A patient with Aicardi syndrome phenotype and DCHS1 gene variants. A new step in the pursuit of the genetic cause of the disease or an incidental finding?

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Abstract

Aicardi Syndrome (AS) is a rare and severe neurodevelopment disorder, usually involving the female gender and characterized by hypogenesis of the corpus callosum, ocular abnormalities (chorioretinal lacunae), a severe, drug-resistant epilepsy, intellectual disability, costovertebral anomalies, other brain malformations and mild facial dysmorphism. The genetic cause of AS has never been found. We report on a female patient, affected by AS spectrum and presenting the classic triad (hypogenesia of the corpus callosum, choriorentinal lacunae, drug-resistant epilepsy) and other brain malformations (polymicrogyria, cortical dysplasia, heterotopias and asymmetric ventricles). A NGS-panel for epilepsy and brain malformations disclosed a compound heterozygosis in DCHS1 gene, which is the cause of Van Maldergem syndrome, characterized by severe face dysmorphism, skeletal abnormalities, respiratory tract malformations and severe brain involvement. We hypothesize that the phenotype of this AS patient may be caused by variants in DCHS1 gene and that the two syndromes may share common genetic causes. Interestingly, DCHS1 is located in proximity to TEAD1 (chromosome 11p15), reported as causative of AS in a single patient, and both the proteins are involved in the hippo-pathway (which regulates cellular growth and apoptosis). Alternatively, the patient could present a new subtype of Van Maldergem Syndrome, without face dysmorphism and skeletal abnormalities.

Introduction

Aicardi Syndrome (AS) [OMIM #304050] is a rare and severe neurodevelopment disorder characterized by agenesis/hypogenesis of the corpus callosum, ocular abnormalities and spasms in flexion (Young et al, 2016). It was named after Jean Aicardì, who first described this nosological entity in 1965 (Aicardi et al, 1965); after his original report, other findings have been included within the spectrum of the syndrome: a nearly exclusive involvement of the female gender, chorioretinal lacunae, costovertebral anomalies (Aicardi, 2005) as well as other brain malformations (i.e. polymicrogyria, periventricular and subcortical heterotopia, intracranial cysts, cerebellar abnormalities, and enlarged cisterna magna) (Lund et al, 2015). Facial dysmorphisms may be absent or subtle and only microphtalmia, prominent premaxilla with upturned nasal tip and sparse lateral eyebrows have been recognized among the "supporting features" for a diagnosis; by the contrary, a severe, drug-resistant epilepsy is often present, together with some non-specific EEG findings including hemispheres asynchrony, hypsarrhytmia, burst suppression (Nascimento et al, 2016). Cognition is usually severely affected and a global intellectual disability reported in almost all patients (Grosso et al, 2007) (Tuft et al, 2017).

AS has never been reported as inherited and no familial cases can be found in literature; a unique genetic cause for this syndrome has never been discovered. It is known that affected individuals are always female or 47,XXY males, and it has been thought, for this reason, that a*de novo* dominant mutation on the X-chromosome may be the (most important) cause of the disease, with a hemizygous lethality in males (Aicardi, 2005).

We herein report on a female patient, affected by AS and presenting the classic triad (anomaly of the corpus callosum, choriorentinal lacunae, drug-resistant epilepsy), other severe brain abnormalities (i.e. polymicrogyria, cortical dysplasia, heterotopias and asymmetric ventricles) and psychomotor delay. Submitted to a NGS analysis of 286 genes associated to epilepsy and brain malformations, a compound heterozygosis (*in trans)* of *DCHS1* variants was found. As this gene has been related to many cerebral malformation anomalies, together with a distinct syndrome (i.e. Van Maldergem syndrome, OMIM #601390) we assume that the phenotype of this AS patient may be caused by her gene variants and that the two syndromes may share some genetic factors in their pathogenesis. Moreover, *DCHS1* location is close to *TEAD1* , a gene reported as causative of AS: beside the other possible explanations of our findings (i.e. an incidental finding, or a new subtype of Van Maldergem Syndrome) it would be possible that an imbalance in the genetic region (11p15) containing *DCHS1* and *TEAD1* could have a critical role in the pathogenesis of AS.

