Molecular Genetics and Metabolism Reports 21 (2019) 100502

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Case Report

Broadening phenotype of adenylosuccinate lyase deficiency: A novel clinical pattern resembling neuronal ceroid lipofuscinosis

Mario Mastrangelo[a], Chiara Alfonsi[a], Isabella Screpanti[b], Laura Masuelli[c], Barbara Tavazzi[d,e], Davide Mei[f], Flavia Giannotti[a], Renzo Guerrini[f], Vincenzo Leuzzi[a,*]

[a] Division of Child Neurology and Infantile Psychiatry, Department of Human Neurosciences, Sapienza University of Rome, Rome, Italy
[b] Department of Molecular Medicine, Sapienza University of Rome, Italy
[c] Department of Experimental Medicine, Sapienza University of Rome, Italy
[d] Institute of Biochemistry and Clinical Biochemistry Catholic University, Rome
[e] Fondazione Policlinico Universitario A. Gemelli IRCCS, Italy
[f] Neuroscience and Neurogenetics Excellence Centre, Children's Hospital Anna Meyer, Florence, Italy

ARTICLE INFO

Keywords:
Purine metabolism
Retinopathy
Neurodegenerative disorders
Drug- resistant epilepsy
Children

ABSTRACT

We describe a 7-year-old boy presenting with a developmental encephalopathy, severe epilepsy, retinopathy with salt and pepper fundus, and ultrastructural skin alterations resembling a neuronal ceroid lipofuscinosis. Whole exome-sequencing detected biallelic variants in the *ADSL* gene (c.65C > T [p.(Ala22Val)] and c.340 T > C [p.(Tyr114His)]). The increase of SAICAR and S-Ado in blood and urine was consistent with the pattern of adenylosuccinate lyase deficiency (OMIM 103050). An unusual increase of AICAR, that was due to a residual ADSL enzyme activity of about 28%, was also detected.

Neither salt and pepper retinopathy nor ultrastructural skin alterations had been reported in ADSL deficiency before. Impaired purinergic signaling inside the retina is probably involved in visual failure. Ultrastructural alterations in fibroblasts suggest a possible damage of autophagic processes, whose role in the pathogenesis of neurological dysfunction deserves further study.

1. Background

Adenylosuccinate lyase (ADSL; EC 4.3.2.2) deficiency (OMIM 103050;) is a rare disorder of purine metabolism involving purinosome assembly and decreasing metabolite fluxes through purine *de novo* synthesis and purine nucleotide recycling pathways [1]. Seventy-six patients are on record in a dedicated disease database (http://www1.lf1.cuni.cz/udmp/adsl). Three main clinical phenotypes have been.

recognized to date, including a severe type I form, a milder type II form, and a fatal neonatal form [1].

We describe a newly diagnosed patient presenting with severe epileptic encephalopathy, visual failure, and ultrastructural skin alterations suggesting the impairment of autophagy machinery.

2. Case report

This 7-year-old boy was born from nonconsanguineous parents with an emergency caesarean section performed due to fetal distress at cardiotocography. Apgar score was 7 [1], 8 [5]. Transient jaundice that required phototherapy was observed after the second day of life. Relevant difficulties in suction and feeding were reported since the first days of life. At the age of three months, a first focal epileptic seizure occurred. Recurrent myoclonic and tonic seizures and frequent episodes of status epilepticus were observed in the following months. EEGs showed multifocal spikes and slow waves, more prominent in the anterior regions, while a burst suppression pattern appeared in the later stages (Fig. 1). A first brain MRI, at the age of 4 months, showed mild hypomyelination, reduced hippocampal volume and moderate dilation of temporal horns and lateral ventricles.

In the following years, several antiepileptic drug associations were used with limited benefit. In particular, vigabatrin was introduced at the age of 6 months and withdrawn two months later because of ineffectiveness.

