A novel *SLC1A4* homozygous mutation causing congenital microcephaly, epileptic encephalopathy and spastic tetraparesis: a video-EEG and tractography-case study.

Erica Pironti[1], Vincenzo Salpietro[2], Francesca Cucinotta[1], Francesca Granata[3], Enricomaria Mormina[3,4], Stephanie Efthymiou[2], Carmela Scuderi[5], Antonella Gagliano[1], Henry Houlden[2], Gabriella Di Rosa[1]

[1]Department of Human Pathology of the Adult and Developmental Age "Gaetano Barresi", Unit of Child Neurology and Psychiatry, University of Messina, Messina 98125, Italy

[2]Department of Molecular Neuroscience, UCL Institute of Neurology, London WC1N 3BG, UK

[3]Neuroradiology Unit - Department of Biomedical Sciences and Morphological and Functional Imaging; University of Messina, Messina 98125, Italy

[4]Department of Clinical and Experimental Medicine, University of Messina, Italy.

[5]Unit of Neuromuscular disorders, IRCCS Oasi Maria SS Troina, Enna, Italy

Running title: SLC1A4 gene and epileptic encephalopathy

Corresponding author: Gabriella Di Rosa, MD, PhD. Department of Human Pathology of the Adult and Developmental Age "Gaetano Barresi"; Unit of Infantile Neuropsychiatry, University Hospital of Messina, Messina, Italy. Via Consolare Valeria, 1 98125 Messina. Tel. 0039-090-221-2908. Fax. 0039-090-2930414. Email: gdirosa@unime.it

ABSTRACT

Biallelic mutations in the SLC1A4 gene have been identified as a very rare cause of neurodevelopmental disorders. L-serine transport deficiency has been regarded as the causal molecular mechanism underlying the neurological phenotype of SLC1A4 mutation patients. To date this genetic condition has been reported almost exclusively in a limited number of Ashkenazi-Jewish individuals and as a result the SLC1A4 gene is not routinely included in the majority of the genetic diagnostic panels for neurological diseases. We hereby report a 7-year-old boy from a Southern Italian family, presenting with epileptic encephalopathy, congenital microcephaly, global developmental delay, severe hypotonia, spasticity predominant at the lower limbs, and thin corpus callosum. Whole exome sequencing identified a novel segregating SLC1A4 gene homozygous mutation (c.1141G>A: p.Gly381Arg) as the likely cause of the disease in our family. In order to deeply characterize the electro-clinical and neurological phenotype in our index patient, long-term systematic video-electroencephalograms (EEG) as well as repeated brain imaging studies (which included tractographic reconstructions) were performed on a regular basis during a 7 years follow-up time.

In conclusion, we suggest to carefully considering SLC1A4 biallelic mutations in individuals presenting an early onset severe neurodevelopmental disorder with variable spasticity and seizures, regardless the patients' ethnic background.

Keywords: SLC1A4 gene, Whole-Exome Sequencing, developmental and epileptic encephalopathy, video-EEG, Tractography.

Contributor Statement

EP conceived of the idea and drafted the article.

VS contributed to the design of the article and to the analysis of the results.

FG collected and analyzed the data.

FC conceived of the idea and drafted the article.

EMM collected and analyzed the data.

SE and AG contributed to the design of the article, to the analysis of the results.

CS collected and analyzed the data.

HH supervised the project and contributed to the final version of the manuscript.

GD supervised the project and contributed to the final version of the manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interests

None of the authors has any conflict of interest to disclose**.**

Acknowledgements

The authors want to thank the patient and his family for the contribution and collaboration.

In this manuscript are included two supplementary files labelled as Figure 2 and Video 1 A-B.

