Ceroid lipofuscinosis type 5 (CLN5) in South America: 16 additional cases, novel pathogenic variants, and novel and unexpected phenotypic findings

Authors: Anderson Rodrigues Brandão de Paiva[1,2], André Pessoa[3,4], Cristiane Araujo Martins Moreno[1,9], David S. Lynch[5], Paulo Ribeiro Nóbrega[1,16], Lucas Mitsuo Taniguti[9], João Paulo Kitajima[9], Fernando Freua[1], Bruno Della Ripa[1], Paulina Cunha[1], Isabella Barcelos[1], Lúcia Inês Macedo-Souza[17] Carlos Takeuchi[6], Antônio Milton Silva Garcia[7], Flávia Nardes dos Santos[8], Sérgio Antônio Antoniuk[10], Monica Troncoso[11], Norma Spécola[12], Consuelo Durand[13], Bianca de Aguiar Coelho Silva Madeiro[14], Maria Juliana Rodovalho Doriqui[15], Diane Vergara[11], Henry Houlden[5], Fernando Kok[1,9].

Affiliations:

1- Neurogenetics Unit, Neurology Department, Hospital das Clínicas da Universidade de São Paulo, São Paulo, Brazil
2- Neurology Department, Hospital São Rafael, Salvador, Brazil
3- Hospital Infantil Albert Sabin, Fortaleza, Ceara, Brazil.
4- Ceara State University – UECE
5- Department of Neuromuscular Disease, UCL Institute of Neurology, London, UK
6- Samaritano Hospital, São Paulo, Brazil
7- Pernambuco
8- Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
9- Mendelics Genomic Analysis, São Paulo, Brazil
10- Universidade Federal do Paraná. Curitiba, PR, Brasil.
11- Children's Neurology Service, San Borja Arriaran Clinical Hospital, University of Chile
12- Hospital de Niños Sor María Ludovica, La Plata, Argentina
13- Laboratorio de Neuroquímica Dr. N.A. Chamoles, Ciudad Autónoma de Buenos Aires, Argentina
14- Hospital Universitário Oswaldo Cruz, Universidade de Pernambuco (UPE), Recife, Brazil
15- Public Health Department, Universidade Federal do Maranhão, São Luís, Brazil.
16- Neurology Department, Universidade Federal do Ceará, Ceará, Brazil
17- Centro de Estudos do Genoma Humano, Universidade de São Paulo, São Paulo, Brazil

Introduction

The neuronal ceroid lipofuscinoses (NCLs) are a heterogeneous group of genetic disorders affecting the brain and the retina. A common mechanism is the intracellular accumulation of autofluorescent material in most cells resembling lipofuscin or ceroid[1–3]. Most NCLs are autosomal recessive, apart from the autosomal dominant CNL4B.

Clinically, most patients present with progressive cognitive- and motor decline, seizures, visual loss, and variable degrees of cerebellar atrophy. NCLs are the most prevalent neurodegenerative disorders of childhood, but adult-onset cases have been increasingly recognized.

Up to 13 genes are known to be associated with this disease. Patients are classified according to their molecular diagnosis and age of onset, but no clear phenotype-genotype correlations among the genes.

CLN5 encodes the ceroid lipofuscinosis neuronal protein 5, a lysosomal soluble protein involved in trafficking lysosomal sorting receptors[4]. The classic presentation is a childhood-onset disorder with motor and visual symptoms after a period of normal development[5–8]. The disease progresses with behavioral problems, cognitive decline, cerebellar ataxia, and myoclonic epilepsy.

The adult-onset form of NCL, also called Kufs disease, makes up approximately 5 to 10% of all NCL cases[9], with the age of onset varying from 17 to 56 years. Two different and overlapping phenotypes of Kufs disease are recognized: type A, characterized by myoclonic epilepsy, ataxia, and dysarthria; and type B, characterized by severe cognitive impairment[10]. Brain MRI typically shows brain atrophy, and retinal involvement is rarely reported[11].

Biallelic *CLN5*-mutations were described mainly in Caucasians, but patients from several populations were already reported, including in South America[7,12–14]

Here we describe 16 South-American NCL patients harboring ten pathogenic variants in *CLN5*, five previously reported, and five novel variants, one of them with a founder effect. This case series broadens the phenotypic spectrum associated with CLN5 disease by describing a very unusual adult-onset patient. This also reinforces that childhood-onset CLN5 disease is indistinguishable from other NCLs on a clinical basis and adds valuable information demonstrating no clear genotype-phenotype relationship.

