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CLINICAL REPORT

CHL1 deletion is associated with cognitive and language disabilities – Case report and review of literature

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Abstract

Background: There is a small, but growing number of reports of pediatric patients with terminal deletions at 3p26.3 involving only the cell adhesion molecule L1-like (*CHL1)* gene that has been found to have language delays and intellectual disability. Here we report a one month of age patient who developed seizures and tone abnormalities, with persistent and prominent gross and fine motor delays. The patient has microcephaly and deficits in language and cognitive delays, similar to what has been seen in previous case reports.

Methods: Chromosome and microarray comparative genomic hybridization (aCGH) analysis was performed to identify clinically significant copy number variants (CNVs). In addition, Fluorescent in-situ hybridization (FISH) was performed to confirm the aCGH findings.

Results: Chromosome analysis revealed an apparently normal (46,XX) female karyotype. Microarray CGH analysis revealed a 639 kb loss at 3p26.3 from 62199 to 701052 base pairs encompassing the whole *CHL1* gene that was confirmed by FISH. Parental follow-up revealed the deletion as maternal in origin.

Conclusion: This case report adds to the limited body of literature that exists on this terminal deletion at 3p26.3 that involves CHL1 gene, and supports prior proposals of an emerging *CHL1* microdeletion syndrome that results in language and cognitive delays. Further studies are needed to understand the degree of phenotypic heterogeneity associated with CHL1 gene deletion and whether the size of the deletion or presence of additional copy number variants (CNVs) which were seen in other case reports help predict the expected phenotype for a patient.

KEYWORDS

3p26.3, *CHL1*, cognitive impairment, developmental delay, language delay, microdeletion

1 | INTRODUCTION

The *CHL1* gene located at 3p26.3 encodes for a member of the L1 gene family of neural cell adhesion molecules, which play a crucial role in neuronal overgrowth and migration in the developing brain (Dong et al., 2002; Katic et al., 2014). There have been several mouse models with partial or complete deletion of *CHL1* that have illustrated cognitive, behavioral, motor, and coordination deficits. To date, there have been five children described with heterozygous deletion of

CHL1 gene who have also demonstrated language and cognitive delays (Bertini et al., 2017; Cuoco et al., 2011; Pohjola et al., 2010; Tassano et al., 2014). We present a sixth patient with a 639 kb loss at 3p26.3 with the youngest reported age of onset of neurologic symptoms. This case report further supports Tassano et al.'s proposal of a *CHL1* microdeletion syndrome resulting in language and cognitive delays, with a broader spectrum of other possible associated clinical features including microcephaly, seizures, dysmorphic features, and short stature (Tassano et al., 2014).

2 | MATERIALS AND METHODS

2.1 | Clinical report

Case 1: She is one of eight children born to healthy, nonconsanguineous Somalian refugee parents. She was the product of an uncomplicated term pregnancy who was delivered via spontaneous vaginal delivery notable for terminal meconium and Apgars of eight and nine at one and five minutes, respectively. Her neonatal course was complicated by two episodes of apnea and hypothermia. She underwent a sepsis evaluation, including a lumbar puncture, which was unrevealing. Birth weight was 2.98 kg (28th percentile), length 48.2 cm (31st percentile), and head circumference 33 cm (23rd percentile). The seven siblings range from three months to nine years in age; all are healthy with normal development. There is no family history of developmental disability, intellectual disability, seizures, spasticity, delayed language, or congenital anomalies. At 30 days of life, the patient had an apneic spell with associated bilateral arm shaking with eyes open. Upon presentation to the local emergency department, she was noted to have stiffening of extremities with left gaze deviation, hypoxia, and repeated apneic spells. Her protracted hospital course of 47 days was complicated by two failed extubations, once in the setting of rhinovirus/enterovirus and tracheitis from non-type B *Haemophilus influenzae*; left temporal seizures confirmed on long term video EEG monitoring manifesting clinically as episodes of tachycardia followed by apnea and desaturations that resolved with lorazepam and initiation of levetiracetam. EEG also showed interictal epileptiform discharges that occurred independently in left more than right temporal regions. During the early portion of her hospitalization, the patient developed appendicular spasticity and symmetric hyperreflexia with sustained clonus at ankles. Over the next several months, her tone exam evolved. At the age of two months, tone normalized and patellar reflexes were normal but with four to six beats of clonus at the ankles. Improvement in her exam temporally correlated to overall clinical improvement. She did not have any further seizures. At nine months of age, she was noted to have truncal and bilateral upper and lower appendicular hypotonia by occupational therapy. However, in neurology follow-up at 13 months of age, the patient had truncal hypotonia with distal greater than proximal symmetric lower extremity spasticity. Repeat routine EEG at that time was normal without focal or epileptiform abnormalities. As she had no further spells concerning for seizure, she was tapered off levetiracetam at that time and continued to remain seizure-free at the time of her last follow-up at 19 months of age. At that time, the patient's exam was notable for microcephaly (HC 43.5 cm, 1st percentile), absence of dysmorphic features, excellent eye contact, and social smile, but without vocalizations (neither babbling nor cooing). She demonstrated axial hypotonia, left greater than right spasticity at the ankles and to a milder degree at the knees. She was able to sit in a tripod stance and was not yet crawling. Occasionally she would perform a "no-no" head shake, and other times would intertwine her hands midline though not consistent with hand wringing. She reached out for objects with either hand with an immature grasp, but did not transfer objects. Deep tendon reflexes were normal and she had no ankle clonus.