INTRODUCTION

SLC1A4 (MIM# 616657) is a neuronal and glial gene encoding the Alanine/Serine/Cysteine/Threonine and Glutamate Transporter 1 (ASCT1). ASCT1 transports L-serine across the blood–brain barrier, from astrocytes to extracellular space and ultimately into neurons (Tabatabaie, Klomp, Berger & de Koning, 2010). SLC1A4 gene mutations are extremely rare and almost exclusively described in the Ashkenazi-Jewish population as a cause of neurodevelopmental disorders, brain malformations and/or progressive spasticity (Heimer et al., 2015; Srour et al., 2015; Conroy et al., 2016). Only one European patient and one Palestinian patients were reported, so far. Epilepsy has been reported in the majority of these patients; however, it mainly showed highly heterogeneous phenotypes. Developmental and epileptic encephalopathies include disorders with pre-existing developmental delay, complicated by plateauing or regression with seizure onset or with prolonged seizures (Scheffer et al., 2017). New-generation sequencing technologies allowed the identification of a growing number of monogenic etiologies of early onset developmental and epileptic encephalopathies, providing insights into their pathophysiological bases and potential etiologically targeted treatments. We hereby report on the electro-clinical features (with systematic video-EEG history and neuroimaging study) of the second European patient with a homozygous SLC1A4 gene pathogenic mutation detected by a trio-based whole exome sequencing.

Case study

The patient is a 7-year-old boy born at 39 weeks of gestation from uneventful pregnancy and delivery. Parents were not frankly consanguineous for their own account but both born in the same small city in Calabria, a region of Southern Italy (400 inhabitants) characterized by high percentages of consanguinity within the community, given the relatively small size of the village and its remote (isolated) location in the Sila mountains. Birth weight was 3230 gr (40th centile), length was 49 cm (25th centile) and head circumference was 32.1 cm (3rd centile). Apgar score was 8–10 at 10 and 50 minutes. No perinatal distress was reported. During the first weeks of life he presented daily episodes of opistotonic posturing associated with apnoea and cyanosis during breastfeeding and respiratory difficulties (recurrent cough) due to gastroesophageal reflux. Early global psychomotor delay and hypotonia were observed since the third month of life with poor head control, absent visual fixation and smiling.

Funduscopy, auditory and visual evoked potentials were normal. The child also showed poor spontaneous motility, ocular nystagmiform jerks and oro-lingual automatisms. Since the fourth month of life he had been presenting daily seizures (occurring both during sleep and daytime) characterized by tonic extensor spasms at the four limbs, and left eyes deviation. Fragmented hypsarrhythmia was depicted by EEG recording during NREM sleep (Figure 1(C, D) and Supplementary Video 1-A). Anticonvulsant treatment initially started with vigabatrin

subsequently adding a number of drugs (levetiracetam, valproic acid, carbamazepine, nitrazepam, clobazam and ACTH) because of poor clinical response. Because of his gastroesophageal reflux, dysphagia and frequent respiratory tract infections (due to aspiration) with failure to thrive, he underwent percutaneous gastrostomy. At the present age of 7 years, the patient shows microcephaly (<0.4th centile), facial dysmorphisms, severe cognitive impairment, poor reactivity to sounds and visual stimuli, absent speech, axial hypotonia (with still absent head control), limbs spasticity and generalized joints contractures and distal dystonic posturing (mainly affecting hands, wrists and ankles) (Figure 1(A, B)). He exhibits daily focal seizures characterized by nystagmiform and opsoclonic fits followed by head version and arms hypertonus, scarcely responsive to combined anticonvulsant treatment with clobazam, valproate and vigabatrin (Supplementary Video 1-B). Latest EEG recording showed diffuse, multifocal, spikes and polyspikes with loss of physiological NREM sleep figures (Figure 1(E, F)). Throughout his clinical history our patient underwent extensive genetic and metabolic work-up including array comparative genome hybridization (array-CGH), all unrevealing.

Neuroimaging findings

Baseline Brain Magnetic Resonance Imaging (MRI) was performed at 3 months of age. Morphological examination (Figure 2, A-C) showed a diffuse, slight hyperintensity on T2-weighted images of biemispheric white matter, related to hypomyelination. Moreover, a severe hypoplasia of the corpus callosum was depicted, especially at trunk and splenium. The anterior commissure was present and enlarged, to compensate the lack of corpus callosum fibers. Tractographic evaluation was performed by diffusion tensor imaging (DTI) technique (Figure 3, A-E). The study was targeted to anterior commissure evaluation. The compensatory enlargement of the anterior commissure was confirmed by DTI reconstruction (Figure 3, A-D). Follow-up Brain MRI examination was performed at four years. A significant hemispheric atrophy was depicted with involvement of both white and gray matter (Figure 2, D). Moreover, brainstem atrophy was showed with enlargement of the cisternal spaces (Figure 2, E, F).

