic	Staring episodes with clonic jerks; generalized epilepsy (intractable)
Seizure frequency	Hundreds per day	10-15 per day	40-60 per day	Daily	Daily
Ataxia	Wide-based unsteady gait with toe walking	Broad-based gait	Ataxic gait, toe walking	Problems with coordination	None

Abstract

Intellectual disability (ID) affects about 3% of the population and has a male gender bias. Of at least 700 genes currently linked to ID, more than 100 have been identified on the X chromosome, including *KIAA2022*. *KIAA2022* is located on Xq13.3 and is expressed in the developing brain. The protein product of *KIAA2022*, X-linked Intellectual Disability Protein Related to Neurite Extension (XPN), is developmentally regulated and is involved in neuronal migration and cell adhesion. The clinical manifestations of loss-of-function *KIAA2022* mutations have been described previously in fifteen males, born from unaffected carrier mothers, but few females. Using whole exome sequencing, we identified a cohort of five unrelated female patients with *de novo* likely gene damaging variants in *KIAA2022* and core phenotypic features of intellectual disability, developmental delay, epilepsy refractory to treatment, and impaired language, of similar severity as reported for male counterparts. This study supports *KIAA2022* as a novel cause of X-linked dominant intellectual disability, and broadens the phenotype for *KIAA2022* mutations.

Keywords:

KIAA2022; whole exome sequencing; intellectual disability; autism; seizures

Introduction

With the availability of whole exome sequencing (WES), our understanding of the genetic causes of neurological conditions such as intellectual disability (ID) and autism, is rapidly expanding [1-3]. As both ID and autism are more frequent in males, with a ratio of 1.3:1 for ID and 4:1 for autism, studying the X chromosome has been informative in identifying genes linked to these conditions, including *KIAA2022* [4-6].

KIAA2022 (OMIM 300524) maps to Xq13.3 and was first described by Cantagrel, et al in 2004 in two related males with severe to profound intellectual disabilities, autism, absent language development, and dysmorphic facial features [7]. Since then, sixteen more patients from nine families have been described with loss-of-function mutations in *KIAA2022* and similar clinical phenotypes [8-13]. Females

with *de novo* loss of function mutations in *KIAA2022* have also recently been reported to be associated with intellectual disabilities and intractable epilepsy [14].

KIAA2022 is expressed in the developing brain. Expression of *Kiaa2022*, the murine ortholog, begins on E11.5, increases during embryonic development, and spreads to areas that later become the hippocampus, entorhinal and piriform cortices, and the hypothalamic ventral premammillary nucleus. Expression tapers off in adulthood [15].

KIAA2022 encodes two protein products: the primary 1516 amino acid protein X-linked Intellectual Disability Protein Related to Neurite Extension (XPN), and an unnamed 118 amino acid protein [15]. Mechanistic insights have been gained through suppression of XPN [16]. XPN suppression leads to an increase in N-cadherin and B1-integrin levels [17]. Through regulation of neural signaling pathways, N-cadherin and B1-integrin impact not only neuronal migration and cell adhesion, but also the growth and proliferation of neurites which are critical for developing neuronal circuits [18-21].

We present five unrelated females with *de novo* likely damaging variants in the protein-coding region of *KIAA2022,* with overlapping core features of global

developmental delay, intellectual disabilities and medically refractory epilepsy, and expand the phenotype of KIAA2022 deficiency to females.

Materials and Methods

The study population is a consecutive case series of patients referred for clinical whole exome sequencing to a clinical diagnostic laboratory. Informed consent was obtained from all individual participants included in this study. This study was approved by the Institutional Review Board of Columbia University. Genomic DNA was extracted from whole blood from the affected children and their family members. Whole exome sequencing (WES) at GeneDx was performed on exon targets isolated by capture using the Agilent SureSelect Human All Exon V4 (50 Mb) kit or Clinical Research Exome kit (Agilent Technologies, Santa Clara, CA). The sequencing methodology and variant interpretation protocol has been previously described [22]. All variants reported were confirmed with Sanger sequencing. The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page.

