Are *CSNK2A1* gene mutations associated with retinal dystrophy? Report of a patient carrier of a novel de novo splice site mutation

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Neurodevelopmental disorders (NDDs) refer to a group of often severe pediatric conditions associated with impaired cognitive, sensory, and/or motor functions stemming from atypical development of the central nervous system. Although the recent development of genetic diagnostic tools, such as exome sequencing, has highlighted the prevalence of genetic anomalies in NDDs, the broad and variable and, at times, evolving clinical manifestations can render their prompt diagnosis difficult.

Recently, de novo mutations in the *CSNK2A1* gene, encoding for the alpha subunit of the casein kinase 2, have been found to cause a novel NDD with multisystemic involvement, termed Okur-Chung disease (MIM 617062). Clinical features include intellectual disability, microcephaly, hypotonia, and ataxia, with high inter-subject variability [1–3]. Here we report, to our knowledge for the first time, a pediatric patient carrier of a "de novo" mutation in the *CSNK2A1* gene initially presenting with isolated retinal dystrophy.

Case report

The patient, now a 12-month-old male, was born from non-consanguineous parents. At birth no pathological features were evident: his weight was 3.725 kg (>75th percentile);

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height 52.5 cm (>90th percentile); and his occipito-frontal circumference (OFC) was 36.5 cm (75th percentile).

The patient was first admitted at the age of 4 months to the Robert Hollman Foundation and Neurophtalmology Service of the Pediatric University Hospital of Padua for infantile nystagmus and suspected low vision. At examination, the OFC was 43.5 cm (50th percentile). The anterior segment of both eyes was normal and vision was in the range of light perception with sluggish pupillary reflexes. The fundus oculi (Fig. 1a) showed salt and pepper appearance with absence of the physiological excavation of the optic disk. The patient also had mild hyperopia and astigmatism in both eyes. The electroretinogram was not recordable under light and dark adaptation, and flash visual-evoked potentials were abnormal, but not absent (Fig. 1b). The neurological examination revealed only mild generalized hypotonia, in the absence of dysmorphic features. Brain magnetic resonance imaging (MRI) was unremarkable showing only mildly delayed myelination and mega cisterna magna. The electroencephalogram was normal. The diagnostic conclusion was isolated Leber's congenital amaurosis and genetic testing was started.

At 8 months of age, the patient's weight was 8.960 kg (50th–75th percentile), height 76 cm (50th–75th percentile), and his OFC was 45.0 cm (50th–75th percentile). At 10 months of age, the patient underwent a subsequent neurological follow-up because of psychomotor regression. The OFC was 46.5 cm (50th–75th percentile) and the neurological examination revealed developmental delay with generalized hypotonia, truncal ataxia, absence of finalized movements and postural changes, stereotyped movements, and sleep-wake cycling disturbance. The ophthalmological work-up was unchanged except for the occurrence of Franceschetti's sign. He underwent an extensive metabolic work-up: plasma acylcarnitines; amino acids; ammonium; lactate and pH; urinary organic acids and amino acids; and cerebrospinal fluid levels of neurotransmitters; all within the normal range.
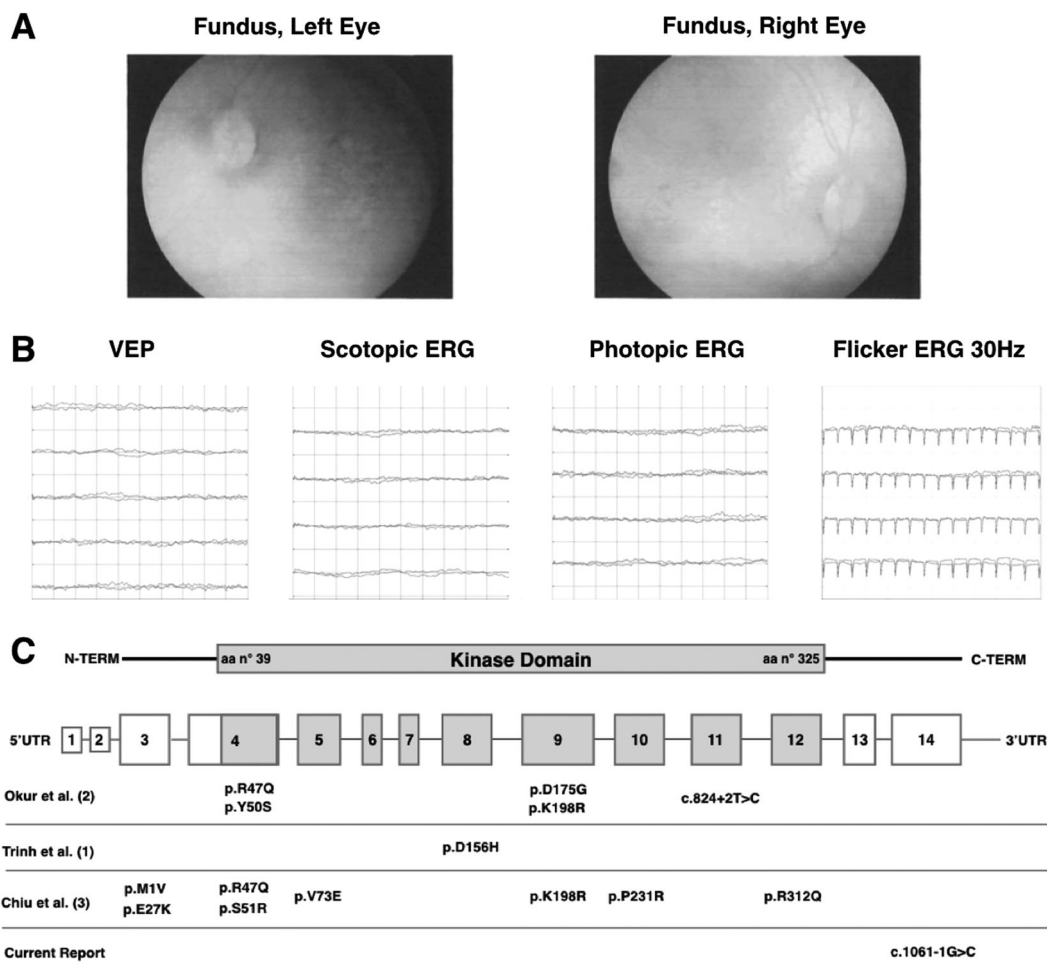
Fig. 1 a Fundus autofluorescence; **b** VEP, ERG, and Flicker ERG electrograms; **c** distribution of the known *CSNK2A1* mutations associated with Okur-Chung disease. Kinase domain (from codon 39 to codon 325) and relative coding exons are represented in gray