Whole exome sequencing

Whole-exome sequencing (WES) was performed in the proband and his unaffected parents and standard investigation and interpretation of the identified variants was performed according to the ACMG guidelines (Richards et al., 2015). Written informed consent was obtained by the patient's parents. Nextera Rapid Capture Enrichment kit (Illumina) was used according to the manufacturer instructions. Libraries were sequenced in an Illumina HiSeq3000 using a 100-bp paired-end reads protocol. Sequence alignment to the human reference genome (UCSC hg19), and variants call and annotation were performed using an in-house pipeline as described

elsewhere (Mencacci et al., 2016). The raw list of single nucleotide variants (SNVs) and indels was then filtered. Only exonic and donor/acceptor splicing variants were considered. In accordance with the pedigree and phenotype, priority was given to rare variants [<1% in public databases, including 1000 Genomes project, NHLBI Exome Variant Server, Complete Genomics 69, and Exome Aggregation Consortium (ExAC v0.2)] that were fitting a recessive or a *de-novo* model and located in genes previously associated with epilepsy or neurological phenotypes. Exome sequencing data were also used for homozygosity mapping analysis of the family. WES of the trio generated a total of 141,968,572, 145,130,142 and 142,150,172 unique reads, with an average on target depth over 160, and >96% of the target bases covered at least 10X. There was a single de novo variant in the gene *ODAM* (NM_017855.3; c.581T>C; p.Ile194Thr) which was predicted as benign/not pathogenic by *in silico* tools and not further considered given that the expression of this gene is confined within the gastrointestinal tract and absent in brain (https://www.proteinatlas.org/ENSG00000109205-ODAM/tissue). We assumed that the causative mutation was inherited in the biallelic state. There were no novel/rare (minor allele frequency < 1%) compound heterozygous variants in the proband, but 2 rare homozygous variants were identified. These were a homozygous missense variant in the *IFNGR1* gene (NM_000416.2; c.1388T>C: p.Leu463Pro), encoding for a interferon-gamma receptor, which was predicted as tolerated and a missense variant in *SLC1A4* (NM_003038.4; c.1141G>A: p.Gly381Arg) which was localized within one of the major critical regions identified by homozygosity mapping and was absent from all the publicly available databases (including 1000 Genomes project and ExAC) as well as from our in-house database containing WES data from over 6,000 individuals. The mutation in *SLC1A4* emerged as the most likely explanation for the disease pathogenesis, as supported by (i) high conservation of the affected residue across species (GERP score 6.17), as well as in-silico analysis (the variant is predicted deleterious by SIFT, PolyPhen2 and Mutation Taster) and high pathogenic scores (Align GVGD, CADD); (ii) protein function (the mutation affects a conserved site within the transmembrane helical domain of the gene), (iii) tissue pattern expression in the brain and, (iv) the published association of this gene with a similar phenotype of progressive microcephaly, infantile seizures, global developmental delay and spasticity (predominantly in Ashkenazi-Jewish patients). The mutation was confirmed by Sanger sequencing as homozygous in the proband and heterozygous in both the healthy parents (detailed conditions of sequencing analysis are available upon request).

Discussion

Our 7-year-old male patient with developmental and epileptic encephalopathy carried a novel p.Gly381Arg homozygous mutation in *SCL1A4* gene identified by trio-based WES analysis. The family came from a small village community in Southern Italy of approximately 400 inhabitants, isolated from the coast, making high the

chance of additional healthy p.Gly381Arg heterozygous mutation carriers in this genetic isolate. The *SLC1A4*-related neurological disease represents a very rare autosomal recessive neurodevelopmental condition with high percentages of heterozygous healthy carriers in Ashkenazi-Jewish population (1:144) (Heimer et al, 2015). In total, 15 *SLC1A4* patients have been reported so far, with only two of them not Ashkenazi-Jewish (but from Irish/European and Palestinian ancestry) (Heimer et al, 2015; Conroy et al, 2016; Damseh, Simonin, Jalas, Picoraro, Shaag & Cho, 2015).