Brain MRI showed no abnormalities at one month of age and on follow-up imaging one month later. Cervical spine MRI was also normal. Ophthalmologic evaluation revealed large optic discs, possibly a normal variant, and no other abnormalities. Metabolic work-up including New York State Newborn Screen, carnitine level, CDT for disorders of glycosylation, ammonia, lactate, plasma acylcarnitine profile, and urine organic acids were all unremarkable. The comprehensive fatty acid profile showed an elevated concentration of docosahexaenoic acid which was of unclear relevance, with the remainder of the profile being essentially normal. Quantitative plasma amino acid analysis initially revealed low plasma serine of 77 (normal 90–300 μmol/L), but repeat testing one year later showed normalization of serine level, with no evidence of any specific inborn error of amino acid metabolism. CSF studies at the time of her hospitalization showed zero nucleated cells, 45 RBCs, and normal glucose (65 mg/dl) and protein (32 mg/dl).

2.2 | Array comparative genomic hybridization (aCGH) analysis

DNA was extracted using QIAamp® DNA Blood Mini Kit (Cat #51106). NanoDrop ND-2000 spectrometer (Thermo Scientific) was used for the determination of DNA concentrations. Microarray experiments were performed on SurePrint G3 ISCA CGH+SNP Microarray Kit, 4x180K v2.0 platform (Agilent Technologies), featuring approximately 110,715 custom oligonucleotides +59,647 SNPs