The patient came to our attention when aged 5 years, after worsening of epileptic seizures. His head circumference was 48 cm (< 3°centile). He was wheelchair bound and had no spontaneous

* Corresponding author at: Division of Child Neurology and Infantile Psychiatry, Department of Human Neurosciences, Sapienza- University of Rome, Via dei Sabelli 108, 00141 Roma, Italy.
E-mail address: vincenzo.leuzzi@uniroma1.it (V. Leuzzi).

https://doi.org/10.1016/j.ymgmr.2019.100502
Received 20 May 2019; Received in revised form 7 August 2019; Accepted 7 August 2019
Available online 21 August 2019

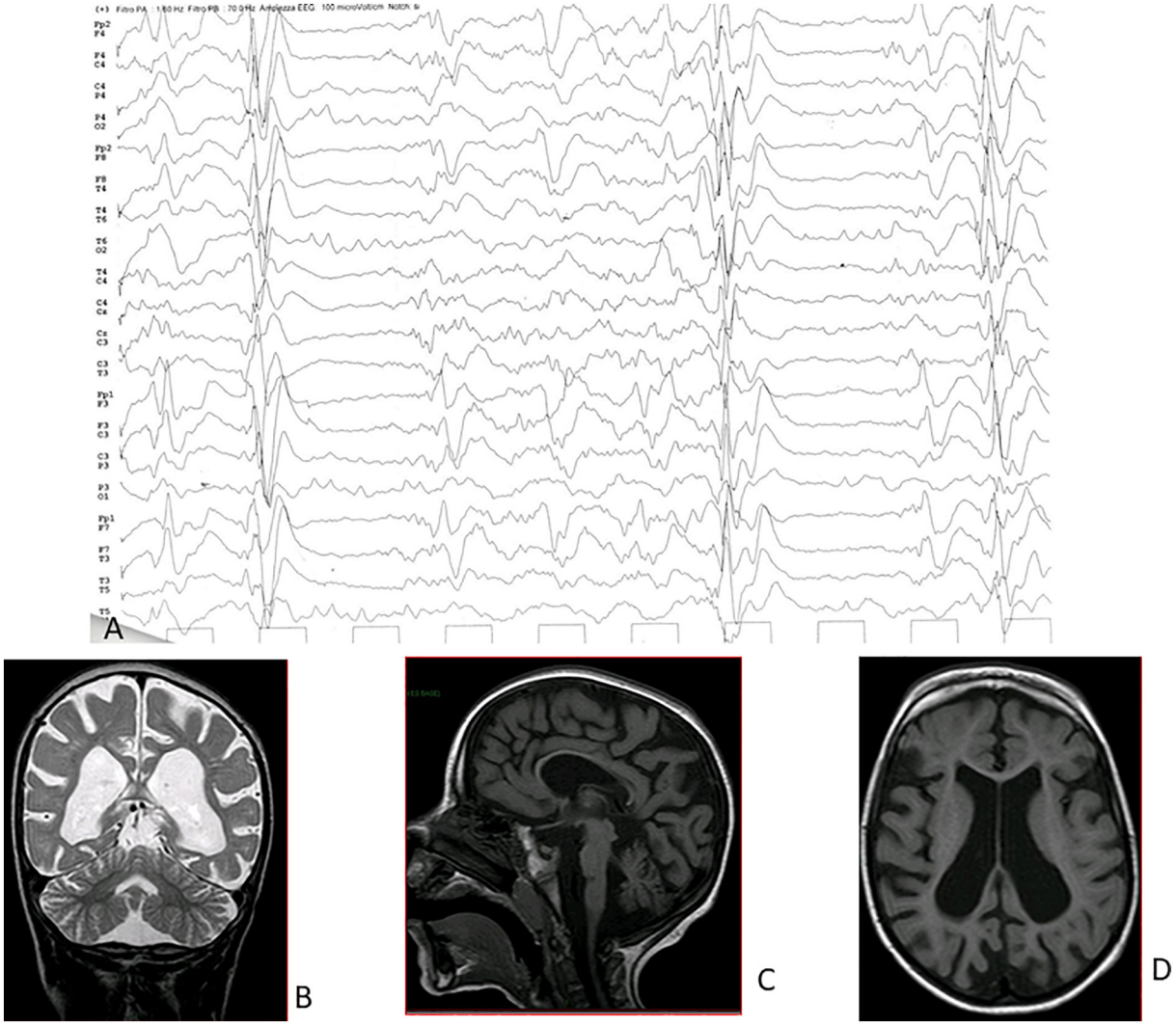

Fig. 1. A) Diffuse epileptiform discharges and a suppression burst pattern at EEG during sleeping in the presented patient. B, C and D) Brain MRI at the age of 5 that evidences a diffuse cortical and subcortical atrophy and a mild callosal hypoplasia.

movements, exhibiting spastic quadriparesis and severe muscular hypotrophy. Eye tracking was extremely limited. Eye fundoscopy revealed a pale optic disc with a salt and pepper retinal pattern. Psychomotor development was severely retarded.