All patients presented with global neurodevelopmental impairment, some of which with epilepsy spanning from simple febrile to intractable seizures with hypsarrhythmia. Only two patients with infantile spasms have been reported so far (Heimer et al, 2015; Srour et al. 2015). In our patient, the electro-clinical picture at onset was suitable with West syndrome, with extensor tonic spasms and suppression-burst pattern mainly during NREM sleep. Notably, our patient presented with microcephaly since birth and subsequent progressive slow head growth. This is an unusual finding given that microcephaly has been mostly reported to be post-natal in the majority of patients with *SLC1A4*-related encephalopathy. Moreover, tendency for febrile seizure has been previously described in *SLC1A4* patients (Heimer et al, 2015). It has been related to the temperature-dependent effect of serine transfer by ASCT1 in both neurons and astrocytes. Despite the frequent respiratory tract infections with fever occurred throughout his life, our patient did not present febrile seizures, suggesting that ASCT1 temperature-dependence might be mostly associated to some but not all *SLC1A4* mutations or that it is influenced by other genetic or environmental factors. The patient presented with early developmental delay with subsequent psychomotor stagnation. Hand stereotypies have been reported in patients from Heimer and Srour, 2015 (Heimer et al, 2015; Srour et al. 2015). In our patient complex hyperkinetic movements were evident within the first months of life involving eyes, mouth and hands resembling other monogenic early-onset epileptic encephalopathies associated to involuntary movements such as those related to *CDKL5* gene or *GRIN1* gene mutations (Kobayashi, Tohyama, Kato, Akasaka, Magara & Kawashima, 2016). Interestingly, we showed evidence of a white matter re-modelling associated to severe hypomyelination and corpus callosum hypoplasia by imaging study. Early connectivity alterations at 3 months of life showed by conventional and tractographic brain MRI examination, might suggest a prenatal effect of *SLC1A4* gene defects in our patient and might explain the congenital microcephaly. These neuroimaging features were not previously reported in patients carrying *SLC1A4* mutations. Genotype-phenotype correlations in previous reports showed that patients with truncating (frame-shift) mutations were more severely affected than those with missense mutations (Srour et al., 2015). *SLC1A4* encodes for ASCT1, Na-dependent transporter of serine and other neutral amino acids, such as alanine, cysteine and threonine and deficiency/inefficiency of this transporter results in a lower level of serine (and other

neutral amino acids) inside neurons (Srour et al., 2015). Given the overlapping features of ASCT1 deficiency with L-serine biosynthesis disorders, it has been proposed that pathophysiological mechanisms underlying *SLC1A4*-related neurodevelopmental/epileptic features implicate abnormal L-serine transport into neuronal cells (Damseh et al., 2015; van der Crabben, et al., 2013). Notably, both serine biosynthesis and transport are active processes in embryonal neurons where 3-phospo-glycerate-dehydrogensase and ASCT1 genes are mainly expressed. Of interest, some authors give the possibility that L-serine supplementation may improve neurological symptoms associated to *SLC1A4* gene defect (Heimer et al., 2015). In conclusion, we reported electro-clinical and neuroradiological features of a patient with a deleterious (novel) *SLC1A4* p.Gly381Arg homozygous mutation, whose parents were their own account but both born in the samesmall city in Calabria, a region of Southern Italy (400 inhabitants), with high percentages of consanguinity within the community given the small size of the village and its geographical characteristics. Our findings provide detailed electroclinical and neuroimaging insights about this rare disorder and encourage SLC1A4 gene mutations' analysis (via sequencing or panels) in patients with severe early onset developmental and epileptic encephalopathy (with associated hyperkinetic movements), regardless of their ethnic background (Conroy et al., 2016).

References

Conroy, J., Allen, N.M., Gorman, K., O'Halloran, E., Shahwan, A., Lynch, B., … King, M.D. (2016). Novel European SLC1A4 variant: infantile spasms and population ancestry analysis. J Hum Genet, 61, 761-764.