Results

Among 2,323 females with developmental delay/intellectual disabilities who were evaluated with clinical WES, we identified six individuals with *de novo* predicted pathogenic variants in *KIAA2022*, one of whom is not included in this paper. WES produced an average of ~9GB of sequence per sample. Mean coverage of captured regions was ~110X per sample, with >96% covered with at least 10x coverage, an average of 89% of base call quality of Q30 or greater, and an overall average mean quality score of >Q36. All reported variants in *KIAA2022* were confirmed with Sanger sequencing. The clinical phenotypes of all five individuals are summarized in Table 1.

Patient 1

Patient 1 is an eight-year-old female and was reported previously (patient 7 by de Lange et al) [14]. There was oligohydramnios during pregnancy, but no other prenatal complications. Birth weight was 2.4 kg (2nd percentile), length was 49.5 cm (50th percentile), and head circumference was 33.7 cm (50th percentile). Her weight later normalized and she was in the 75th percentile at six years old. She was developmentally delayed and did not talk until twenty-four months. She is now able to speak in two word phrases. She has a limited vocabulary, global developmental delay with an intelligence quotient (IQ) of 56 on the Wechsler Intelligence Scale for Children, and hypotonia. She began having generalized and absence seizures at

twenty-two months and continues to have hundreds of seizures per day. Behaviorally, she is hyperactive, inattentive and anxious, has frequent tantrums, and is unable to dress and feed herself. She has a wide-based unsteady gait and toe walks. Her only dysmorphic features are low-set ears. Initial brain MRI was normal, but MRI two years later showed generalized atrophy and generalized volume loss. Other medical issues include chronic constipation, use of a gastrostomy tube due to feeding difficulties, anemia and eczema. She has a *de novo* 14-nucleotide deletion (c.3053_3066del14, p.G1018Dfsx2) in *KIAA2022*, predicted to produce a truncated protein product of 1018 amino acids.

Patient 2

Patient 2 is an eleven-year-old girl and was reported previously (patient 12 by de Lange et al) [14]. Her mother had an uneventful pregnancy. Birth weight was 3.2 kg (50^{th} percentile) and length was 48.3 cm (25^{th} percentile). She started walking at 15 months, and developmental delays were clearly identified by 20 months. She did not start talking until 4.5 years and currently only speaks in two word phrases. Her IQ is < 60. She began having generalized tonic-clonic seizures at two years old and continues to have between ten and fifteen seizures/day. Her development regressed with the onset of her seizures. She is unable to dress or feed herself and is continent of urine only during the day. She is hypotonic, has autism spectrum

disorder, severe attention deficit hyperactivity disorder, and problems with impulse control. Brain MRIs have been normal. She has a broad-based gait. She had cardiac rhabdomyomas in the first few years of life and hypopigmented macules with no mutations identified in *TSC1* or *TSC2*. She has a *de novo* nonsense c.652 C>T, p.R218X mutation in *KIAA2022*, predicted to produce a truncated 217 amino acid protein product.

Patient 3

Patient 3 is an eleven-year-old girl. Her mother had elevated antibodies to parvovirus during the pregnancy, but no symptoms of parvovirus were demonstrated and weekly prenatal ultrasonography was normal.

Birth weight was 3.5 kg (75th percentile) and length was 49.5 cm (50th percentile). OFC measurements increased from 36.8 cm at 1 month old (50th percentile) to 48.3 cm at 3 years old (50th percentile). She was developmentally delayed, particularly in her limited speech. Currently, she babbles and makes sounds, but has no verbal speech. Other neurological abnormalities include esotropia, hypotonia, hyperactivity and autistic features. She has myoclonic, atonic drop, tonic and tonic-clonic seizures. These seizures are medically refractory and occur an average of forty to sixty times per day, with up to 100 seizures/day. After she began having seizures, she experienced regression in her vocalization skills. She has limited self-