A written informed consent has been obtained by the patient's parents in order to report her clinical case.

Clinical Report

A previously healthy 3-month-old baby girl presented to our hospital with a 3-week history of episodes of loss-of-contact of brief duration, then evolved into focal seizures, characterized by deviation of the head and gaze to the right and hypertonia of the ipsilateral limbs. Parents were healthy, non-consanguineous Caucasians. Maternal and paternal undefined family history of epilepsy was reported. Pregnancy and delivery were normal. She had normal motor milestones. Vaccinations were not on schedule and she did not have a significant health problem up to date. At the initial examination in the paediatric unit, she was in the 50^{th} percentile for weight, 90^{th}percentile for length and 10-25^{th} percentile for head circumference. No hypo- or hyperchromic patches were evident. Vital signs were within normal ranges according to her age (body temperature, heart rate, breath rate, blood pressure, oxygen saturation). She was fully awake with eye contact, without apparent nystagmus. There were no skeletal anomalies and muscular trophism was normal. On detailed neurological examination, pupils were isocoric with a bilateral normal light reflex. Cranial nerves' function was normal, with no facial asymmetry. She had a mild hypotonia of the trunk. Patellar reflexes were present bilaterally. Laboratory investigations, including complete blood count, biochemical parameters, arterial blood gas and coagulation profile, were normal. Tandem mass spectrometry, urine and blood amino-acid profile and urine organic acid profile, carried-out in order to exclude inborn errors of metabolism, were all normal; as well as ECG, echocardiography and abdomen ultrasound. A first EEG examination showed spikes/spikes waves starting from the temporal region with secondary generalization. Therapy with Levetiracetam and vitamin B6 was started without benefit. In the following days, she started to presented frequent seizures (i.e. 15/day) which evolved in spasms in flexion, with duration varying from two to ten minutes. A video-EEG was then performed and a right hemi-hypsarrhythmia was noticed. Brain MRI disclosed a complex brain malformation, with asymmetry of the hemispheres (right>left) with asymmetric ventricles, polymicrogyria of the right anterior mesial frontal cortex, cortical dysplasia, multiple focal areas of cortical heterotopias along the walls of the right lateral ventricle, the largest in the paratrigonal area (Figures 1-2). A marked and uniform thinning of the corpus callosum was also documented, with considerable hypotrophy of the knee (Figure 3). There was also a distorted and dysmorphic appearance of the supratentorial ventricular cavities with extension of the bifrontal-bitemporal periencephalic liquor spaces. ACTH therapy was then performed (0,2 mg im once a day and then every other day) with initial resolution of seizures and significant improvement of control EEGs. When ACTH was withdrawn, focal seizures reappeared. They were characterized by deviation of the ocular globes to the right and loss of contact of variable duration, especially evident on awakening and falling, often in cluster. A new EEG was performed and it showed asymmetric basic activity with low-turned sharp elements on frontal regions of the right hemisphere. Topiramate was then added to the therapy, at the initial dosage of 1 mg/kg. Due to pressure values at the upper limits, Levetiracetam was suspended and Vigabatrin therapy undertaken. To complete the diagnostic work-up, an ophthalmological evaluation was performed with evidence of pathological excavation of the right optical disc with small inferior coloboma and peripapillary retinal areas of chorioretinal atrophy. Visual and auditory evocative potentials were normal. The baby girl was discharged from our department with frequent follow-up controls as an outpatient. At the age of 9 months the child presented a delay in psychomotor development

and she does not maintain the sitting position on its own. To clinical re-evaluation we noticed a dystonic posture of the hand. A new ophthalmological evaluation showed a peripapillary pigmentary ring and areas of pigmentend atrophy (corioretinal gaps). She was on therapy with Topiramate (5mg/kg/day) and Vigabatrin (100mg/kg/day) with moderate seizures control. Submitted to array-CGH analysis and to a NGS-gene panel for epilepsy and cortical malformations, the girl was found as harboring two variants *in trans* (compound heterozygosis) of the*DCHS1* gene, never reported in literature and databases.