Damseh, N., Simonin, A., Jalas, C., Picoraro, J.A., Shaag, A., Cho, M.T., … Edvardson, S. (2015) . Mutations in SLC1A4, encoding the brain serine transporter, are associated with developmental delay, microcephaly and hypomyelination. J Med Genet, 52, 541-547

Heimer, G., Marek-Yagel, D., Eyal, E., Barel, O., Oz Levi D., Hoffmann, C., Ruzzo, E.K., … Ben Zeev B (2015). SLC1A4 mutations cause a novel disorder of intellectual disability, progressive microcephaly, spasticity and thin corpus callosum. Clin Genet, 88, 327-235

Kobayashi, Y., Tohyama, J., Kato, M., Akasaka, N., Magara, S., Kawashima, H., … Matsumoto, N. (2016). High prevalence of genetic alterations in early-onset epileptic encephalopathies associated with infantile movement disorders, Brain Dev, 38, 285-292.

Mencacci, N.E., Kamsteeg, E.J., Nakashima, K., R'Bibo, L., Lynch, D.S., Balint, B., … Bhatia K.P. (2016). De Novo Mutations in PDE10A Cause Childhood-Onset Chorea with Bilateral Striatal Lesions. Am J Hum Genet, 98, 763-771.

Ohba, C., Shiina, M., Tohyama, J., Haginoya, K., Lerman-Sagie, T., Okamoto, N., … Matsumoto, N. (2015). GRIN1 mutations cause encephalopathy with infantile-onset epilepsy, and hyperkinetic and stereotyped movement disorders, Epilepsia, 56:841-848.

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., … Rehm, H.L.; ACMG Laboratory Quality Assurance Committee. (2015). 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. May;17(5):405-24.

Scheffer, I.E., Berkovic, S., Capovilla, G., Connolly, M.B., French, J., Guilhoto, L., … Zuberi, S.M. (2017). ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology, Epilepsia, 58: 512-521

Srour, M., Hamdan, F.F., Gan-Or, Z., Labuda, D., Nassif, C., Oskoui, M., … Michaud, J.L. (2015). A homozygous mutation in SLC1A4 in siblings with severe intellectual disability and microcephaly, Clin Genet, 88, e1-4.

Tabatabaie L., Klomp L.W., Berger R., de Koning T.J (2010). L-serine synthesis in the central nervous system: a review on serine deficiency disorders, Mol Genet Metab, 99, 256-262.

van der Crabben, S.N., Verhoeven-Duif, N.M., Brilstra, E.H., Van Maldergem, L., Coskun, T., Rubio-Gozalbo, E., Berger, R., de Koning, T.J (2013). An update on serine deficiency disorders, J Inherit Metab Dis, 36, 613-619.

