

Aicardi Syndrome Associated with Autosomal Genomic Imbalance: Coincidence or Evidence for Autosomal Inheritance with Sex-Limited Expression?

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Key Words

Aicardi syndrome · Autosomal inheritance · Genomic disorder · Sex-limited expression

Abstract

Aicardi syndrome (AIS), a rare neurodevelopmental disorder thought to be caused by an X-linked dominant mutation, is characterized by 3 main features: agenesis of corpus callosum, infantile spasms and chorioretinal lacunae. A genome-wide study of a girl with AIS lead us to identify a 6q deletion;12q duplication, derived from a maternal 6q;12q translocation. The two intellectually impaired brothers of the proband showed the same genomic anomalies, but not the constellation of features characterizing the AIS. This could be either a coincidental observation of 2 rare conditions, but can also suggest an alternative hypothesis for the genetic etiology of AIS, indicating the existence of a subset of autosomal genes whose mutation could act in a sex-confined manner.

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Aicardi syndrome (AIS, OMIM 304050), identified by Dr. Jean Aicardi [Aicardi et al., 1965], is a rare neurodevelopmental disorder that affects almost exclusively females, defined by the triad of partial or complete absence of the corpus callosum, infantile spasms and typical chorioretinal lacunae. Revised criteria include: characteristic facial features, such as prominent premaxilla, upturned nasal tip, decreased angle of the nasal bridge, sparse lateral eyebrows, coloboma of the optic nerve, in addition to the chorioretinal lacunae, microcephaly, periventricular heterotopias, microgyria, enlarged ventricles or porencephalic cysts. Moreover, moderate-to-severe developmental delay and intellectual disability is found in almost all patients [Aicardi et al., 1965; Bertoni et al., 1979; Aicardi, 1999, 2005; Roser, 2003; Sutton et al., 2005; Hopkins et al., 2008].

The etiology of AIS is still unknown, even if a genetic etiology due to a de novo X-linked dominant mutation lethal in hemizygous male embryos is strongly suspected [Wetke-Schäfer and Kanter, 1983; Donnenfeld et al.,

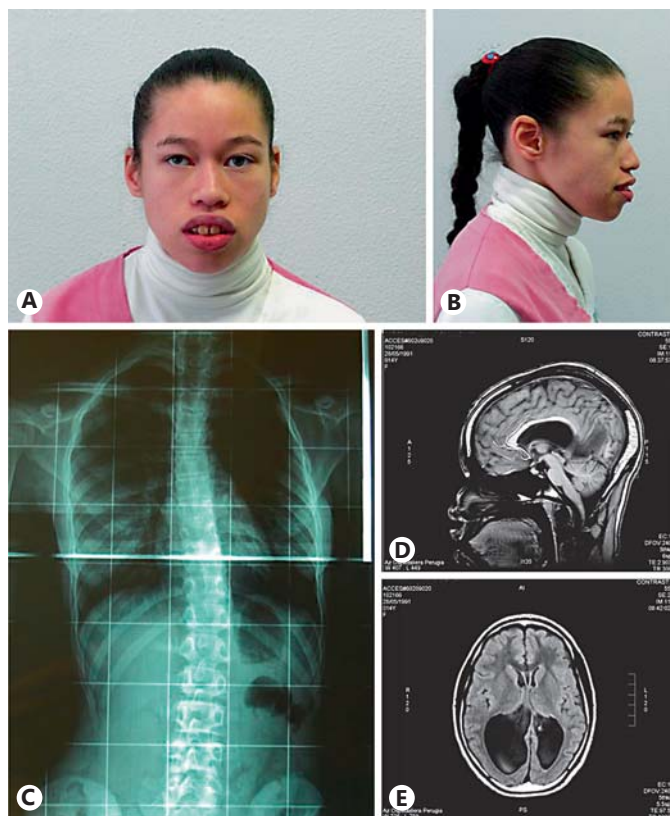


Fig. 1. **A, B** Note the facial dysmorphisms of the patient, in particular the strabismus, high forehead, fullness of periorbital region, prominent premaxilla, prognathism, short philtrum, open-mouth appearance, long mandible, thick lips. **C** Anterior-posterior X-ray projection of the vertebral column showing the moderate scoliosis. The MRIs show the partial agenesis of corpus callosum (**D**) and ventriculomegaly with gross cerebral asymmetry (**E**).