Methods

According to the local ethics committee, this study was approved by the local IRB and the written informed consent from all patients/legal representatives. Patients were seen in several centers, and the assistant physician for each case provided all the information requested by the authors.

Patients were obtained through a search for homozygous/compound heterozygous pathogenic variants in the *CLN5* gene in the genomic database of a commercial laboratory (Mendelics) and by contacting reference centers in Neurogenetic diseases and Epilepsy from all South America for CLN5 disease confirmed cases. The assistant physician was contacted and invited to be part of the study for all the selected patients. History, physical exam, and additional studies were reviewed according to availability.

Genetic analysis

Genetic analysis was performed in a CLIA-certified laboratory using next-generation sequencing, either through whole-exome sequencing or targeted epilepsy gene panel, according to clinical need and availability. Variants were classified according to the ACMG guidelines.

Haplotype analysis was performed in 4 phased-VCFs from 9 patients using a custom script.

Results

We identified 18 patients from 17 previously unpublished families. Two of them were excluded because of insufficient clinical data. A summary of the available clinical details and the genetic profile is shown in Table 1.

We analyzed one adult-onset patient (described in detail below) and 15 patients from 14 families aged between 1 and 10 years. There were 5 males and 11 females, with an average of 4.5 years for the early onset cases.

All patients presented with cognitive and motor decline, cerebellar ataxia, and seizures. Epilepsy presented with myoclonus, focal and generalized pharmaco-resistant seizures. Two patients had no visual loss until the last visit. Fundoscopic examination was performed in 11 patients and disclosed retinitis pigmentosa in three patients and nonspecific retinopathy in two. Only one patient (2) showed signs of autism spectrum disorder.

Seizures were the presenting feature in eight patients, and language disorders in other six. Low visual acuity and developmental delay were also common first symptoms. Motor decline due to cerebellar ataxia was noted with first symptoms in only four cases (3, 4, 7, and 10). All childhood-onset cases had epilepsy. The adult case presented hypogonadotropic hypogonadism.

Severe cerebellar and moderate to severe cortical and subcortical atrophy were seen in all cases. Scattered posterior periventricular white matter hyperintensities were consistently found in T2-weighted MRI images (Figure 1). Ancillary tests and cerebrospinal fluid analysis were unremarkable in all patients.

All patients had confirmed diagnosis by molecular analysis through next-generation sequencing. Four patients were compound heterozygous. We have found six missense mutations: three novel and three previously reported (p.Asn143Ser; p.Arg63Pro; p.Asp230Asn)[7,8]; two frameshift variants, one novel and one previously reported (p.Ser98Leufs*)[13]; two nonsense variants (p.Leu126*; p.Arg145*)[13,5] ; and one new splicing site variant (c.340-1delG). Patient 13 had two missense variants in the homozygous state belonging to the same previously reported pathogenic haplotype p[Arg63Pro; Asp230Asn][8]. This patient lost follow-up and it is not possible to definitely infer if this haplotype conferred a worse prognosis.

The variant p.Arg63Cys was found in a homozygous state in six patients and was also present as compound heterozygous in one patient. This variant is present in only one individual in the population databank GnomAD v2 (around 140,000 individuals). Among 54,000 exomes performed in a Brazilian commercial laboratory (Mendelics Genomic Analysis, São Paulo, Brazil), it was found in a heterozygous state in 10 unaffected individuals. Patients with this variant came from different regions of Brazil and an isolated Amerindian community of Northern Argentina.

Haplotype analysis of the affected cases detected the same haplotype present in all Brazilian samples, suggesting a possible founder effect. However, the haplotype was not shared by the Argentinian case (number 8). The genomic region containing the variant in the Argentinian cases was smaller than the genomic region of the Brazilian patients. Thus, it is unlikely that this variant is of Amerindian origin.