Figures

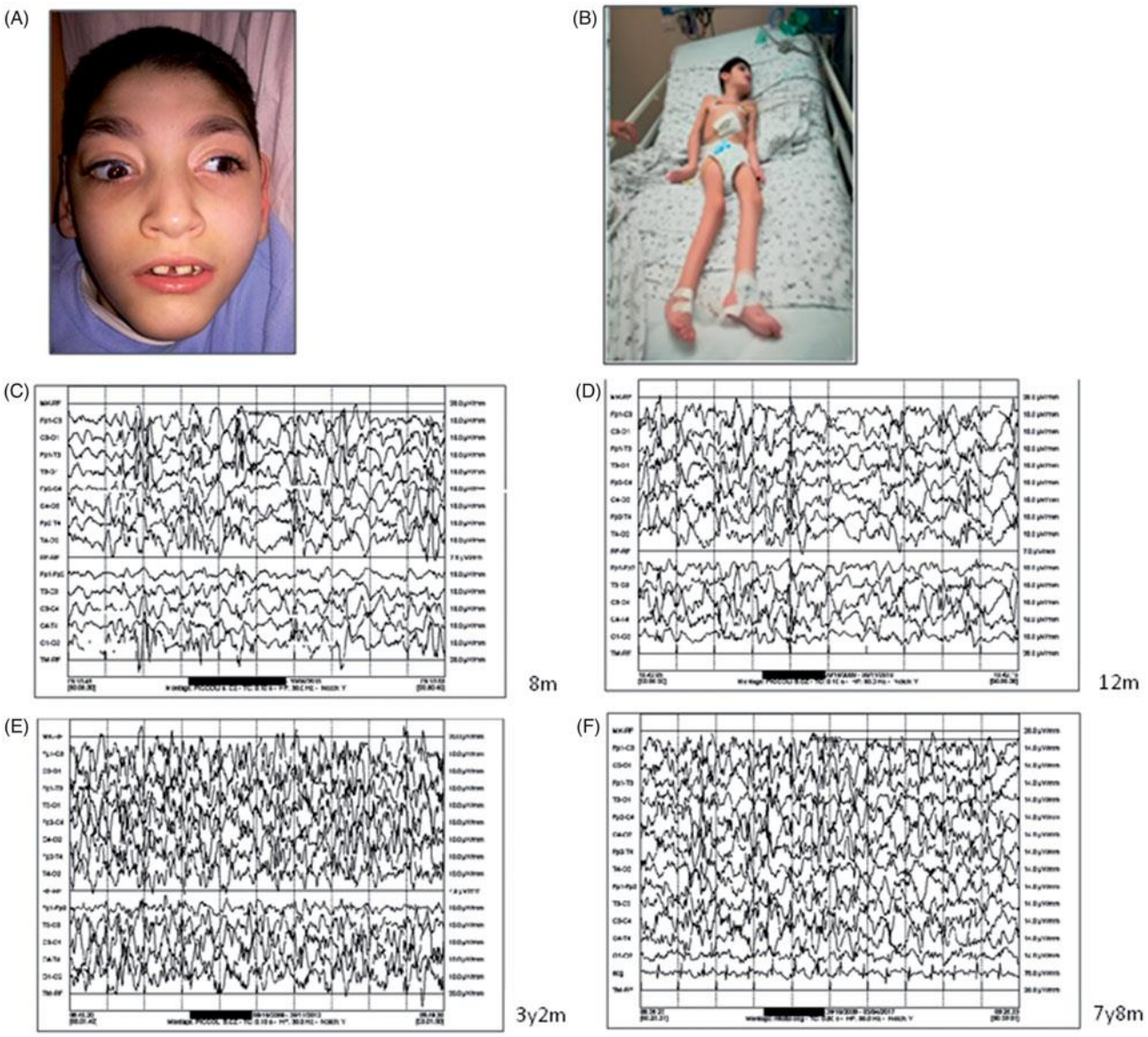

Figure 1: pictures of the patient at the age of 7 years. A: mild facial dysmorphisms (large nose root, low-implanted and wide auricles; B: bedridden, extended limbs with pronated hands and equinus feet. Left dorso-lumbar convex scoliosis). EEG patterns and evolution from the first year of life up to the present age (7y8m). The EEG showed recurrent discharges of bilateral, asymmetric, high-voltage spikes on C-T regions with main left amplitude, interposed with diffuse slowing of the background activity or flattening featuring fragmented hypsarrhythmia (C, D). Multifocal diffuse spikes and polyspikes with loss of physiological stage 2 sleep figures became evident at the latest EEG recordings (E, F).