Genetic Analysis

The girl was submitted to a NexSeq (Illumina) analysis of a 286-gene panel for epilepsies and brain malformations. The sequencing was preceded by a selective enrichment of the regions of the DNA analyzed, through hybridation with probes selectively designed (Sureselect, Agilent). Average coverage of the target bases <100X.

The resulting variants were therefore analyzed by the software BWA (V.0.7.7-r441), Picard (v 1.109) and GATK (v 3.1), and annotation performed by Annovar (v. 17June15). In the analysis were considered all the non-synonimous exonic variants and splice-site (+/- 2 nucleotides of the codyging exons) with frequencies lower than 0.1% dominant genes and lower than 1% for recessive genes (ExAC, GnomAD). The data obtained through NGS sequencing have then been analyzed with the tool CoNVaDING in order to identify variants of the copy number of the exons (Johansson et al, 2016) and the variants reported with the Human Genetic Mutation Database (HGMD) nomenclature.

In the same occasion, an array-CGH analysis was also performed in the patient and in her parents recombination breakpoints were detected using high-resolution 8x60K Human Genome CGH microarray (Agilent Technologies, Santa Clara, CA). Array experiments were performed as recommended by the manufacturer (Agilent Technologies, Santa Clara, CA). Proband DNA was labeled with Cy5 and reference DNA (human DNA Promega) with Cy3. Purification, hybridization and washing steps were performed according to the manufacturer's instructions. Data visualization and analysis were performed with Sure Scan microarray Scanner G2600D (Agilent), Feature Extraction software (Agilent) and Agilent Cytogenomics Software Edition 2.5.

Results

A compound heterozygosis of variants in the *DCHS1* gene were found in the child. The first, inherited by the mother, was c.7408G>T; the second, c.3512G>A, was inherited by the father. The variants were confirmed also by Sanger sequencing both in the child and in the parents.

In silico analysis of the variants (performed by online tool mutations-taster and Alamut Visual Software) found both to be pathogenic (Schwarz et al, 2014). The first (c.7408G>T) is an unknown, never reported, variant occurring in a well-conserved domain (in this position, a different aminoacid is present only in Xenopus Tropicalis), which causes an amino acid sequence change, affecting protein structure and changing one splice site. The second (c.3512G>A) is a known, already-reported variant (dbSNP Short Genetic Variations Database, 2019; gnomeAD Browser, 2020) which occurs in a semi-conserved domain for several species (but absent in *Felix Catus* ,*Gallus Gallus* and *Clostridium Elegans*), and causes amino acid sequence change, and subsequent alteration of the protein features.

Other mutation analysis software have shown some contrasting results. Running the variants through the VarCards server (VarCards Database, 2020) (which applies up to 23 different pathogenicity predictions tools), it has been suggested that the variant c.3512G>A is more likely to be pathogenic with 16 out of 23 tools predicting pathogenicity. In contrast for the variant c.7408G>T, only 7 out of 23 tools predicted this variant to be pathogenic.

No other variants were found in the gene panel analysis.

The array-CGH analysis gave a normal 46 (XX) result in the girl, and 46 (XY) and 46 (XX) results in the parents.

Discussion

The present girl came to our observation at the age of 3 months for severe epileptic seizures, started 3 weeks early with episodes of loss-of-contact of brief duration, then evolved in severe drug-resistant epilepsy mainly characterized by spasms in flexion; brain MRI disclosed a complex brain malformation, with thin corpus callosum, polymicrogyria, cortical dysplasia, heterotopias and asymmetric ventricles. These findings, together with a chorioretinal atrophy and optic disc coloboma, allowed to perform a clinical diagnosis of Aicardi syndrome (Aicardi, 2005). Submitted to a NGS-gene panel for epileptic encephalopathies and brain malformation syndromes, the girl was found as harboring two variants *in trans* (compound heterozygosis) of *DCHS1* gene: c.7408G>T: and c.3512G>A. In a *in silico* evaluation, both showed pathogenic significance, even with different grade of pathogenicity, as demonstrated by the analysis through VarCards server, which reports the results from 23 distinct prediction tools, including SIFT, Polyphen2 and CADD (Varcards Database, 2020). These variants are unlikely to affect splicing (only one splice site is altered by the variant c.7408G>T) and are both missense variants, therefore unlikely to result in a truncated protein. However they could affect stability and localization of the protein, or its interaction with other proteins of the Hippo-pathway; in this aspect, western blots and/or immunofluorescence microscopy would be, in future, helpful to clarify more the pathogenic role of such variants.