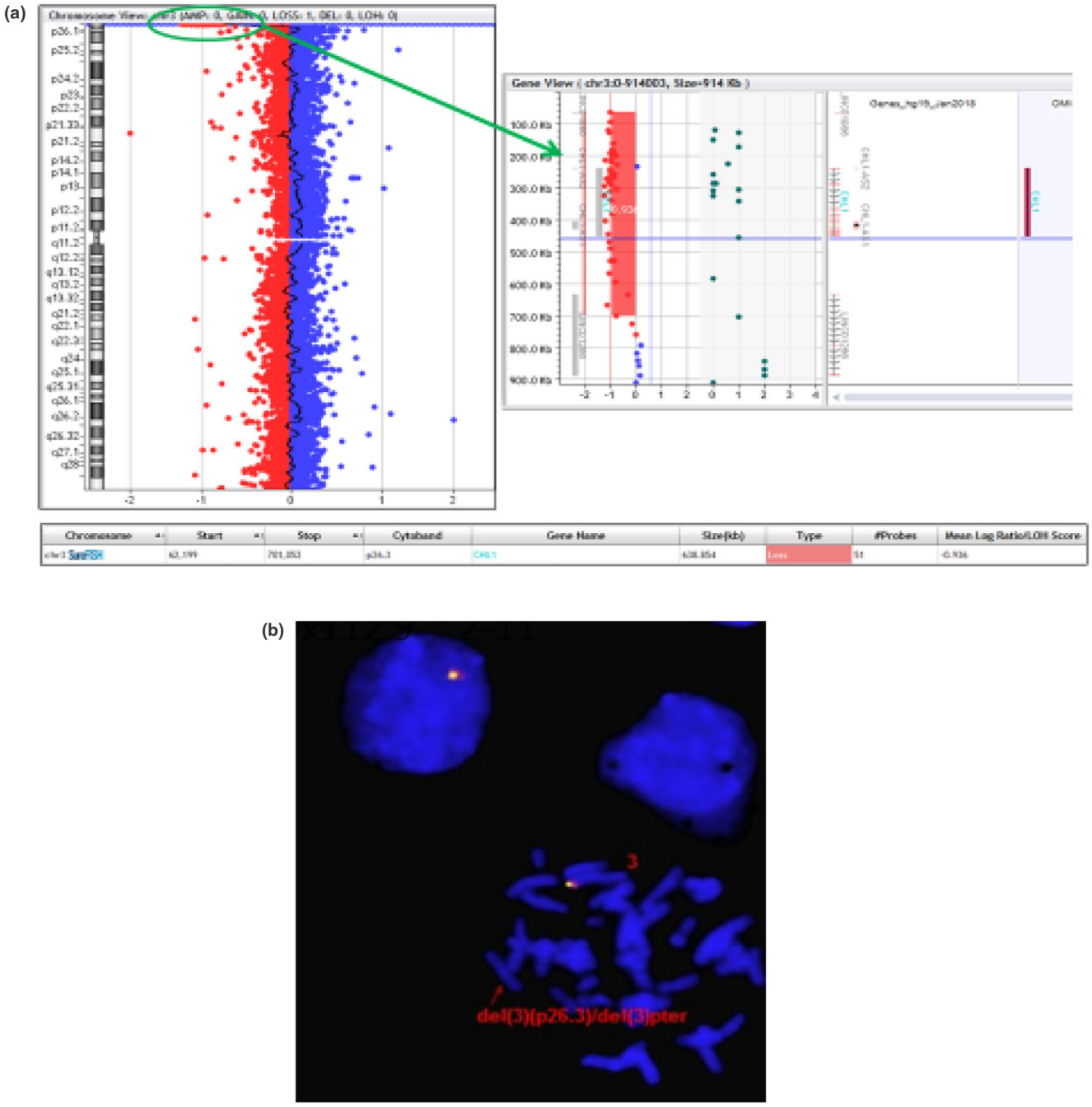

FIGURE 1 (a) Chromosome view of chromosome 3 showing the deletion in the 3p26.3 region, expanded view of the deletion and the log2 ratio below the figures. (b) Fluorescence in situ hybridization (FISH) confirmation of chromosome microarray (CMA) finding, RP11-964P7 (3p26.3;SO) with TelVysion 3p (SG)

(60-mers) and covering 1282 ISCA regions, resulting in a 25.3 kb resolution. The patient DNA was referenced against Agilent Human Reference DNA male/female (5190-4370/4371) using Agilent's SureTag Complete DNA labeling Kit (Cat # 5190-4240) as per the manufacturer's recommendations. Patient data were scanned (Agilent Model #G2505C) at 3μm resolution, and visualized (Cytogenomics Software) with log2 threshold ratios of −0.25 for losses and 0.25 for gains.

2.3 | Fluorescence in situ hybridization (FISH) analysis

FISH analysis was carried out to confirm the aCGH findings. The BAC and subtelomeric probes RP11-964P7 (3p26.3;SO) with TelVysion 3p (pter;SG) was labeled according to manufacturer's instructions (Cat #05J03-003, Abbott Laboratories). Hybridization was performed as per the manufacturer's instructions and standard protocols. Slides were analyzed using

a Nikon (Eclipse 80i) fluorescence microscope attached with a CCD camera; Cytovision (Leica systems) software was used for image acquisition and analyses. Ten metaphase and 100 interphase cells were analyzed for confirmation of aCGH findings.