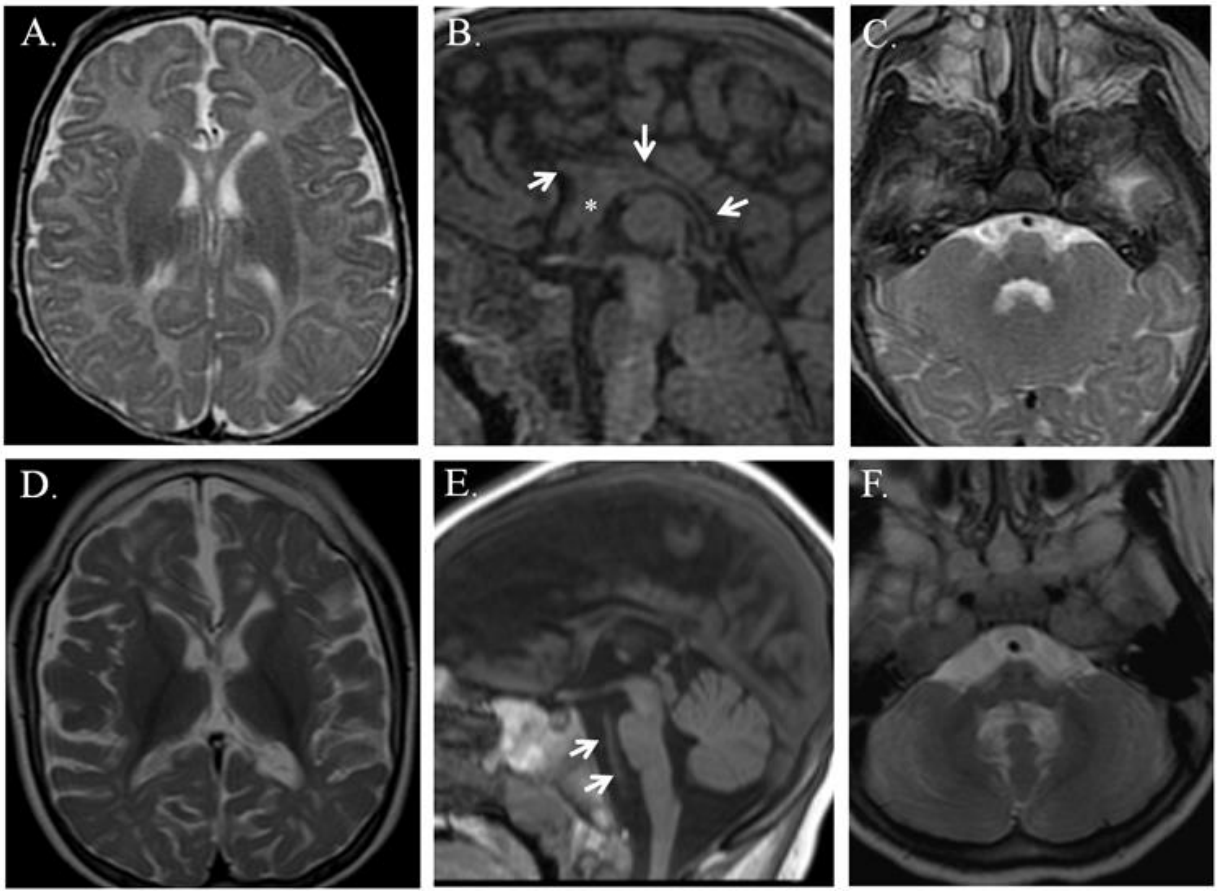

Figure 2: Baseline Magnetic Resonance Imaging (MRI) examination performed at 3-months (A,B,C). A: Axial T2-weighted image showed a diffuse slight hyperintensity of biemispheric white matter related to hypomyelinization. B: Sagittal T1-weighted Magnetization-Prepared Rapid Gradient Echo (MP-RAGE) image well depicted the severe hypoplasia of the corpus callosum (arrows); the anterior commissure is enlarged (asterisk). C: Axial T2-weighted image showing a quite normal appearance of posterior fossa structures. Follow-up MRI examination at the age of 4 years (D, E, F). D, F: Axial T2-weighted images showing gray matter/white matter volume loss at both supratentorial and infratentorial region. E: Sagittal T1-weighted MP-RAGE image better depicts brainstem atrophy with enlargement of the cisternal spaces (arrows).