A childhood-onset typical case

This 9-year-old girl (case 2), born after uneventful pregnancy and labor, was the only child of a consanguineous, first-cousin couple. Her developmental milestones were normal in the first year of life. However, language acquisition was delayed, and she had some learning disabilities. At seven years of age, she developed pharmacoresistant epilepsy, with focal and generalized motor seizures. She presented

incoordination and cognitive decline. At nine years old, she had global cerebellar ataxia and was wheelchair-bound with severe intellectual disability and autistic features. Brain MRI showed moderate cortico-subcortical and cerebellar atrophy and hypersignal in T2 and FLAIR sequences in the peritrigonal regions. Genetic analysis detected the variant c.188C>T;p.Arg63Cys in homozygous state.

An atypical adult-onset case

This 61-year-old man (case 1) developed slowly progressive visual complaints suggestive of metamorphopsia when he was 40 years old. By 43, he could no longer drive or read due to low visual acuity. By 45, he developed a slowly progressive unsteady gait and dysarthria. Shortly after the onset of gait problems, he reported anterograde amnesia, difficulties with naming, paraphasias, and problems recognizing people, even close relatives. Long-term memory was well preserved at that point. He was born from a non-consanguineous family and had two younger brothers, one with similar symptoms. On his first evaluation at our institution, at age 55, he was alert, apathetic, spatially and temporally disoriented, with anterograde amnesia, anomia, and semantic paraphasias; he was inattentive, with motor perseveration, ideomotor apraxia, and simultanagnosia. His strength was preserved, deep tendon reflexes were brisk in the four limbs, he had flexor plantar responses and mild spasticity on the lower limbs. There was global cerebellar ataxia and severe cerebellar dysarthria. The sensory exam was unreliable. Fundoscopic findings were suggestive of retinitis pigmentosa. Ancillary tests showed hypogonadotropic hypogonadism and hypothyroidism. Brain MRI revealed global cortical atrophy (with posterior predominance), global cerebellar atrophy, and no white matter involvement. He started using a wheelchair by the age of 60, and on his last office visit, he was dependent on others for basic activities of daily living, complaining of dysphagia, and had severe visual loss. He passed away one year later from acute respiratory distress. Whole-exome sequencing was performed, and two pathogenic variants were found in *CLN5*. The first one, c.428A>G, is a missense variant that changes asparagine for a serine (p.Asn143Ser) and has already been associated with juvenile CLN5 disease[7]. The second one, c.427G>T, is a missense variant that changes a valine for phenylalanine (p.Val94Phe) in a highly conserved residue, predicted by all *in silico* techniques to be damaging.

Discussion

After the initial description of Finnish patients with CLN5-disease[5], most cases described had European origin. This series adds an essential contribution to all patients coming from South America.

The classic form of late infantile variant CLN5 disease has a relatively uniform phenotype, regardless of ethnicity or genetics, quite similar to our case series. The age of onset in our series ranged from 1 to 40 years, with one patient younger than two years (infantile) and one adult-onset. The remaining 14 patients' ages ranged from 2 to 10 years (average 4,5 years, standard deviation 2,3), characterizing the late infantile variant, close to previously reported patients[6–8,12,15–18].

As observed in other series, most patients presented with language impairment[7,8,18]. Seizures, found in eight patients, were also a common presenting feature, contrary to the observed later onset of this symptom (median age 6,5 years) in

the literature[7,8,18]. Interestingly, four bear the new variant (p.Arg63Cys), which we believe has a founder effect in South America. It might be a distinctive feature of this genotype.

In most cases, visual symptoms started in the later stage of the disease, similar to what is described for the late infantile forms of NCLs[18]. However, three patients (cases 5, 6, and 7) had visual decline in the first year of symptoms. Motor symptoms were also a common feature in the first years of disease (age of onset ranged from 3 to 10 years, median 6,5 years), in concordance with the literature[5–8,18].

The disease evolved with behavioral problems, cognitive decline, cerebellar ataxia, and epilepsy in all patients. Myoclonus was common but not a universal feature. It was absent in five patients. The most consistent brain image findings were cortical and cerebellar atrophy, observed in all patients, very similar to other reported series[19].

Using next-generation sequencing techniques, we identified ten different variants, and five of them have never been published. Ten patients were homozygous, five were compound heterozygous, and one was homozygous for two pathogenic variants (a pathogenic haplotype). There were six missense, two frameshifts, two nonsense, and one splice-site variant (Table 1). We could not find any particular genotype-phenotype relationships, but for the possible association of p.Arg63Cys with early-onset seizures, as previously stated.