DCHS1 gene encodes a transmembrane cell adhesion molecule that belongs to the protocadherin superfamily. DCHS1 is a ligand for FAT4, which is another protocadherin; DCHS1 and FAT4 form an apically located adhesive complex in the developing brain, but their functions encompasses also an important role in cell-cell adhesion of fibroblasts, which is thought to be necessary for wound healing (Cappello et al, 2013). It has been shown that mutations in genes encoding the receptor-ligand cadherin pair DCHS1 and FAT4 lead to periventricular neuronal heterotopia, both in humans and in experimental mice models, with reduced expression of *Dchs1* or *Fat4* (Beste et al, 2016). In particular, within mouse embryonic neuroepithelium, it has been observed an increased progenitor cell numbers and a reduction of their differentiation into neurons, resulting in the heterotopic accumulation of cells below the neuronal layers in the neocortex, reminiscent of the human phenotype. A concurrent knockdown of Yap, a transcriptional effector of the Hippo signaling pathway, was also observed, thus confirming that Dchs1 and Fat4 proteins are upstream of Yap as key regulators of mammalian neurogenesis (Cappello et al, 2013) (Beste et al, 2016).

DCHS1 gene is the causative factor of a rare (less than 20 patients reported so far) and complex malformation syndrome, Van Maldergem type 1, which is characterized, in its classical form, by intellectual disability, typical craniofacial features, auditory malformations, hearing loss, skeletal and limb malformations, brain abnormalities with periventricular neuronal heterotopia and other variable anomalies (van Maldergem et al, 1992) (Sotos et al, 2017) (Ulubas et al, 2018) (Ivanovski et al, 2018). The function of*DCHS1* gene product in fibroblast adhesion can explain why some allelic variants of this gene have been found in a familiar form of mitral valve prolapse, with high penetrance (Durst et al, 2015).

An exact cause of Aicardi Syndrome has never been precisely elucidated and its pursuit is still matter of debate (Wong et al, 2018). In the last 15 years, many studies have suggested a potential role of X-chromosome (Xp22) or other autosomal (1p, 3q, 6q, 12q) translocations. At the same time, male (46XY) patients have been reported as affected by severe microcephaly (that is not a distinctive sign of the disease), thus suggesting that the (possible) mutation in X-chromosome may not be always lethal but (in some cases) related to a very severe phenotype in males or that other genes may be involved in the pathogenesis of the disease (Chappelow et al, 2008). Modern array-CGH studies, however, have failed in reporting (small or large) CNVs in X-Chromosome, with only one patient out of a total of 156 subjects found to have a de novo 157-kb deleted region in Xq25, in which no known codifying exons of any known gene were found (Wong et al, 2018).

A monogenic origin of the disease has been also investigated with potential candidate genes. In particular *FLNA* gene, which encodes for a protein (Filamin A) involved in brain cytoskeleton organization and whose mutations are related to brain heterotopias; however, no disease-causing mutations for this variants have been found in AS patients (Anderson et al, 2009). Another gene whose variants were potentially associated with AS was *CDKL5* , which plays a role in synaptic formations and is implicated in the etiology of early onset

drug-resistant epilepsies (Nemos et al, 2009), but a genetic analysis of this gene in 10 patients affected by AS gave negative results. With the development of NGS gene panels and the use of whole exome sequencing (WES) or Whole genome sequencing (WGS) in large cohort of AS patients, two novel variants in previously unidentified genes have been found (Schrauwen et al, 2015): the genes involved, in two unrelated girls, were *TEAD1* (11p15.3) and *OCEL1* (19p13.11): however a further Sanger sequencing of these two genes in other 38 AS patients failed to find pathogenic variants of these genes (Wong et al, 2017). Hypomethylation of *KCNAB3* (17p13.1) was also proposed among the causes of the disease (Piras et al. 2017), but no consistent finding has been found. More recently, a WES-based study on 11 patients affected by AS has failed to find any significant variant in these patients (the Authors analyzed in particular *TEAD1* and *OCEL1*) (Lund et al, 2016).