care skills, but can undress herself and feed herself with a spoon. She has an ataxic gait, toe walks and has tight heel cords. Her dysmorphic features include a short philtrum, prognathism, low-set hairline, frontal bossing, widely spaced teeth, high-arched palate, and slightly short fingers (Figure 1). Brain MRI was normal. Other medical conditions include gastroesophageal reflux disease, constipation, decreased pain sensitivity and difficulty with chewing and swallowing. She has a *de novo* frameshift mutation, c.422delA, p.Q141RfsX7 in *KIAA2022*, predicted to produce a truncated 146 amino acid protein product.

Patient 4

Patient 4 is a six-year-old girl. Her mother had an uneventful pregnancy. Birth weight was 3.3 kg (50th percentile), height was 51 cm (57th percentile) and OFC was 33.5 cm (10th percentile). Her OFC at 4.5 years was 50.5 cm (53rd percentile) and increased to52.2 cm at 5.8 years (81st percentile). She had normal motor milestones but is developmentally delayed. While she began to talk at 8-9 months, she currently only speaks in short three word phrases. She also has esotropia, attention difficulties and tantrums. She began having "shuddering" episodes at four months old, which progressed into myoclonic seizures diagnosed at 1.5 years old. She continues to have daily, uncontrolled seizures. Her gait is normal, but she has poor coordination. Her only dysmorphic feature is inverted nipples. Other medical issues

include hypotonia, gastroesophageal reflux disease, and easy bruising. She has a *de novo* duplication, c.625dupC, p.L209PfsX3 mutation in *KIAA2022*, predicted to produce a truncated 210 amino acid protein product.

Patient 5

Patient 5 is a seven year old girl. Her mother had an uneventful pregnancy. Birth weight was 3.6 kg (75th percentile), length was 52 cm (75th-90th percentile), and OFC was 38.1 cm (50th percentile). She walked without support and said single words at 18 months. She currently speaks in sentences with language skills of a three to four year-old. Her IQ is 48. She also has anxiety and tantrums. Seizures started at two and a half years of age, primarily described as head drops and staring episodes with multiple events per day. Recent electroencephalograms show generalized epilepsy. She has myoclonus and absence-like events that have been medically refractory including failure of the ketogenic diet. She has not experienced significant regression. Her self-care abilities reflect those of a three year-old. She is non-dysmorphic. Other medical conditions include gastroesophageal reflux in infancy, constipation, encopresis and a kidney infection that required hospitalization. She has a *de novo* nonsense c.C937T, p.R313X mutation in *KIAA2022*, producing a truncated 312 amino acid product.

Discussion

We present phenotypic data from five females with *de novo* likely gene damaging variants in *KIAA2022*. All of our patients have intellectual disabilities, medically refractory seizures and impaired language development; two of them have autism spectrum disorders and a third has some autistic features. Three exhibit abnormal or ataxic gait, and a fourth has problems with motor coordination.

Previously described *KIAA2022* mutations include microdeletions, duplications, translocations in the non-coding regions of the gene, frameshifts and nonsense mutations and are predicted to act through loss of function (Table 2).

Fifteen of the eighteen previously described patients with *KIAA2022* mutations were male. Many of them shared intellectual disabilities, absent or limited language, and autism spectrum disorders. Less consistently observed were dysmorphic facial features (including hypotonic face, narrow forehead, anteverted nostrils, open mouth, and large ears), seizure disorder, and delayed or absent walking. Brain imaging studies in nine patients from six families were normal except for patients 1 and 2 in Table 2. Patient 1 also had a small cerebellar vermis [7-10]. Based on inheritance patterns of these male patients, there are fourteen unaffected female

obligate carriers in these families. All were described as clinically normal although they were not evaluated for subtle neurocognitive differences. The mother of patient 2 expressed normal levels of the *KIAA2022* gene unlike her son [7-10].