A further MRI examination, at the age of 5 years, revealed diffuse brain atrophy with a more relevant involvement of hippocampal regions, and a thin corpus callosum (Fig. 1B, C and D). Visual evoked potentials were normal while electroretinogram evidenced lack of rods responses bilaterally.

Ultrastructural analysis by transmission electron microscopy was performed on a 3 mm punch skin biopsy (Fig. 2). Microscopic observation showed lipopigments in few epithelial cells in eccrine sweat glands and in a dermal fibroblast (Fig. 2). “Fingerprint- like” formations were present in the cytoplasm of dermal fibroblasts and mast-cells (Fig. 2). A distension of the endoplasmic reticulum was also evident, together with multiple empty vacuoles of large size, of which some were surrounded by a double membrane (Fig. 2). Osmiophilic deposits were visible in unmyelinated nervous fibers (Fig. 2).

An NGS panel for genes associated with all the 14 known neuronal ceroid lipofuscinosis was mutation negative.

3. Materials and methods

3.1. Whole exome sequencing

Whole exome-sequencing and parallel sequencing were performed on genomic DNA obtained from leukocytes. Enrichment of the whole exome was attained using the SureSelect Clinical Research Exome V2 (Agilent, Santa Clara, CA, USA) and the library was sequenced on an Illumina NextSeq 500 platform (Illumina, San Diego, CA, USA). WES data analyses were performed using the BaseSpace OnSite platform (Illumina, San Diego, CA, USA). Reads were aligned to human genome build GRCh37/UCSC hg19 and variants were quality-filtered according to GATK's best practices, annotated with VarSeq (Golden Helix, Bozeman, MT, USA), and filtered against public (gnomAD V.2.0) (http://gnomad.broadinstitute.org/) and *in-house*.

databases to retain private and rare variants (Minor Allele Frequency $< 0.1\%$) located in exons with any effect on the coding sequence, and within splice site regions. Variants with depth > 10 and genotype quality > 20 were filtered according to the *de novo*, homozygous recessive and compound heterozygous models. Among the private or rare variants with an impact on the transcripts, two biallelic *ADSL* variants from the compound heterozygous model were retained and validated by Sanger sequencing using the Big Dye Terminator V3.1 chemistry (Thermo Fisher, MA, USA) on a 3500 DX Genetic Analyzer