It is worthwhile to note that the genetic location of the mutated gene of the present patient (*DCHS1*) is close to *TEAD1.* Given the proximity of *TEAD1* and *DCHS1* , it cannot be excluded that an imbalance in this region may be among the causes of AS, which could be hypothetically caused by *DCHS1* variants and not by *TEAD1* mutations. If *DCHS1* has a pivotal role, however, it cannot be excluded that some (still unknown) gene regulators or modifiers localized in X-chromosome may contribute to the phenotype.

By contrast, there are more than 50 genes in the region containing *DCHS1* and *TEAD1* , many of which are involved in autoimmunity, therefore it is unlikely that mutations in all genes in this region will share the same phenotype. A further *in-silico* analysis through geneMANIA tool (Warde-Farley et al, 2017) to assess potential interactions between the two proteins suggested no physical interaction, co-expression, co-localisation, shared pathway, protein domains or genetic interactions. The only evidence found for any links between the two proteins is that they are both involved in the Hippo signaling pathway, causing (similar) structural brain changes.

Another hypothesis is that this patient could be the first report of a new syndrome or a variant of Van Maldergem syndrome, presenting with the same clinical characteristic triad of the AS syndrome, absence of facial dysmorphism and only the dystonic posture of the hand as the clinical symptoms of Van Maldergem syndrome (apart from some brain anomalies present both in AS and Van Maldergem syndrome, as periventricular heterotopia, brain asymmetry, corpus callosum hypoplasia) (table 1). This hypothesis may be supported by the lower pathogenicity of c.7408G>T variant, which resulted "pathogenic" only in 7 out of 23 prediction tools. Lastly, it cannot be excluded that this variants in *DCHS1* can be an incidental finding, and the phenotype of the girl caused by other genetic anomalies.

Further researchers of variants in *DCHS1* gene in patients affected by Aicardi or Van Maldergem syndromes may contribute to elucidate the role of this and other genes involved in the hippo-pathway in the pathogenesis of the diseases, together with new studies on low-level mosaicism and balanced rearrangements, as well as platforms examining changes at the DNA and chromatin level affecting regulatory regions, in particular for AS (Wong et al, 2018).

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Aicardi, J. (2005). Aicardi syndrome. *Brain & Development,27*, 164-171 Aicardi, J., Lefebvre, J., Lerique-Koechlin, A. (1965). A new syndrome: spasms in flexion, callosal agenesis, ocular abnormalities.*Electroencephalogr. Clin. Neurophysiol, 19*, 609–610. Anderson, S., Menten, B., Kogelenberg, M., Robertson, S., Waginger, M., Mentzel, H.J., Brandl, U., Skirl, G., Willems, P. (2009). Aicardi syndrome in a male patient. *Neuropediatrics, 40,* 39–42. Beste, C., Ocklenburg, S., von der Hagen, M., Di Donato, N. (2016). Mammalian cadherins DCHS1-FAT4 affect functional cerebral architecture.*Brain Struct Funct, 221*, 2487-2491. Cappello, S., Gray, M.J., Badouel, C., Lange, S., Einsiedler, M., Srour, M., et al. (2013). Mutations in genes encoding the cadherin receptor-ligand pair DCHS1 and FAT4 disrupt cerebral cortical develop-