3 | RESULTS

Chromosome analysis in case 1 revealed an apparently normal (46,XX) female karyotype. Microarray CGH analysis revealed a 639 kb loss at 3p26.3 from 62199 to 701052 base pairs encompassing the whole *CHL1* gene that was confirmed by FISH (Figure 1a,b). Parental follow-up FISH testing revealed the deletion at 3p26.3 as maternal in origin. Unfortunately, parents declined FISH testing of their seven other children and trio whole-exome sequencing was not available. The deletion was inherited from an apparently normal mother (data not shown). The nomenclature is described as:

46,XX.ish del(3)(p26.3p26.3)(RP11-964P7-,pter-). arr[GRCh37/hg19] 3p26.3(62199_726704)x1 mat

4 | DISCUSSION

The 3p deletion syndrome is a rare but well-characterized syndrome caused by deletions of varying sizes in the 3p25-pter region that has a known but variably expressed clinical phenotype including developmental delay, intellectual disability, dysmorphic features, ptosis, microcephaly, and growth retardation. This syndrome often involves deletion of large regions that include multiple genes, including *CHL1*, contactin 4 (*CNTN4*), leucine-rich repeat neuronal 1 (*LRRN1*), SLIT-ROBO Rho GTPase activating protein 3 (*SRGAP3*), cereblon *(CRBN*), making it difficult to assess whether a particular gene is responsible for a particular aspect of the phenotype. However, there is a growing number of reports of terminal deletions at 3p26.3 involving only the *CHL1* gene associated with language delays and intellectual disability. The association between CHL1 deletion and this clinical phenotype is further supported by this case report. Table 1, adapted from Tassano et al., (2014), summarizes the clinical presentation of five previously published patients and includes the new case in this report. All five published patients were heterozygous for this terminal deletion with one unaffected parent carrying the variant. Our patient presented with neurologic symptoms at the youngest age reported to date in the literature. The remainder of cases presented more insidiously, with delays not typically noted until well over one year of age. Our patient had an evolving tone exam and remained spastic in the lower extremities. Although she continued to make slow gains in development, she remained significantly delayed in gross and fine motor domains. This degree of motor delay was not a prominent feature in other reported patients, except for the patient reported by Bertini et al., (2017). The neuropsychological evaluation had not yet been performed. Her "no-no" head shake and midline hand movements may be stereotypies, but further observation will be needed; stereotypies or other movement disorders had not been previously described. At 4 year old follow-up visit, she presented with global developmental delays, slow motor progress, proper crawling, and sitting independently. She is reaching out and grasping with both hands. Her parents are feeding her a soft, pureed diet, not yet advanced to any foods that require chewing at home, she is not yet self-feeding.

The reason for the phenotypic heterogeneity among reported cases is unclear. It may be indicative of an even broader spectrum of associated clinical features and degree of severity of impairments than previously recognized. Alternatively, the size of the deletion and/or the presence of additional CNVs, which may act in an epistatic manner, may play a role in determining the overall clinical phenotype of each patient (Girirajan et al., 2010). *CHL1* partial or whole gene deletions are also reported in few unpublished cases in DECIPHER (https://decipher.sanger.ac.uk) with variable phenotypic expression and inheritance. Bertini et al., (2017) give a summary of cases observed in both DECIPHER and DGV. The cases in DECIPHER listed in Bertini et al., (2017) do support the argument presented in this manuscript.

As we continue to work towards gaining a better understanding of the role of *CHL1* deletion on the developing brain, there is also burgeoning literature describing a clinical phenotype of language delay and cognitive deficits associated with 3p26.3 microduplication encompassing the *CHL1* gene only that may provide more insight (Li et al., 2016; Palumbo et al., 2015; Shoukier et al., 2013). To date, there are three cases that have been reported. The first described a boy with mild hypertelorism, down-slanting palpebral fissures, long philtrum with thin upper lip, and mildly prominent ear lobes who had cognitive deficits, hyperactivity, short attention span, and language delay. He had a 0.85 Mb de novo microduplication on 3p26.3 that solely encompassed the *CHL1* gene (Palumbo et al., 2015). The second described a girl with a maternally inherited 1.07 Mb microduplication at 3p26.3 who had intellectual disability and epilepsy (Shoukier et al., 2013). The third case was of a male toddler with autism spectrum disorder, developmental disability, and minor dysmorphic facial features who had a maternally inherited 690 kb deletion. He had delays in cognitive, social, language, and motor domains. This was the first case with associated autism spectrum disorder (Li et al., 2016).