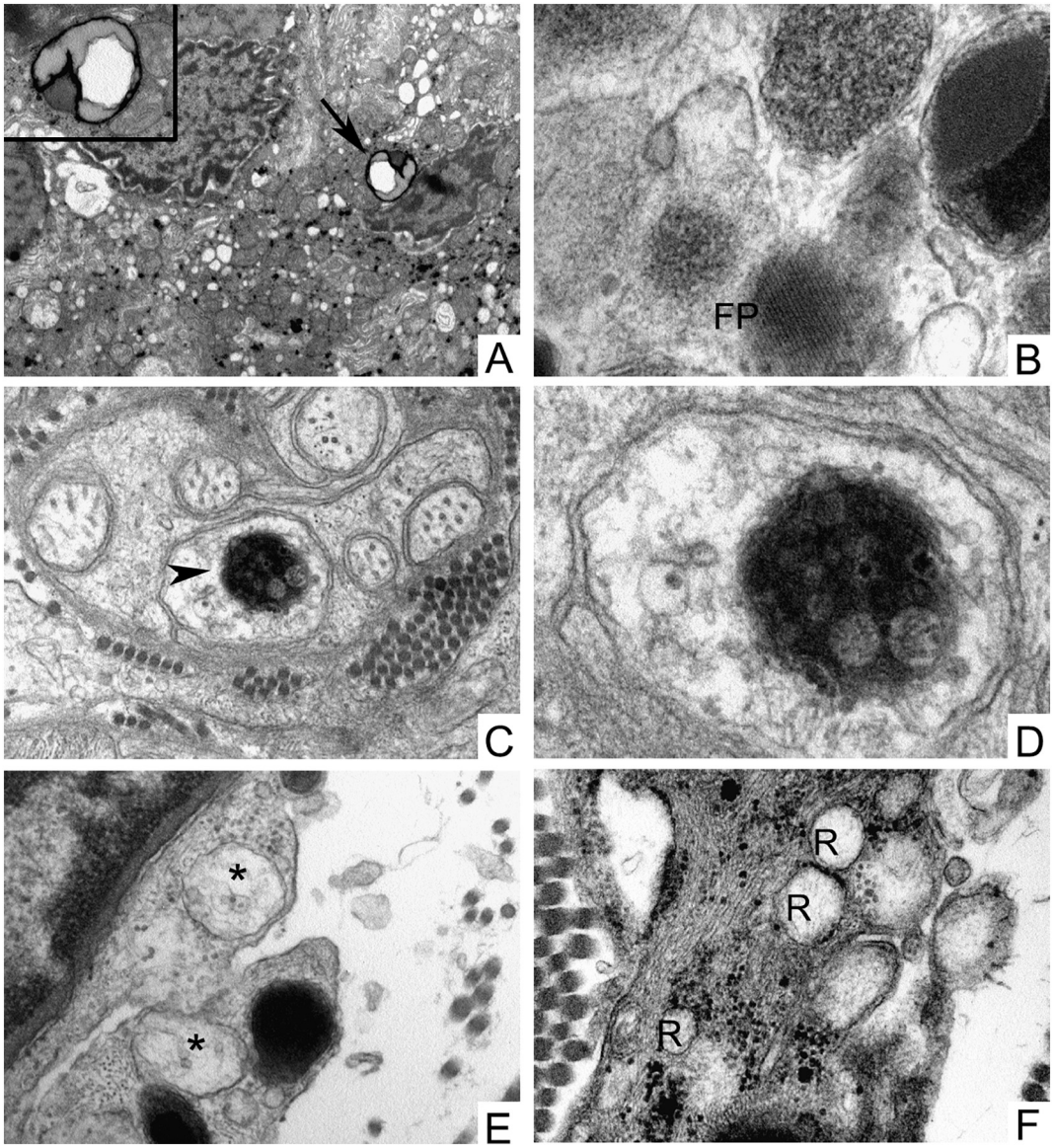

Fig. 2. Ultrastructural analysis of skin biopsy: Ultrastructural analysis was performed by transmission electron microscopy as previously described [21].
A Lipofuscin accumulation in glandular epithelium (arrow). Higher magnification is reported in the upper square. Original magnification (O.M.) x7000; B "Fingerprint- like" (FP) formation in the cytoplasm of a dermal fibroblast. O.M. x71000; C-D Osmiophilic deposits (arrowhead) in unmyelinated nervous fibers. O.M. x36000 and 89,000 respectively; E. Multiple empty vacuoles surrounded by double membrane (*) suggesting an autophagic origin in dermal fibroblasts. O.M. x44000 F. Distension of rough endoplasmic reticulum (R). O.M. x 44000.

(Thermo Fisher, MA, USA). No variants were retained from *de novo* and homozygous recessive models.

3.2. Biochemical investigations

An aliquot of the whole heparinized blood sample of the patient was centrifuged at 1860*g*/10 min, to separate erythrocytes and plasma; the red blood cells were washed 3 times with abundant volumes of PBS-glucose, and a weighed aliquot of packed erythrocytes was then hemolized by adding HPLC-ultrapure water (1:5; v:v).

Enzyme activity was measured by HPLC analysis of AMP, S-Ado and AICAR formed from SAMP and SAICAR respectively, used as substrates of the ADSL enzyme.

Assays were performed at 35 °C in 20 mM Tris–HCl (pH 7.4) with varying concentrations of SAMP and SAICAR (30–250 μM), and with 5 mL of the hemolysate. For every substrate concentration, were used three aliquots of the reaction mixture in order to determine the ADSL activity at the zero time and after 10 and 20 min of incubation. At the established times, all of the aliquots were extracted by addition of 100 μL 70% perchloric acid on ice to stop the reaction, and after the solution were neutralized by adding 100 μL of 5 M potassium carbonate. Experiments were run in duplicate and samples stored overnight at −80 °C.

After centrifugation, 100 mL of the supernatants were analyzed according to a slight modification of the HPLC method of Tavazzi B, et al. to evaluate the concentration of compounds produced by the ADSL activity during incubation [2]; the product concentrations obtained permitted to extrapolate the enzyme activity expressed as International Units related to the volume of red blood cells (IU/L rbcs).