ment. *Nature Genetics, 45*, 1300-1308 Chappelow, A.V., Reid, J., Parikh, S., Traboulsi, E.I. (2008). Aicardi syndrome in a genotypic male. *Ophthalmic Genet, 29*, 181-183 dbSNP Short Genetic Variations Database. (2012, December 12). Retrieved from:*https://www.ncbi.nlm.nih.gov/snp/rs547827281*, Durst, R., Sauls, K., Peal, D., deVlaming, A., Toomer, K., Leyne, M., et al. (2015). Mutations in DCHS1 cause mitral valve prolapse.*Nature, 525*, 109-113. gnomeAD Browser: DCHS1 (dachosuos cadherin-related 1). (2020, Janaury 15) retrieved from:*https://gnomad.broadinstitute.org/gene/ENSG00000166341?dataset=gnomad_r2_1*Grosso, S., Farnetani, M.A., Bernardoni, E., Morgese, G., Balestri, P. (2007). Intractable reflex audiogenic seizures in Aicardi syndrome.*Brain & Development, 29*, 243-246. Ivanovski, I., Akbaroghli, S., Pollazzon, M., Gelmini, C., Caraffi, S.G., Mansouri, M., et al. (2018). Van Maldergem syndrome and Hennekam syndrome: Further delineation of allelic phenotypes. *Am J Med Genet A*, 176, 1166-1174. Johansson, L.F., van Dijk, F., de Boer, E.N., van Dijk-Bos, K.K., Jongbloed, J.D., van der Hout, A.H., Westers, H., Sinke, R.J., Swertz, M.A., Sijmons, R.H., Sikkema-Raddatz, B. (2016). CoNVaDING: Single Exon Variation Detection in Targeted NGS Data. *Human Mutations, 37*, 457-464. Lund, C., Bjørnvold, M., Tuft, M., Kostov, H., Røsb, O., Selmer, K.K. (2015). Aicardi syndrome: an epidemiologic and clinical study in Norway.*Pediatr Neurol, 52*, 182-186 Lund, C., Striano, P., Sorte, H.S., Parisi, P., Iacomino, M., Sheng, Y., Vigeland, M.D., Øye, A.M., Møller, R.S., Selmer, K.K., Zara, F. (2016). Exome Sequencing Fails to Identify the Genetic Cause of Aicardi Syndrome. *Mol Syndromol,7*, 234-238 Nascimento, F.A., Aljaafari, D., Rahim, M., Krings, T., Andrade, D. (2016). Aicardi syndrome: when to suspect the unexpected.*Epileptic Disord, 18*, 216 Nemos C, Lambert L, Giuliano F, et al. (2009). Mutational spectrum of CDKL5 in early-onset encephalopathies: a study of a large collection of French patients and review of the literature. *Clin Genet, 76*, 357–371. Piras, I.S., Mills, G., Llaci, L., Naymik, M., Ramsey, K., Belnap, N., Schrauwen, I. (2017). Exploring genome-wide DNA methylation patterns in Aicardi syndrome. *Epigenomics,9*,1373–1386. Schrauwen, I., Szelinger, S., Siniard, A.L., et al. (2015). A De Novo Mutation in TEAD1 Causes Non-X-Linked Aicardi Syndrome. Invest Ophthalmol Vis Sci.; 56(6):3896–3904. Schwarz, J.M., Cooper, D.N., Schuelke, M., Seelow, D. (2014) MutationTaster2: mutation prediction for the deep-sequencing age.*Nat Methods, 11*, 361-362. Sotos, J., Miller, K., Corsmeier, D., Tokar, N., Kelly, B., Nadella, V., Zhong, H., Wetzel, A., Adler, B., Yu, C.Y., White, P. (2017). A patient with van Maldergem syndrome with endocrine abnormalities, hypogonadotropic hypogonadism, and breast aplasia/hypoplasia. *Int J Pediatr Endocrinol, 12*. Tuft, M., Østby, Y., Nakken, K.O., Lund, C. (2017). Aicardi syndrome and cognitive abilities: A report of five cases. *Epilepsy Behav, 73*, 161-165. Ulubas Isik, D., Unal, S., Erol, S., Arslan, Z., Bas, A.Y., Demirel, N. (2018). A newborn diagnosed with van Maldergem syndrome. *Clin Dysmorphol, 27*, 63-65. van Maldergem, L., Wetzburger, C., Verloes, A., Fourneau, C., Gillerot, Y. (1992). Mental retardation with blepharo-naso-facial abnormalities and hand malformations: a new syndrome? *Clin Genet, 41*, 22-24. VarCards: an integrated genetic and clinical database for coding variants in the human genome; (2020, January 19) Retrieved from:*http://varcards.biols.ac.cn*. Warde-Farley, D., Donaldson, S.L., Comes, O., Zuberi, K., Badrawi, R., Chao, P., Franz, M., Grouios, C., Kazi, F., Lopes, C.T., Maitland, A., Mostafavi, S., Montojo, J., Shao, Q., Wright, G., Bader, G.D., Morris, Q. (2010). The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function.*Nucleic Acids Res, 38 Suppl*, W214-20 Wong, B.K., Sutton, V.R., Lewis, R.A., Van den Veyver, I.B. (2017). Independent variant analysis of *TEAD1* and *OCEL1* in 38 Aicardi syndrome patients. *Molecular Genetics & Genomic Medicine,5*, 117–121. Wong, B.K.Y., & Sutton, V.R. (2018). Aicardi syndrome, an unsolved mystery: Review of diagnostic features, previous attempts, and future opportunities for genetic examination. *Am J Med Genet C Semin Med Genet, 178*, 423-431. Young, R.J., Francis, J.H., Abramson, D.H. (2016). Aicardi Syndrome.*Ophthalmology, 123,* 1645.