However, three unrelated female patients with *de novo KIAA2022* mutations were recently reported [11-13]. In Table 2, patient 16 had severe intellectual disabilities, autism spectrum disorder, absent language and dysmorphic facial features; patient 17 had mild intellectual disabilities, and patient 18 had severe intellectual disabilities, autistic behavior, limited language, epilepsy, walking difficulties, dysmorphic features, microcephaly and hypotonia. Presumably, an X-linked dominant inheritance pattern with skewed X-inactivation towards the normal allele in the brain could explain why some females with these mutations are affected while others are asymptomatic carriers [11-13]. There likely has been bias of ascertainment in the recognition and reporting of rare variants in *KIAA2022* and greater recognition of the role of *KIAA2022* in neurodevelopment and behavior should lead to a more complete and less biased description of the full spectrum of phenotypes in females with either inherited or *de novo* predicted pathogenic variants in *KIAA2022.* The variability in phenotype of female carriers could also be partially explained by other additive factors including other interacting genetic factors including possibly tuberous sclerosis in one of our patients.

Compared to previously reported female patients, our patients are younger, and show phenotypic overlap with the more severely affected end of the spectrum. For instance, the female with the p.R628X mutation (patient 17 in Table 2) has a milder phenotype relative to our patients. Our patients and the female with the p.R322X mutation (patient 18 in Table 2) have more severe and refractory epilepsies relative to other reported females. In several neurologic conditions, severity and age at onset of epilepsy is a major morbidity factor, usually related to severity of phenotype, i.e. intelligence quotient or speech [23]. A larger number of patients may allow for establishing similar correlations in this condition.

Our study expands the phenotype of *KIAA2022* mutations and demonstrates that females with *de novo* predicted loss-of-function mutations in *KIAA2022* may exhibit similar neurological phenotypes to their male counterparts, including medically refractory seizures and severe intellectual disability.

Table 1. Clinical characteristics of females with *de novo* likely gene damaging mutations in *KIAA2022*. WT= weight. HT=height, OFC=occipital frontal circumference. GERD= gastroesophageal reflux disease. ID = intellectual disability. ADD=attention deficit disorder. ADHD=attention deficit hyperactivity disorder. PDD-NOS= pervasive developmental disorder not otherwise specified.

Patient	1	2	3	4	5
Mutation	g.183982_183995del14 c.3053_3066del14 p.G1018Dfsx2	g.181581 C>T c.C652T p.R218X	g.181351_181351delA c.422delA p.Q141RfsX7	g.181554_181555dupC c.625dupC p.L209PfsX3	g.181866 C>T c.C937T p.R313X
Current age (years)	8	11	11	6	7
Gender	Female	Female	Female	Female	Female
Prenatal issues	Oligohydramnios	None	Elevated parvovirus antibodies	None	None
Congenital anomalies	None	None	None	None	None
Birth WT, HT, OFC	WT = 2.4 kg (2%) HT = 49.5 cm (50%) OFC = 33.7 cm (50%)	WT = 3.2 kg (50%) HT = 48.3 cm (25%)	WT = 3.5 kg (75%) HT = 49.5 cm (50%)	WT = 3.3 kg (50%) HT= 51 cm (57%)	WT = 3.58kg (75%) HT = 52 cm (75-90%) OFC = 38.1 cm (50%)
Current WT, HT	At 6 yo WT = 23.1 kg (75%) HT = 114.3 cm (50%)	WT = 34.0 kg (25%) HT = 147.3 cm (75%)	WT = 51.7 kg (90%) HT = 143.4 cm (50%)	At 5 yo WT = 23.2 kg (95%) HT = 111.5 cm (75%) OFC = 52.2 cm (81%)	WT = 36.6kg (95%) HT =130.1cm (80%)
Gastrointestinal /Growth issues	Chronic constipation, gastrostomy tube due to feeding difficulties	None	GERD, constipation	GERD	GERD, constipation
Ophthalmologic issues	None	None	Esotropia	Esotropia	None
Developmental delay/ID	Yes	Yes	Yes	Yes	Yes
Age at sitting	6 months	5.5 months	"on time"	4 months	Not specified