The ion-pairing HPLC method used for these analyses is highly sensitive and reproducible, and very valid for the analysis of a broad spectrum of purines. This is demonstrated by the fact that, as reported in the previous work [2], also for AICAR,

SAICAR, S-Ado and AMP, the values of LLOD (evaluated with a signal to noise ratio > 3) were 0.05, 0.04, 0.05 and 0.05 μM, respectively; those of LOQ (evaluated with a signal to noise ratio > 5) 0.08,

0.08, 0.07 and 0.08 μM respectively; the correlation coefficients were in a range of 0.94–0.98.

4. Results

4.1. Whole exome sequencing

WES detected the compound heterozygous mutations c.65C > T [p.(Ala22Val)] and c.340 T > C [p.(Tyr114His)] in the *ADSL* gene. The c.65C > T [p.(Ala22Val) variant was paternally inherited and was not reported in the GnomAD database (107,580 alleles from European non-Finnish individuals) while the c.340 T > C [p.(Tyr114His) variant was maternally inherited and it was previously reported as pathogenic [3–5]. The novel p.(Ala22Val) missense substitution, detected *in trans* with the known pathogenic p.(Tyr114His) missense substitution, was predicted as damaging by several bioinformatic tools (DANN, GERP, FATHMM, LRT, MutationAssessor, MutationTaster, FATHMM-MKL and SIFT) assessed by VarSome (http://varsome.com) [6]. The Ala22 residue was highly conserved across species (data not shown) and a p.(Ser23Arg) mutation, located near the Ala22 residue, has been demonstrated to result in a decreased stability of the ADSL homomeric complexes [5]. These observations suggested a likely pathogenic role of the p.Ala22Val substitution according to the ACMG guidelines [7].

4.2. Biochemical investigations

Urinary and blood purine measurements detected high levels of SAICAR, S-Ado and AICAR (SAICAR 37.59, S-Ado 236.11 and AICAR 19.96 μmol/mmol creatinine, respectively, in urine; SAICAR 4.52, S-Ado 5.92 and AICAR 1.88 μmol/L, respectively, in plasma). It is important to underline that, in biological fluids of healthy patients, all of the three metabolites are undetectable.

Moreover, in order to explain either the value of SAICAR, S-Ado and AICAR, it was performed the analysis of the ADSL activity on blood sample. As reported above, the enzymatic activity has been analyzed considering that ADSL is a bifunctional protein that is involved in both purine *de novo* synthesis and purine interconversion. The results obtained showed that the ADSL activity was 95 IU/L rbcs (320–450 IU/L rbcs is the reference range measured in 50 control subjects, aged between 2 and 15 years), corresponding to about a 28% of residual enzymatic activity.

5. Discussion

This report highlights that ADSL deficiency can be manifested with a previously unrecognized clinical pattern resembling neuronal ceroid lipofuscinosis, including a developmental encephalopathy with drug-resistant epilepsy, retinopathy and some ultrastructural features resembling those observed in neuronal ceroid lipofuscinoses.

Epileptic seizures, severe developmental delay and stagnation or regression, likewise observed in all neuronal ceroid lipofuscinoses, have been observed in most published patients with ADSL deficiency, making these symptoms in our patient consistent with the known phenotypic spectrum of the disease [1]. The correlated pathogenic mechanisms are still under discussion and include the toxic effects of intermediates, the deficient synthesis of purine nucleotides and the subsequent impairment of neuronal ceroid energy metabolism through displacement of the ATP/AMP ratio [1].

No retinal involvement has been previously reported in ADSL deficiency. Rod impairment and the correlated defect in scotopic vision observed in our patient may be interpreted as the consequence of a reduced stimulation of purinergic ionotropic receptors $P2X_3$ and $P2X_7$, resulting from the decreased production of purine nucleosides [8]. This hypo-stimulation might induce disempowerment of gabaergic transmission from A17 amacrine cells onto rod bipolar cells, with disorganization of the structural integrity of photoreceptors [8,9]. The dysregulation of purinergic signaling could also result in a subsequent decreased visual information flow within the inner retina or in a $P2X_7$receptors-mediated excitotoxicity in ganglionic cells [1,8–10].