1989, 1990; Van der Veyver, 2002; Rosser, 2003; Aicardi, 2005]. About 200 girls with AIS have been reported in the literature [Donnenfeld et al., 1989; Rosser, 2003; Aicardi, 2005; Glasmacher et al., 2007; Grosso et al., 2007]. Chromosome and genomic (e.g. array-CGH) studies of AIS patients are normal in the majority of the cases with only few exceptions [Ropers et al., 1982; Naritomi et al., 1992; Bursztejn et al., 2009; Wang et al., 2009]. Ropers et al. [1982] reported a presumably balanced X;3 translocation in a girl with features of AIS, indicating gene or genes located within the Xp22.2p22.3 region as possible candidates. Bursztejn et al. [2009] reported an 8-year-old girl with an initial diagnosis of AIS who subsequently found to carry a de novo 11.73-Mb terminal deletion of 1p36 chromosome band and emphasized the phenotypic overlap between the 2 disorders.

In this study, we report a sibship of a girl with AIS and her 2 intellectually impaired brothers, all having the same 6q deletion;12q duplication, derived from the malsegregation of a maternal balanced translocation.

Clinical Report

Clinical History

Personal and familial histories of the parents were unremarkable. The proband, a 21-year-old girl, had 3 brothers of 16, 8 and 6 years, respectively. She was born at 39 weeks gestation after a dystocia delivery, weighing 3,550 g. She showed global neurodevelopmental delay and was able to walk alone and use a few words at 2 years of age. The mother referred that from the fourth to the sixth month and then later on, at 2 years of age, she showed episodes of spasms, mainly characterized by rigidity with stiffening of the arms and legs. At 4 years of age, she developed an epileptic partial crisis, characterized by head and eye rotation, preceded by vomiting, nausea and cyanosis and followed by unconsciousness. The EEG showed multifocal, paroxysmic alteration, bioelectric activity disorganization, but not hypsarrhythmia. Similar EEG findings were recorded during the first and second decades of life, with a crises frequency of 3–4/month. The epilepsy was resistant to pharmacologic treatment until the introduction, 6 years ago, of oxcarbazepine, lamotrigine and sodium valproate which led to a reduction of the crises frequency. The most recent EEG, performed at 20 years of age, revealed slow and paroxysmic temporo-occipital alteration, mainly in the right hemisphere, always associated with general bioelectric activity disorganization. The MRI disclosed partial corpus callosum agenesis, mainly of the splenium, colpocephaly, ventriculomegaly, gross cerebral asymmetry, periventricular cerebral atrophy, and right temporal lobe cortical dysplasia (fig. 1D, E).

Previous ophthalmologic evaluation, performed at 4 years, disclosed a congenital coloboma of the right optic nerve and yellow-white, well-circumscribed, depigmented areas of the retinal pigment epithelium (chorioretinal lacunae). The visual acuity was 3/10 in the right eye and 1/60 in the left. At present, the left eye is completely blind. She also developed a severe scoliosis, confirmed by X-ray examination (fig. 1C). The neuropsychological evaluation with the LEITER-R test, at 14 years of age, ascertained a moderate intellectual disability.

The 20-year-old patient presented in our Medical Genetics Unit for the first time. The dysmorphic assessment revealed: height 164 cm (50th centile), weight 45 kg (10th centile) and OFC 52 cm (3th centile), microcephaly, prominent premaxilla, prognathism, short philtrum, open-mouth appearance, long mandible, thick lips, highly arched palate, scoliosis, and proximally placed thumbs (fig. 1A, B).

Neuropsychological assessment with LEITER-R test disclosed mild intellectual delay in 2 out of the 3 brothers; however, their clinical history was very different from that of the sister. In fact, they did not show infantile spasms, visual defects (normal ophthalmologic evaluations), scoliosis, or facial dysmorphisms, and the brain MRI revealed that they both displayed a mild ventricular dilation, but no corpus callosum agenesis or other structural brain defects.