Table 1 – Differential diagnosis between Van Maldergem Syndrome type 1 and Aicardi Syndrome, together with the clinical features presented by the present patient.

Clinical features	Van Maldergem Syndrome	Aicardi Syndrome	**Present Patient**
Both sexes affected	+		

Clinical features	Van Maldergem Syndrome	Aicardi Syndrome	**Present Patient**
Severe face dysmorphisms (i.e. large forehead, flat face – maxillary hypoplasia, hypertelorism, Epicanthal folds, Narrow palpebral fissures, Pear shaped nose, Dental malocclusion, ear abnormalities, small deformed ears)	+		
Mild face dysmorphisms (i.e. microphtalmia, prominent premaxilla with upturned nasal tip and sparse lateral eyebrows)		+	+/- (only mild microphtalmia)
Multiple skull abnormalities (i.e. Sclerotic base of the skull, thickened frontal bone maxillary hypoplasia)	+		
Skeletal abnormalities (i.e. narrow thorax with short clavicles, limb bone abnormalities [metacarpals, phalanges, others], abnormal pattern of ossification, scoliosis, generalized osteopenia, narrow thorax with short clavicles)	+	+/-	
Hyperlaxity of joints	+		
Camptodactyly and/or palmar, plantar interphalangeal dystonia	+		+
Respiratory tract malformations (i.e. upper airway obstruction, choanal atresia/stenosis, tracheomalacia)	+		
Small kidneys	+		
Chorioretinal lacunae		+	+
Hearing loss	+		
Brain asymmetry	+		+

Clinical features	Van Maldergem Syndrome	Aicardi Syndrome	**Present Patient**
Abnormal corpus callosum	+	+	+
Subcortical/Periventricular heterotopias	+	+	+
Polymicrogyria		+	+
Large lateral ventricles	+		+/-
Reduced volume of pons & cerebellum	+		
Thin optic nerves	+		
Drug-resistant epilepsy		+	+
Infantile Hypotonia	+		+/-
Developmental delay	+	+	+
Early feeding difficulties	+		
Genetics	*DCHS1* mutations	Mainly unknown. X chromosome anomalies? Somatic Mosaicism? Two cases monogenic (other than X-chromosome)	*DCHS1* compound heterozygosis c.7408G>T and c.3512G>A

Legend for Figures

Figure 1 – Brain MRI of the patient, at the age of 6 months. T2-weighted axial Sequences. A slight predominance of the right hemisphere can be noticed, as well as frontal polymicrogyria (white arrows) and peri-ventricular nodular heterotopia (black arrows).

Figure 2 - Brain MRI of the patient, at the age of 6 months. T1-weighted sagittal sequences. An hypotrophic corpus callosum of the patient is evident (white arrows).

Figure 3 - Brain MRI of the patient, at the age of 6 months. FLAIR axial sequences. Asymmetry of the brain hemispheres is noticeable, together with a dilatation of the frontal portion of the right ventricle (black arrows). Periventricular nodular heterotopia is also present in the posterior horn of the right ventricle (white arrow).