In our patient, a potential confounding effect on retinal function played by vigabatrin treatment is unlikely since this medication was only administered for two months [11,12]. Other mechanisms responsible for potential retinal dysfunctions resulting from an abnormal purinergic signaling in ADSL deficiency include a possible deregulation of regional blood flow through an impairment of retinal pericytes and the inhibition of the transepithelial transport of nutrients, ions, and water in the subretinal space [9].

The fundoscopy finding of a salt and pepper retina in our patient remains unexplained. A possible hypothesis implies an abnormal storage of lipofuscin in retinal pigment epithelium resulting from the dysregulation of lysosomal activity, due to an altered ratio between ATP and adenosine mediated by a dysfunction in purinergic neurotransmission in these cells [8,13,14].

The presence of lipopigments in the eccrine sweat glands and dermal fibroblasts of our patient, associated with the detection of “fingerprint-like” formations and of osmiophilic inclusions in unmyelinated nervous fibers, is another relevant point of contact with morphologic features typical of neuronal ceroid lipofuscinoses [15–17]. These findings could be related to the unusual high levels of AICAR and the minimal residual ADSL enzyme activity that were detected in our patient and never reported before in the literature. AICAR has been reported to stimulate, but also to inhibit autophagic mechanisms through complex and incompletely known interactions18. Its increase could result in a dysregulation of autophagy [18].

The severity of the pathological findings detected in our patient is not measurable since skin biopsies were not reported in previously published observations. Further studies are needed to clarify morphological characteristics allowing differential diagnosis with neuronal ceroid lipofuscinosis. For example, no curvilinear profiles were observed in our patient, which could be distinctive feature from neuronal ceroid lipofuscinosis.

The few available neuropathological data in ADSL, could not find impaired autophagic processes or lipofuscin accumulation in the cerebral hemispheres, brain stem and cerebellum [19,20]. The main detected abnormalities included widespread damages and subsequent loss of neurons accompanied by microspongiosis of neuropile and diffuse destruction of myelin sheets with severe astroglial reaction resulting from the toxic effects of intermediated metabolites [19] [20].

6. Conclusions

The history of the reported patient evidenced the importance to consider urinary and plasmatic measurements of purine and subsequent genetic assessment for ADSL deficiency in subjects in which a developmental epileptic encephalopathy, with visual dysfunction and ultrastructural features resembles neuronal ceroid lipofuscinosi.

Financial disclosures

None of the authors have any disclosure to declare.

Data avaialability statement

The present paper does not contain other data apart from the ones that were already inserted (Data availability not applicable).

Editorial policies and ethical considerations

All the reported investigations and a specific permission for the publication of the results was obtained through a written informed parental consent.

Acknowledgements

We would acknowledge Dr. Elena Parrini and Dr. Claudia Bianchini (Neuroscience and Neurogenetics Excellence Centre, Children's Hospital Anna Meyer, Florence, Italy) for their role in the realization of whole exome sequencing and Dr. Giacomo Lazzarino (Institute of Biochemistry and Clinical Biochemistry Catholic University- Rome/ Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome) for his role in the realization of biochemical analysis.