TABLE 1 Summary of clinical phenotype in *CHL1* deletion cases

	Index patient (case 1)	Tassano et al.	Cuoco et al., patient 1	Cuoco et al., patient 2	Pohjola et al., patient B	Bertini et al.
Age at presentation	1 month	6 years[a]	8 years[b]	4.5 years	10 months	6 years
Gender	F	M	M	M	M	F
Birth weight (g)	2980 (28th %ile)	2970	2400 (10th−25th %ile)	2080 (3rd−10th %ile)	3070	3222 (25th−50th %ile)
Birth length (cm)	48 (31st %ile)	50 (25th−50th %ile)	NA	44 (3rd−10th %ile)	48	52 (75–90th %ile)
Birth HC (cm)	33 (23rd %ile)	34	NA	31.5 (10^{th} %ile)	31.5	35
Postnatal microcephaly	+	+	−	−	+ (−3 SD at 12 yo)	−
Motor delay	+	−	−	−	+ ("slow physical development")	+
Cognitive delay/ Learning difficulties	Delayed; no formal testing	Mild intellectual disability (IQ 66)	Borderline IQ level (WISC-R)	N	+	Mild ID
Language delay	+	+	+	+	+[c]	+
Deficit in visual-perceptual organization	NA	+	+	NA	NA	−
Neuro-behavioral symptoms	−	Aggression, relation difficulties	−	−	Temper tantrums	−
Abnormal neurologic exam findings	Axial hypotonia, L>R lower extremity spasticity	Hypotonia, mild clumsiness	−	−	−	Axial hypotonia
Dysmorphic features	−	Epicanthal folds	Epicanthal folds, joint hyperlaxity	Straight eyebrows, short and smooth philtrum, right single palmar crease	Hypotelorism, low forehead, long thin and pointed nose, tapered fingers	Unremarkable
Associated systemic involvement	−	−	−	Shallow scrotum	Sleep apnea	−
MRI abnormalities	−	−	+ Mild ectopia of cerebellar tonsillar -at the foramen magnum	NA	−	−
Seizures	+	−	+	−	−	−
Neurocutaneous stigmata	−	−	Abdominal café-au-lait spots	Café-au-lait, dry skin	Reticular hyperpigmentation	−
Ophthalmologic abnormalities	Large optic discs, possibly normal variant	Mild bilateral convergent strabismus	Divergent strabismus OD, myopia, and retinal spots	−	−	−

(Continues)

TABLE 1 (Continued)

	Index patient (case 1)	Tassano et al.	Cuoco et al., patient 1	Cuoco et al., patient 2	Pohjola et al., patient B	Bertini et al.
Family history of developmental delay	–	Paternal cousin with language delay	Brother (Cuoco, patient 2)	Brother (Cuoco, patient 1)	(–) but maternal grandfather was microcephalic	–
Size of deletion at 3p26.3	639 kb	956 kb	555 kb	555 kb	1.1 mb	966 kb
Pattern of inheritance	Maternal	Maternal	Paternal	Paternal	Maternal	Maternal
Additional CNVs/ Parental origin/Gain or Loss of Function	–	209 kb duplication at 21q22.3/Paternal/ Gain	696 kb duplication at 1q44/Maternal/Gain	696 kb duplication at 1q44/ Maternal/Gain	–	–

[a]Although there was minimal language acquisition obtained between 12 months and 3 years, information from a neurological evaluation is only available from 6 years of age.
[b]Patient had a seizure at 8 years of age. School learning difficulties prompting neuropsychological evaluation is noted, but the age at which these concern arose is not available.
[c]A severe learning disability in language and mathematics is noted, but further details are not available.

The overlap of cognitive and language impairments seen between deletions and duplications of 3p26.3 specifically involving *CHL1* further supports that this gene likely plays a major role in language and cognitive development. It is possible that parents and probands could be heterozygous for the same deletion and yet the parents will remain unaffected as a result of incomplete penetrance. Frints et al., (2003) did find that reduction in *CHL1* expression by 50% in mice resulted in a wide range of clinical phenotypes that spanned from those seen in wild-type to knock out mice; it has been suggested by this group that there could be a gene dosage effect at play. The role that additional CNVs may contribute to the clinical phenotype as mentioned previously remains uncertain. While the further investigation into this is still needed, *CHL1* microdeletion syndrome as a cause for language and cognitive delay, sometimes accompanied by a constellation of other neurologic issues such as seizures, deficits in other developmental domains, and postnatal microcephaly should become a more recognized entity among developmental pediatricians, geneticists, and child neurologists.

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CONFLICT OF INTEREST

The authors declared no conflicts of interest.

AUTHOR CONTRIBUTIONS

MAI and MT contributed to the conception of this work. MT performed the clinical evaluation. MAI performed the genetic analysis. MT wrote the draft of the manuscript. All authors contributed to manuscript revision and approved the final submitted version.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this work.