The variant p.Arg63Cys was found in homozygosity in five patients and compound heterozygosity in patient 6. This is a scarce variant in population databases (GnomAD and Abraom) and has maximal conservation in all species. Two other variants affecting the same codon have been reported as probably pathogenic. The variant p.Arg63His was reported in Colombian patients[12], and the variant p.Arg63Pro was reported in Portuguese patients[8] – and also found in this case series. All *in silico* programs predict this variant to be pathogenic. These facts and these six new cases with a compatible phenotype make this variant meet American College of Medical Genetics criteria for definitely pathogenic[20].

We speculated that this variant might be of Amerindian origin, as it was found in a patient from an isolated Amerindian community in the Charcot region, North Argentina (patient 8). We compared the haplotypes of all patients with this variant. We found that the Argentinian patient had a shorter haplotype than the Brazilian patient, making the possibility of an Amerindian origin unlikely. However, given the rarity of this variant in population databases and the relatively higher frequency in this series, we believe this variant has a founder effect in South America.

Finally, we present a sporadic case of adult-onset CLN5 disease. Adult-onset form of NCL corresponds to approximately 5 to 10% of all NCL cases[9]. The age of onset varies from 17 to 56 years. Retinal involvement is not common in Kufs disease[11]. Brain MRI typically shows brain atrophy. Kufs disease is autosomal recessive, and it has been described as associated with the following genes: *CTSD, PPT1, CLN3, CLN5, CLN6, CTSF, GRN.*

This adult patient had a unique presentation with visual symptoms and associated endocrine manifestations. Two previously described siblings with adult-onset CLN5 disease presented with visual loss due to severe glaucoma[21], but no retinal abnormalities like our adult patient with retinitis pigmentosa. The cognitive profile is also different, with patient one presenting with involvement of all cognitive domains, especially higher-order visual symptoms, while previously reported patients had preserved memory and orientation[21]. Severe cerebellar ataxia, dysarthria, and brain atrophy with severe cerebellar atrophy were shared among these patients. The

hypogonadotropic hypogonadism found in our patient is unexpected, and it is difficult to define if it was due to CLN5 mutations or a coincidental finding.

Conclusion

In conclusion, we presented a large case series of South American patients with CLN5 disease with a recurrent missense variant with a possible founder effect. This study broadens the phenotypic and genotypic spectrum associated with CLN5 disease, with seizures presenting in patients with this new recurrent variant. We also described hypogonadotropic hypogonadism and early-onset dementia with higher-order visual symptoms in an adult-onset case with biallelic pathogenic variants in *CLN5*. We suggest that CLN5 disease should be considered in the differential diagnosis of infantile and juvenile patients with cognitive decline, ataxia, and epilepsy. CLN5 disease should also be considered in adult patients with early-onset dementia, ataxia, and epilepsy.

References:

1. Williams RE, Mole SE. New nomenclature and classification scheme for the neuronal ceroid lipofuscinoses. *Neurology*. 2012;79(2):183-191. doi:10.1212/WNL.0b013e31825f0547
2. Nita DA, Mole SE, Minassian BA. Neuronal Ceroid Lipofuscinoses. *Epileptic Disord*. 2016;18(Suppl.2):S73-S88. doi:10.1016/B978-0-12-385157-4.00106-8
3. Johnson TB, Cain JT, White KA, Ramirez-Montealegre D, Pearce DA, Weimer JM. Therapeutic landscape for Batten disease: current treatments and future prospects. *Nat Rev Neurol*. 2019;15(3):161-178. doi:10.1038/s41582-019-0138-8
4. Mamo A, Jules F, Dumaresq-Doiron K, Costantino S, Lefrancois S. The Role of Ceroid Lipofuscinosis Neuronal Protein 5 (CLN5) in Endosomal Sorting. *Mol Cell Biol*. 2012;32(10):1855-1866. doi:10.1128/mcb.06726-11
5. Savukoski M, Klockars T, Holmberg V, Santavuori P, Lander ES, Peltonen L. CLN5, a novel gene encoding a putative transmembrane protein mutated in Finnish variant late infantile neuronal ceroid lipofuscinosis. *Nat Genet*. 1998;19(3):286-288. doi:10.1038/975
6. Holmberg V, Lauronen L, Autti T, et al. Phenotype-genotype correlation in eight patients with Finnish variant late infantile NCL (CLN5). *Neurology*. 2000;55(4):579-581. doi:10.1212/WNL.55.4.579
7. Xin W, Mullen TE, Kiely R, et al. CLN5 mutations are frequent in juvenile and late-onset non-finnish patients with NCL. *Neurology*. 2010;74(7):565-571. doi:10.1212/WNL.0b013e3181cff70d
8. Bessa C, Teixeira CAF, Mangas M, et al. Two novel CLN5 mutations in a Portuguese patient with vLINCL: Insights into molecular mechanisms of CLN5 deficiency. *Mol Genet Metab*. 2006;89(3):245-253. doi:10.1016/j.ymgme.2006.04.010
9. Sadzot B, Reznik M, Arrese-Estrada JE, Franck G. Familial Kufs' disease presenting as a progressive myoclonic epilepsy. *J Neurol*. 2000;247(6):447-454. doi:10.1007/s004150070174
10. Berkovic SF, Carpenter S, W EVAA, Wolfe LS. KUFS ' DISEASE : A

CRITICAL REAPPRAISAL. Published online 1988:27-62.
11. Schulz A, Kohlschütter A, Mink J, Simonati A, Williams R. NCL diseases - clinical perspectives. *Biochim Biophys Acta - Mol Basis Dis*. 2013;1832(11):1801-1806. doi:10.1016/j.bbadis.2013.04.008
12. Pineda-Trujillo N, Cornejo W, Carrizosa J, et al. A CLN5 mutation causing an atypical neuronal ceroid lipofuscinosis of juvenile onset. *Neurology*. 2005;64(4):740-742. doi:10.1212/01.WNL.0000151974.44980.F1
13. Kousi M, Lehesjoki AE, Mole SE. Update of the mutation spectrum and clinical correlations of over 360 mutations in eight genes that underlie the neuronal ceroid lipofuscinoses. *Hum Mutat*. 2012;33(1):42-63. doi:10.1002/humu.21624
14. Kohan R, Pesaola F, Guelbert N, et al. The neuronal ceroid lipofuscinoses program: A translational research experience in Argentina. *Biochim Biophys Acta - Mol Basis Dis*. 2015;1852(10):2301-2311. doi:10.1016/j.bbadis.2015.05.003
15. Cannelli N, Nardocci N, Cassandrini D, et al. Revelation of a novel CLN5 mutation in early juvenile neuronal ceroid lipofuscinosis. *Neuropediatrics*. 2007;38(1):46-49. doi:10.1055/s-2007-981449
16. Lebrun AH, Storch S, Ruschendorf F, et al. Retention of lysosomal protein CLN5 in the endoplasmic reticulum causes neuronal ceroid lipofuscinosis in asian sibship. *Hum Mutat*. 2009;30(5). doi:10.1002/humu.21010
17. Al-Kowari MK, Hassan S, El-Said MF, et al. Neuronal ceroid lipofuscinosis in qatar: Report of a novel mutation in ceroid-lipofuscinosis, neuronal 5 in the arab population. *J Child Neurol*. 2011;26(5):625-629. doi:10.1177/0883073810387298
18. Simonati A, Williams RE, Nardocci N, et al. Phenotype and natural history of variant late infantile ceroid-lipofuscinosis 5. *Dev Med Child Neurol*. 2017;59(8):815-821. doi:10.1111/dmcn.13473
19. Biswas A, Krishnan P, Amirabadi A, Blaser S, Mercimek-Andrews S, Shroff M. Expanding the neuroimaging phenotype of neuronal ceroid lipofuscinoses. *Am J Neuroradiol*. 2020;41(10):1930-1936. doi:10.3174/ajnr.A6726
20. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. doi:10.1038/gim.2015.30
21. Mancini C, Nassani S, Guo Y, et al. Adult-onset autosomal recessive ataxia associated with neuronal ceroid lipofuscinosis type 5 gene (CLN5) mutations. *J Neurol*. 2015;262(1):173-178. doi:10.1007/s00415-014-7553-y