Age at walking	12 months	15 months	16 months	15 months	18 months
Age at talking	24 months	4.5 years	N/A	8-9 months	Single words 18 months
Current speech abilities	Limited language; 2 word phrases, unable to name common objects	Limited language; 2 word phrases	Babbling, some specific sounds	Short 3-word phrases	Can speak in sentences; language on the CELF is at ~ 4 year level
Muscle tone	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Normal
Autism	Yes	Yes	Autistic features, but no formal diagnosis	No	No
ADD/ADHD	Hyperactive and inattentive	Yes	Hyperactive	Attention difficulties	Not hyperactive, but distracted in classroom environment
Full scale IQ	56	<60	Not tested	Not tested	48
Notable behaviors	Hyperactivity, temper tantrums, anxiety	Severe ADHD, impulsive control disorder	Self-injurious behaviors, destructive, stares at objects, licks things, turns in circles	Tantrums	Anxiety, tantrums
Regression	Yes	Yes, before onset of seizures	Had more vocalization previously; after seizures often would lose and regain skills	None	None
Seizures	Yes – onset at 22 mos	Yes – onset at 2 years	Yes	Yes – first at 4 mos	Yes
Seizure type / EEG results	Generalized epilepsy; absence seizures (intractable)	Generalized, tonic clonic seizures (intractable)	Myoclonic, atonic drop seizures, absence, tonic, tonic-clonic (intractable)	Myoclonic epilepsy - diagnosed at age 1.5 yo; myoclonic astatic	Staring episodes with clonic jerks; generalized epilepsy (intractable)
Seizure frequency	Hundreds per day	10-15 per day	40-60 per day	Daily	Daily

Ataxia	Wide-based unsteady gait with toe walking	Broad-based gait	Ataxic gait, toe walking	Problems with coordination	None
Other neurological or neuromuscular disorder(s)	None	None	Tight heel cords	None	None
Self-care abilities	None	Unable to dress or feed herself; can urinate in toilet during the day; wears diaper at night	Emerging skills: can undress self, feeds self with spoon	Not provided	Consistent with that of a 3 year old
Brain MRI/CT results	Generalized atrophy on MRI	2 normal MRIs	Normal MRI	Normal MRI	Normal MRI
Dysmorphic features	Low set ears	None	Short philtrum, prognathism, low-set hairline, frontal bossing, widely spaced teeth, highly arched palate, slightly short fingers	Inverted nipples	None
Additional medical problems	Anemia, eczema	Cardiac rhabdomyoma as found in first few years of life with negative *TSC 1* and *TSC2* gene testing. No brain tubers or renal lesions. Hypopigmented macules.	Decreased pain sensitivity, chewing and swallowing difficulties	Bruises easily	Significant kidney infection in past

Table 2: Clinical characteristics of previously described patients with mutations in *KIAA2022*. GERD=gastroesophageal reflux disease; NA= data not available