Genetic results

Whole-exome sequencing was, following obtainment of informed consent from the parents, performed on genomic DNA isolated from a blood sample withdrawn from the patient. Exomes were captured using the the Agilent SureSelect Clinical Research Exome kit and sequenced on a HiSeq2500 platform (Illumina) at an average coverage of 100× (supplementary table 1).

Variant prioritization and interpretation [4] revealed the presence of the variation c.1061-1G > C in the putative splice site of exon 14 of the *CSNK2A1* gene (NM_177559). This variation, confirmed by Sanger sequencing, is de novo (family segregation analysis, supplementary figure 1) and absent from the major allele frequency databases (ExAC, EVS, and 1000 Genome Project). In silico computational pathogenicity tools predict that the mutation is not tolerated (Mutation Taster = 1; CADD score = 15) and NNSplice and GeneSplicer predictions indicate with high confidence a deleterious effect on mRNA splicing. Conservation tools, such as PhyloP, GERP, and PhastCons, show that the variation is located in an highly conserved position. Accordingly, in line with the American College of Medical Genetics and Genomics guidelines, the de novo variation c.1061-1G > C of the *CSNK2A1* gene can be classified as likely pathogenic [5]. No mutations were detected in those genes associated with retinal dystrophies nor in those listed in Human Phenotype Ontology database (HPO) in association with vision impairment. Also, no significant variations were found in other genes associated with NDDs (HPO).

Discussion

To date, 14 patients, ranging from 2 to 14 years of age, have been described to be carriers of de novo mutations in the *CSNK2A1* gene. Clinical features of the affected patients (Okur-Chung disease) include the following: microcephaly (8/14 patients); brain MRI anomalies (9/14 patients); intellectual disability (14/14 patients); hypotonia (9/14 patients);

ataxia (3/14 patients); seizures (4/14 patients); sleep problems (4/14 patients); and attention deficit/hyperactivity disorder (4/14 patients) as well as dysmorphisms (e.g., round face, prominent forehead, low-set ears, epicantal folds, broad nasal bridge, and ear fold abnormality) with high inter-subject heterogeneity [1–3].

The patient here reported is a carrier of a de novo mutation in the *CSNK2A1* gene. He presented at the age of 4 months with isolated retinal dystrophy and only at the age of 10 months with symptoms indicative of a NDD. None of the previously described patients carrying mutations in the *CSNK2A1* gene has been reported to display retinal disturbances. However, recent evidence indicates that CK2 kinase regulates, at least in Drosophila, eye morphogenesis via phosphorylation of E(spl)M8 suggesting a potential role of CK2 during retinal patterning [6], in addition to photoreceptor formation [7].

Also, unlike the previously reported patients, our patient does not display microcephaly nor recognizable dysmorphisms or multisystemic involvements. However, because of his still very young age (12 months), we cannot exclude that he will develop these and/or other clinical features in the future. In fact, at birth, the OFC measures of our patient were, although within normal range, lower relative to weight and length and, from 4 to 10 months, changed the percentile curve, raising the suspicion of a lower than expected growth. Microcephaly has been reported to present in at least one patient carrier of a de novo mutation in the *CSNK2A1* gene in the first 1–2 years of life, indicating that symptoms presenting in Okur-Chung disease are evolutive [1].

Previously reported mutations found in the *CSNK2A1* gene are mostly missense mutations located within the protein kinase domain, with only two reported mutations outside the kinase domain (Fig. 1c). For all these mutations a dominant negative effect has been proposed [3]. The mutation c.1061-1G > C here found is, instead, located in the canonical splice site of the last coding exon not transcribing for the catalytic domain. This exon contains the 3′ untranslated region, which is essential for mRNA maturation and stability [6]. The absence of this region in the transcribed RNA may lead to mRNA degradation raising

the possibility that *CSNK2A1* haploinsufficiency may be a disease-causing mechanism [8].

In conclusion, we here report a patient presenting with retinal dystrophy and neurodevelopmental delay carrier of a de novo mutation in the *CSNK2A1* gene. Future genotype–phenotype correlation studies are warrented to define whether the retinal abnormalities are representative of a larger phenotypic spectrum of Okur-Chung disease or, alternatively, part of a novel *CSNK2A1*-related disorder.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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