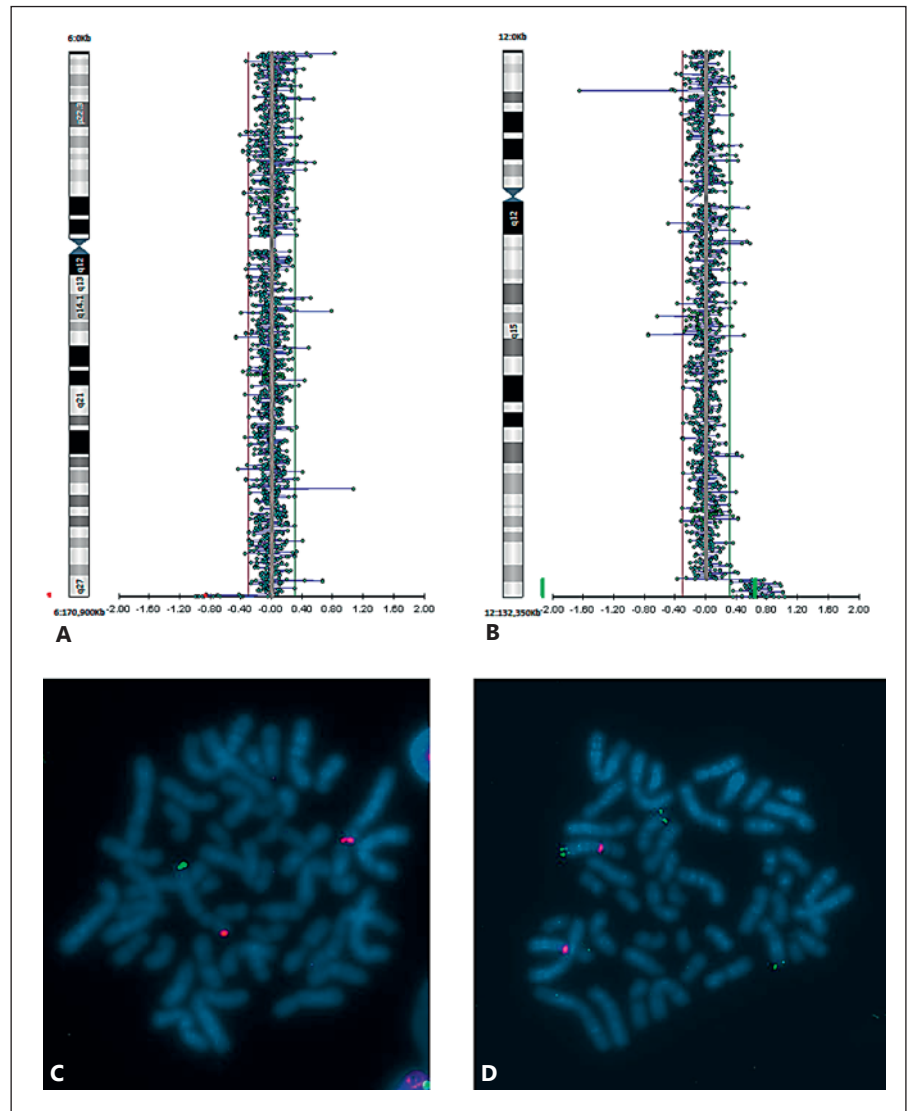


Fig. 2. Results of the array-CGH analysis (CytoChip ISCA 4x44K v1.0 BlueGnome) showing the 6q27 deletion (**A**) and the 12q24.32q24.33 duplication (**B**). FISH on metaphase cells: **C** subtelomere specific 6qtel probe (green) and subtelomere specific 6ptel control probe (red) showing the 6q deletion. **D** Subtelomere specific 12qtel probe (green) and α -satellite chromosome 6 probe (red) as control, showing the 12q duplication on chromosome 6.

Genetic Testing

Array-CGH analyses of the proband and her intellectually impaired brothers were performed on DNA extracted from whole blood (PerfectPure DNA Blood Kit, 5Prime GmbH, Hamburg, Germany), using commercially available arrays (CytoChip ISCA 4x44K v1.0 BlueGnome, Cambridge, UK) according to the manufacturer's instructions as previously described [Prontera et al., 2009].

The array-CGH revealed the presence of a ~550-kb deletion in the 6q27 region and a ~4.2-Mb duplication in the 12q24.32q24.33 region [arr 6q27(170.244.093×2,170.330.025–170.921.059×1).arr 12q24.32q24.33(129.407.053×2,129.491.848–133.767.956×3)] (Ensembl version 54, Genome build NCBI 37, hg19) in all subjects (fig. 2A).