Patient	Genotype	Sex	Age	ID	Autism/Autistic Behaviors	Absent/Limited Language	Limited/Delayed Walking	Seizure Disorder	Facial Features	Hypotonia	Imaging
1	Inherited; Translocation (Intron 1)	M	13	Severe-Profound		X	X	None	Dysmorphic	X	MRI: moderate brain atrophy, small cerebellar vermis, thick calvarium
2	Inherited; Translocation (Intron 1)	M	20	Severe-Profound	X	X	X	Generalized	Dysmorphic	X	CT: frontal cortical atrophy
3	Inherited; Duplication (p.S1200YfsX5)	M	6	Severe-Profound	X	X		Flexor spasms	Dysmorphic	X	NA
4	Inherited; Duplication (p.S1200YfsX5)	M	4	Severe-Profound	X	X	X	Flexor spasms	Dysmorphic	X	NA
5	Inherited; 70kb Microdeletion (Exon 1)	M	8	Mild	X	X		None	Dysmorphic	X	MRI: normal @ 9yo
6	Inherited; Deletion (p.R62EfsX22)	M	14	Moderate -Severe		X		None	Dysmorphic	X	MRI: normal
7	Inherited; Deletion (p.R62EfsX22)	M	10	Moderate -Severe	X	X		Lennox-Gastaut	Dysmorphic	X	MRI: normal
8	Inherited; Deletion (p.R62EfsX22)	M	40	Moderate		X		Generalized	Dysmorphic	NA	NA
9	Inherited; Duplication (Xq13.3: whole gene + intergenic sequences)	M	NA	Moderate	X			None	Normal	NA	MRI: normal
10	Inherited; Duplication (Xq13.3: whole gene + intergenic sequences)	M	NA	Severe	X			Generalized paroxysmal seizures on EEG	Normal	NA	MRI: normal
11	Inherited; Duplication (Xq13.3: whole gene + intergenic sequences)	M	NA	NA		X		None	NA	NA	NA
12	Inherited; Duplication (Xq13.3: whole	M	NA	X				NA	NA	NA	NA

	gene + intergenic sequences)										
13	Inherited; Duplication (Xq13.3: whole gene + intergenic sequences)	M	NA	X				NA	NA	NA	NA
14	De novo; Nonsense (p.Q705X)	M	3	Severe	X	X	X	None	Dysmorphic	X	MRI: normal
15	De novo; Nonsense (p.R322X)	M	5	Severe	X	X	X	None	Dysmorphic	X	MRI: normal
16	De novo; Translocation (Intron 1)	F	26	Severe	X	X		None	Dysmorphic	NA	NA
17	De novo; Nonsense (p.R628X)	F	13	Mild				None	Normal	NA	MRI: normal
18	De novo; Nonsense (p.R322X)	F	17	Severe	X	X	X	Absence, atonic drop, tonic & generalized seizures	Dysmorphic	X	Normal
19	De novo; Nonsense (p.K1396fs)	F	26	X	X		X	Generalized tonic-clonic, myoclonic	Normal		Normal
20	De novo, Nonsense (p.C146*)	F	9	X	X		X	Generalized and focal; tonic-clonic, myoclonic, absence, atonic	Dysmorphic	X	Normal
21	De novo; Nonsense (p.G681fs)	F	25	X			X	Generalized tonic-clonic, myoclonic, absence, atonic	Normal		Normal
22	De novo; Nonsense (p.R322*)	F	11	X	+			Generalized, and focal myoclonic, tonic atonic	Normal		Normal
23	De novo; Nonsense (p.K734Sfs*24)	F	36	X				Generalized tonic-clonic, myoclonic, absence	Normal		Normal
24	De novo; Nonsense (p.R481*)	F	2	X		X	X		Dysmorphic	X	Normal
25	De novo; Deletion (Exon 1)	F	18	X	X	X	X	Focal clonic	Normal		Normal
26	De novo; Nonsense (p.R528Qfs*4)	F	5	X		X	X	Generalized myoclonic, absence, tonic-clonic, atonic	Normal		Normal

27	De novo; Nonsense (p.R628*)	F	2	X		X	X		Normal	X	Normal
28	De novo; Nonsense (p.A909Pfs*13)	F	53					Generalized, absence	Normal		N/A
29	De novo; Nonsense (p.Q318*)	F	51	X		X	X	Generalized tonic-clonic, myoclonic, atonic, tonic-clonic,	Dysmorphic		Normal
30	De novo; Nonsense (p.K1199Nfs)	F	8	X		X	X	Generalized tonic-clonic, myoclonic, atonic, tonic-clonic,	Normal	X	Normal

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Online resources:

Mutation Taster. *MutationTaster.org.*
http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/

Legends:

Figure 1: Facial characteristics of Patient 3 including a short philtrum, prognathism, low-set hairline, frontal bossing, and widely spaced teeth

KIAA2022 Figure 1.jpg