References

[1] A. Jurecka, M. Zikanova, S. Kmoch, et al., Adenylosuccinate lyase deficiency, J. Inherit. Metab. Dis. 38 (2015) 231–242.
[2] B. Tavazzi, G. Lazzarino, P. Leone, et al., Simultaneous high performance liquid chromatographic separation of purines, pyrimidines, N-acetylated amino acids, and dicarboxylic acids for the chemical diagnosis of inborn errors of metabolism, Clin. Biochem. 38 (2005) 997–1008.
[3] S. Kmoch, H. Hartmannová, B. Stibůrková, et al., Human adenylosuccinate lyase (ADSL), cloning and characterization of full-length cDNA and its isoform, gene structure and molecular basis for ADSL deficiency in six patients, Hum. Mol. Genet. 9 (10) (2000) 1501–1513.
[4] A. Jurecka, M. Zikanova, A. Tylki-Szymanska, et al., Clinical, biochemical and molecular findings in seven polish patients with adenylosuccinate lyase deficiency, Mol. Genet. Metab. 94 (4) (2008) 435–442.
[5] M. Zikanova, V. Skopova, A. Hnizda, J. Krijt, S. Kmoch, Biochemical and structural analysis of 14 mutant adsl enzyme complexes and correlation to phenotypic heterogeneity of adenylosuccinate lyase deficiency, Hum. Mutat. 31 (4) (2010) 445–455.
[6] Kopanos C1, Tsiolkas V1, Kouris A1, et al., VarSome: the human genomic variant search engine, Bioinformatics. 35 (11) (2019) 1978–1980.
[7] S. Richards, N. Aziz, S. Bale, 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. 17 (2015) 405–424.
[8] J. Sanderson, D.A. Dartt, Trinkaus-Randall vet al purines in the eye: recent evidence for the physiological and pathological role of purines in the RPE, retinal neurons, astrocytes, Müller cells, lens, trabecular meshwork, cornea and lacrimal gland, Exp. Eye Res. 127 (2014) 270–279.
[9] A. Guzman-Aranguez, C. Santano, A. Martin-Gil, B. Fonseca, J. Pintor, Nucleotides in the eye: focus on functional aspects and therapeutic perspectives, J. Pharmacol. Exp. Ther. 345 (2013) 331–341.
[10] C.H. Mitchell, D. Reigada, Purinergic signalling in the subretinal space: a role in the communication between the retina and the RPE, Purinergic Signal 4 (2008) 101–107.
[11] N. Niyadurupola, P. Sidaway, N. Ma, J.D. Rhodes, D.C. Broadway, J. Sanderson, P2X7 receptor activation mediates retinal ganglion cell death in a human retina model of ischemic neurodegeneration, Invest. Ophthalmol. Vis. Sci. 54 (2013) 2163–2170.
[12] C.A. Westall, T. Wright, F. Cortese, et al., Vigabatrin retinal toxicity in children with infantile spasms: an observational cohort study, Neurology 83 (2014) 2262–2268.
[13] T. Wright, A. Kumarappah, A. Stavropoulos, A. Reginald, J.R. Buncic, C.A. Westall, Vigabatrin toxicity in infancy is associated with retinal defect in adolescence: a prospective observational study, Retina. 37 (2017) 858–866.
[14] K.I. Mazzitello, C.M. Arizmendi, F. Family, H.E. Grossniklaus, Formation and growth of lipofuscin in the retinal pigment epithelium cells, Phys. Rev. E Stat. Nonlinear Soft Matter Phys. 80 (5 Pt 1) (2009) 051908.
[15] S. Guha, E.E. Coffey, W. Lu, J.C. Lim, J.M. Beckel, A.M. Laties, C.H. Mitchell, Approaches for detecting lysosomal alkalinization and impaired degradation in fresh and cultured RPE cells: evidence for a role in retinal degenerations, Exp. Eye Res. 126 (2014) 68–76.
[16] P.K. Puri, C. Leilani Valdes, M. Angelica Selim, R.C. Bentley, Neuronal ceroid lipofuscinosis diagnosed via skin biopsy, J. Clin. Neurosci. 17 (2010) 1585–1587.
[17] G.W. Anderson, H.H. Goebel, A. Simonati, Human pathology in NCL, Biochim. Biophys. Acta 1832 (11) (2013 Nov) 1807–1826.
[18] T. Israeli, Y. Riahi, A. Saada, et al., Opposing effects of intracellular versus extracellular adenine nucleotides on autophagy: implications for β-cell function, J. Cell Sci. 131 (15) (2018).
[19] H. Mierzewska, B. Schmidt-Sidor, Lewandowska E et al clinical, biochemical, neuropathological and molecular findings of the first polish case of adenylosuccinase deficiency, Folia Neuropathol. 46 (2008) 81–91.
[20] H. Mierzewska, B. Schmidt-Sidor, E. Jurkiewicz, A. Bogdańska, K. Kuśmierska, T. Stepień, Severe encephalopathy with brain atrophy and hypomyelination due to adenylosuccinate lyase deficiency–MRI, clinical, biochemical and neuropathological findings of polish patients, Folia Neuropathol. 47 (2009) 314–320.
[21] L. Masuelli, M. Granato, M. Benvenuto, R. Mattera, R. Bernardini, M. Mattei, G. d'Amati, G. D'Orazi, A. Faggioni, R. Bei, M. Cirone, Chloroquine supplementation increases the cytotoxic effect of curcumin against Her2/neu overexpressing breast cancer cells in vitro and in vivo in nude mice while counteracts it in immune competent mice, Oncoimmunology. 6 (11) (2017) e1356151.