Fluorescence in situ hybridization (FISH) was performed, as previously reported [Prontera et al., 2009], on chromosome metaphases using subtelomere specific 6qtel probe (clone 57H24),

subtelomere specific 12qtel probe (clone 221K18) and, as controls, α -satellite chromosome 6 probe (locus D6Z1) and subtelomere specific 6ptel probe (clone 62I11) (Aquarius CytoCell, Cambridge, UK) (fig. 2B). Multiple ligation probe amplification (MLPA) (SALSA MLPA kit P036-E1 Human Telomere-3 probe-mix; SALSA MLPA kit P070-B2 Human Telomere-5; SALSA MLPA kit P277-B1 Human Telomere-10; SALSA MLPA kit P286-B1 Human Telomere-11 – MRC-Holland, Amsterdam) was carried out on DNA extracted from peripheral blood, following the manufacturer's protocols, to better delineate the boundaries of the rearrangements (data not shown). Taken together, the array-CGH and MLPA results permitted establishing that, in all subjects, the last genes deleted on chromosome 6q and duplicated on chromosome 12q were *DLL1* and *GLT1D1*, respectively, while the first genes not deleted on chromosome 6q and not duplicated on chromosome 12q were *C6ORF208* and *SLC15A4*, respectively. There are no other genes mapped between the first

Table 1. Genes involved in the deletion and duplication

Chromosome location	Gene symbol (OMIM)	Gene name	Human phenotype (OMIM)
Del 6q27	DLL1 (606582)	delta-like 1	no
Del 6q27	<i>FAM120B</i> (612266)	family with sequence similarity 120, member B	no
Del 6q27	<i>PSMB1</i> (602017)	proteasome subunit, beta-type 1	no
Del 6q27	<i>TBP</i> (600075)	TATA-box binding protein	spinocerebellar ataxia 17 (607136), Parkinson disease, susceptibility to (168600)
Del 6q27	<i>PDCD2</i> (600866)	programmed cell death 2	no
Dup 12q24.32	GLT1D1	glycosyltransferase 1 domain containing 1	no
Dup 12q24.32	<i>TMEM132D</i> (611257)	transmembrane protein 132D	no
Dup 12q24.33	<i>FZD10</i> (606147)	frizzled, <i>Drosophila</i> , homolog of 10 with frizzled family receptor 10	no
Dup12q24.33	<i>PIWIL1</i> (605571)	PIWI, <i>Drosophila</i> , homolog of with piwi-like RNA-mediated gene silencing 1	no
Dup12q24.33	<i>RIMBP2</i> (611602)	RIMS-binding protein 2	no
Dup12q24.33	<i>STX2</i> (132350)	epimorphin (syntaxin 2)	no
Dup12q24.33	<i>RAN</i> (601179)	ras-related nuclear protein	no
Dup12q24.33	<i>GPR133</i> (613639)	G protein-coupled receptor 133	no
Dup12q24.33	<i>SFSWAP</i> (601945)	splicing factor, arginine/serine-rich 8	no
Dup12q24.33	<i>MPP17</i> (602285)	matrix metalloproteinase 17	no
Dup12q24.33	<i>ULK1</i> (603168)	UNC51-like kinase 1	no
Dup12q24.33	<i>PUS1</i> (608109)	pseudourine synthase 1	mitochondrial myopathy and sideroblastic anemia 1 (600462)
Dup12q24.33	<i>EP400</i> (606265)	p400 SWI2/SNF2-related protein	no
Dup12q24.33	<i>SNORA49</i>	small nucleolar RNA, H/ACA box 49	no
Dup12q24.33	<i>EP400NL</i>	EP400 N-terminal like	no
Dup12q24.33	<i>DDX51</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 51	no
Dup12q24.33	<i>NOC4L</i> (612819)	nucleolar complex-associated protein 4, <i>Saccharomyces cerevisiae</i> , homolog of with nucleolar complex associated 4 homolog (S. cerevisiae)	no
Dup12q24.33	<i>GALNT9</i> (606251)	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetyl-galactosaminyl transferase 9	no
Dup12q24.33	<i>MUC8</i> (601932)	mucin 8, tracheobronchial	no
Dup12q24.33	<i>FBRSL1</i>	fibrosin-like 1	no
Dup12q24.33	<i>P2RX2</i> (600844)	purinergic receptor P2X, ligand-gated ion channel 2	no
Dup12q24.33	<i>POLE</i> (174762)	polymerase (DNA directed), epsilon	no
Dup12q24.33	<i>PXMP2</i>	peroxisomal membrane protein 2, 22 kDa	no
Dup12q24.33	<i>PGAM5</i>	phosphoglycerate mutase family member 5	no
Dup12q24.33	<i>ANKLE2</i>	ankyrin repeat and LEM domain containing 2	no
Dup12q24.33	<i>GOLGA3</i> (602581)	Golgi autoantigen, golgin subfamily A3	no
Dup12q24.33	<i>CHFR</i> (605209)	checkpoint protein with FHA and ring-finger domains	no
Dup12q24.33	<i>ZNF605</i>	zinc finger protein 605	no
Dup12q24.33	<i>ZNF26</i> (194537)	zinc finger protein 26 (KOX20)	no
Dup12q24.33	<i>ZNF84</i>	zinc finger protein 84	no
Dup12q24.33	<i>ZNF140</i> (604082)	zinc finger protein 140	no
Dup12q24.33	<i>ZNF10</i> (194538)	zinc finger protein 10 (KOX 1)	no
Dup12q24.33	<i>ZNF268</i> (604753)	zinc finger protein 268	no