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REFERENCES

Bertini, V., Azzara, A., Toschi, B., Gana, S., & Valetto, A. (2017). 3p26.3 terminal deletions: a challenge for prenatal genetic counseling. *Prenatal Diagnosis*, *37*, 197–200. https://doi.org/10.1002/pd.4978

Cuoco, C., Ronchetto, P., Gimelli, S., Benaz, F., Divizia, M. T., Lerone, M., Mirabelli-Badenier, M., Mascaretti, M., & Gimelli, G. (2011).

Microarray based analysis of an inherited terminal 3p26.3 deletions, containing only the *CHL1* gene, from a normal father to his two affected children. *Orphanet Journal of Rare Diseases*, *6*, 1–6.

Dong, L., Chen, S., Richter, M., & Schachner, M. (2002). Single-chain variable fragment antibodies against the neural adhesion molecule CHL (close homolog of L1) enhance neurite outgrowth. *Journal of Neuroscience Research*, *69*(4), 437–447.

Frints, S. G. M., Marynen, P., Hartmann, D., Fryns, J. P., Steyaert, J., Schachner, M., Rolf, B., Craessaerts, K., Snellinx, A., Hollanders, K., D'Hooge, R., De Deyn, P. P., & Froyen, G. (2003). CALL interrupted in a patient with non-specific mental retardation: Gene dosage-dependent alteration of murine brain development and behavior. *Human Molecular Genetics*, *12*(13), 1463–1474. https://doi.org/10.1093/hmg/ddg165

Girirajan, S., Rosenfeld, J. A., Cooper, G. M., Antonacci, F., Siswara, P., Itsara, A., Vives, L., Walsh, T., McCarthy, S. E., Baker, C., Mefford, H. C., Kidd, J. M., Browning, S. R., Browning, B. L., Dickel, D. E., Levy, D. L., Ballif, B. C., Platky, K., Farber, D. M., … Eichler, E. E. (2010). A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. *Nature Genetics*, *42*, 203–209. https://doi.org/10.1038/ng.534

Katic, J., Loers, G., Karl, N., Schmidt, C., Buck, F., Zmijewski, J. W., Jakovcevski, I., Preissner, K. T., & Schachner, M. (2014). Interaction of the cell adhesion molecule CHL1 with vitronectin, integrins, and the plasminogen activator inhibitor-2 promotes CHL1-induced neurite overgrowth and neuronal migration. *Journal of Neuroscience*, *34*(44), 14606–14623.

Li, C., Liu, C., Zhou, B., Hu, C., & Xu, X. (2016). Novel microduplication of *CHL1* gene in a patient with autism spectrum disorder: A case report and a brief literature review. *Molecular Cytogenetics*, *9*(51), 1–7. https://doi.org/10.1186/s13039-016-0261-9

Palumbo, O., Fischetto, R., Palumbo, P., Nicastro, F., Papadia, F., Zelente, L., & Carella, M. (2015). De novo microduplication of *CHL1* in a patient with non-syndromic developmental phenotypes. *Molecular Cytogenetics*, *8*(66), 1–6. https://doi.org/10.1186/s13039-015-0170-3

Pohjola, P., de Leeuw, N., Penttinen, M., & Kaariainen, H. (2010). Terminal 3p deletions in two families – correlation between molecular karyotype and phenotype. *American Journal of Medical Genetics*, *152A*, 441–446. https://doi.org/10.1002/ajmg.a.33215

Shoukier, M., Fuchs, S., Schwaibold, E., Lingen, M., Gartner, J., Brockmann, K., & Zirn, B. (2013). Microduplication of 3p26.3 in nonsyndromic intellectual disability indicates an important role of *CHL1* for normal cognitive function. *Neuropediatrics*, *44*(5), 268–271. https://doi.org/10.1055/s-0033-1333874

Tassano, E., Biancheri, R., Denegri, L., Porta, S., Novara, F., Zuffardi, O., Gimelli, G., & Cuoco, C. (2014). Heterozygous deletion of *CHL1* gene: detailed array-CGH and clinical characterization of a new case and review of the literature. *European Journal of Medical Genetics*, *57*, 626–629. https://doi.org/10.1016/j.ejmg.2014.09.007

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