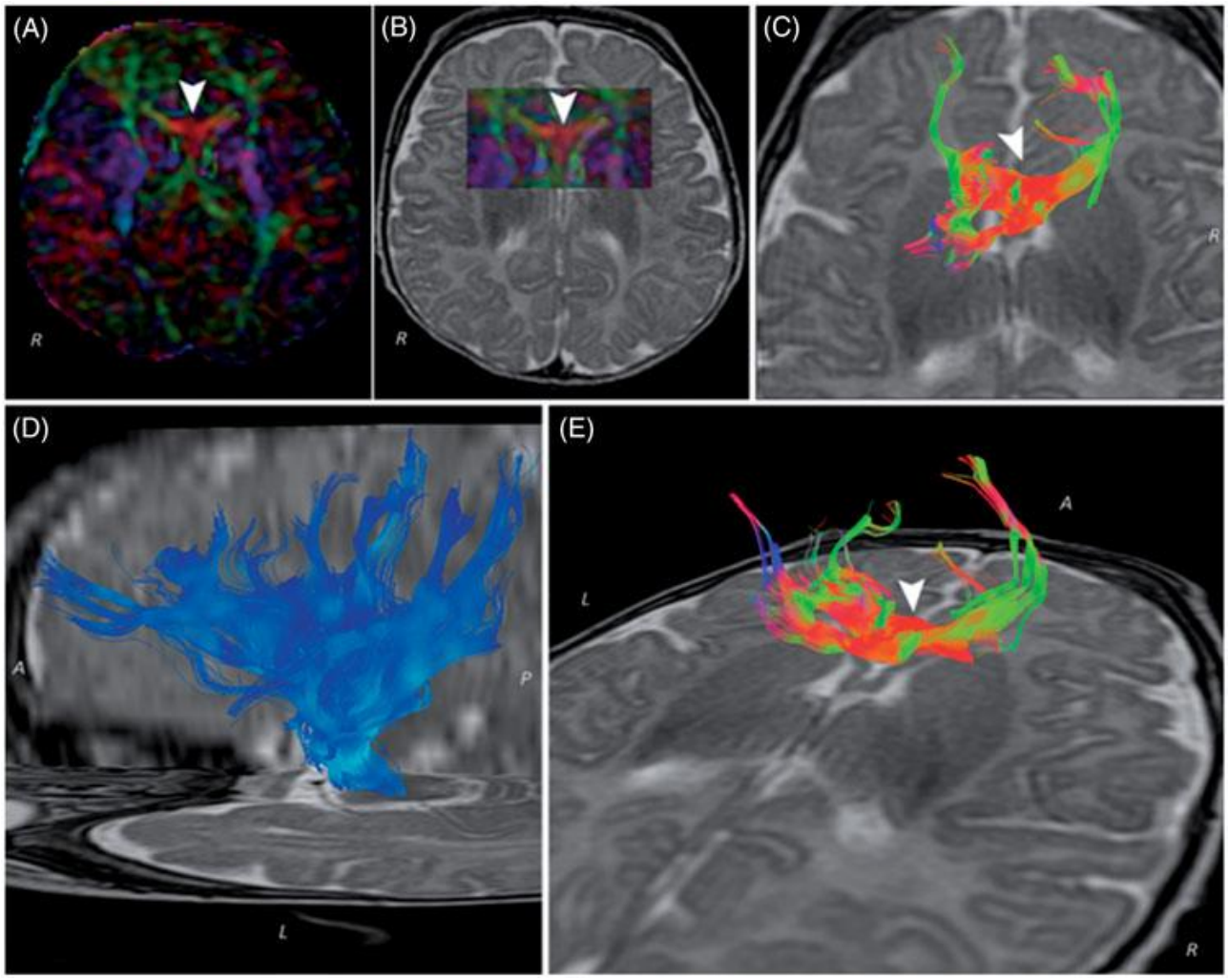

Figure 3: Diffusion Tensor Imaging (DTI) tractography performed at 3-months. A: Axial color coded Fractional Anisotropy (FA) map at the level of the anterior commissure (arrowhead). Please note that the anterior commissure, which in our case is enlarged (arrowhead), is conventionally red-colored since it is mainly composed of white matter fibers with a latero-lateral course at the midline level.B: Axial color coded Fractional Anisotropy (FA) map on top of an axial T2-weighted image at the level of the anterior commissure (arrowhead). C, D: Diffusion Tensor Imaging (DTI) fiber tractography of the enlarged anterior commissure on top of a T2-weighted image, respectively C) axial and D) oblique axial viewed from the top. The reconstruction of fiber tracts followed the FA color-coded convention. Please note that the enlarged anterior commissure is red-colored at the midline level (arrowhead). E: Diffusion Tensor Imaging (DTI) fiber tractography of left corticospinal tract does not reveal any gross morphological abnormality (right corticospinal tract not showed). Fiber tracts were arbitrarily blue-colored.

Video Legend

Video 1: interictal EEG videoEEG recording showing recurrent discharges of bilateral, asynchronous, high-voltage, C-T spikes, interposed with brief tracts of diffuse background slowing or flattening. Spontaneous motility of the child is characterized by oral automatisms.

Video 2: videoEEG recorded during a cluster of seizures characterized by sudden staring and oculogyric movements, grimacing, masticatory and lingual automatisms associated with crying. The electrical correlate is poorly evaluable due to artefactual activity, diffuse multifocal discharges could be evidenced.