The first deleted and duplicated genes are shown in bold type. The last column shows the associated human phenotype.

deleted or duplicated and the first normal gene. The complete list of the genes deleted and duplicated in our patients are shown in table 1.

Cytogenetics and FISH analyses performed using the subtelomere specific probes for the chromosomes 6 and 12 terminal long arms, disclosed the presence of an apparently balanced reciprocal 6/12 translocation [t(6;12)(q27;q24.33)] in the mother and normal karyotypes in the father and healthy brother.

Discussion

Chromosomal and genomic rearrangements are important findings when encountered in patients who show phenotypic overlap with, presumably, monogenic syndrome of still unknown etiology. In this study analyzing AIS patients, it is hypothetically expected to find X chromosome abnormalities even if the possibility remains

that AIS could be caused by a new mutation on an autosome with gender-limited expression.

To the best of our knowledge, there are no cases in the literature of patients with definitive AIS diagnosis and an autosomal genomic imbalance. The patient reported by Bursztejn et al. [2009], with suspected AIS and 1p36 deletion, does not show the chorioretinal lacunae which is a salient feature of the syndrome, being present in about 90–100% of cases [King et al., 1998; Aicardi, 2005]. Therefore, the authors concluded a possible phenocopy.

The female patient reported here, not only meets the AIS diagnostic criteria, but also has 2 male brothers with the same autosomal genomic imbalance, which show a clinical phenotype similar to each other, but completely different from that of the sister.

Various hypotheses can be proposed to explain this intrafamilial variability: (a) the dosage effect (gain and/or loss) of a gene, or gene subsets, acts in a sex-confined manner leading to AIS in the female and a different phenotype in males; (b) the deletion has uncovered a recessive allele only in the female or the recessiveness acts in a sex-confined manner; (c) the genomic imbalance generated an AIS phenocopy, and (d) the female patient harbors a single gene mutation, which is unknown (leading to AIS), and the genomic imbalance is merely a coincidence. The hypothesis of a recessive allele is in contrast with the inheritance model of AIS, while the hypothesis of a coincidence of 2 such rare conditions (genomic imbalance and single gene de novo mutation) is possible, even if statistically unlikely.

We did not find reports of patients with the same complex rearrangement involving chromosome 6 and 12 in the literature, so that it is not possible to compare our case with previous data. Moreover, patients with an exclusive deletion 6q or duplication of 12q are very rare, being in the majority of cases associated with a second rearrangement. The few patients reported with terminal deletion of 6q27 displayed facial dysmorphisms, intellectual disabili-

ties, autistic behavior, hirsutism, and general abnormalities of the vision (myopia), while those with similar 12q24.32 duplication showed facial dysmorphisms, intellectual disabilities, hydrocephalus, heart and kidney defects (Ecaruca database at <http://umcecaruca01.extern.umcn.nl:8080/ecaruca/ecaruca.jsp>; Decipher database at <http://decipher.sanger.ac.uk/>). The very few cases described are not comparable with our study at a molecular level, in order to state that these genomic regions are not associated with the AIS phenotype. Obviously, we cannot exclude that our complex and unique rearrangement results in a phenocopy; however, why such a wide phenotypic variability exists among female and males patients remains an unanswered question. In this study, we hypothesized that the dosage alteration of gene(s) in the 6q/12q regions have had a female-limited expression and that the same gene(s) are potential candidates for AIS (table 1).

In conclusion, even if genetic heterogeneity is possible in the AIS, and the 2 proposed inheritance models (X-linked dominant de novo and autosomal de novo with sex-limited expression) are not mutually excludable, we believe that the extensive molecular analysis of the genes depicted in table 1 could provide new insight into the still unknown etiology of the syndrome. Finally, our findings advise clinicians to perform genome-wide analysis of patients with clinical diagnosis of AIS, since the evidence of a genomic imbalance could change the patient and family management following the diagnosis drastically.

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