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Short clinical report

Constitutional 11q14-q22 chromosome deletion syndrome in a child with neuroblastoma MYCN single copy

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ABSTRACT

Constitutional 11q deletion is a chromosome imbalance possibly found in MCA/MR patients analyzed for chromosomal anomalies. Its role in determining the phenotype depends on extension and position of deleted region. Loss of heterozygosity of 11q (region 11q23) is also associated with neuroblastoma, the most frequent extra cranial cancer in children. It represents one of the most frequent cytogenetic abnormalities observed in the tumor of patients with high-risk disease even if germline deletion of 11q in neuroblastoma is rare. Hereby, we describe a 18 months old girl presenting with trigonocephaly and dysmorphic facial features, including hypotelorism, broad depressed nasal bridge, micrognathia, synophrys, epicanthal folds, and with a stage 4 neuroblastoma without *MYCN* amplification, carrying a germline 11q deletion (11q14.1-q22.3), outside from Jacobsen syndrome and from neuroblastoma 11q critical regions. The role of 11q deletion in determining the clinical phenotype and its association with neuroblastoma development in the patient are discussed.

1. Introduction

Constitutional 11q deletion is a chromosome imbalance possibly found in Multiple Congenital Anomaly/Mental Retardation (MCA/MR) patients analyzed for chromosomal anomalies. Its role in determining the phenotype depends on extension and position of the deleted segment. Deletions of region 11q14.1-q14.2 seem to determine developmental delay and/or mental retardation associated with minor dysmorphic features, as found in patients reported by Kariminejad et al. [1] and Wincent et al. [2], showing deletions 11q14.1-q22.1 and 11q13.4-q14.3, respectively. On the other hand, deletions involving region 11q14.3-q22.1 or 11q14.3-q21.1 could have no clinical effects, as reported by Goumy et al. [3] and Li et al. [4], respectively.

Neuroblastoma (NB) is the most frequent extra cranial cancer in children [5–10]. The primary tumor is usually located in the adrenal medulla, abdominal or thoracic sympathetic ganglia [5,9]. The prognosis of NB is mainly influenced by patient's age, disease

Abbreviations: ARSH, ARSE, ARSD, arilsulphatases genes; aCGH, array comparative genomic hybridization; CR, critical region; DECIPHER, database of chromosomal imbalance and phenotype in humans using ensembl resources; DGV, database of genomic variants; *DCN1*, defective in cullin neddylation 1; *DCUN1D5*, domain containing 5; ECARUCA, European cytogeneticists association register of unbalanced chromosome aberrations; FISH, fluorescence in situ hybridization; *GYG2*, glycogenin 2 gene; INSS, International NB Staging System; ^{123}MIBG, 123-iodine metaidobenzoguadinyl scintigrafy; *MMPs*, matrix metalloproteinase genes; *MMP13*, matrix metalloproteinase 13; MCA/MR, multiple congenital anomaly/mental retardation; MLPA, multiplex ligation-dependent probe amplification; NB, neuroblastoma; PAR1, pseudo autosomal region 1; SLE, systemic lupus erythematosus; CNVs, structural copy number variations.

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extension, tumor histology and *MYCN* oncogene status [7,8]. Moreover, nonrandom structural copy number variations (CNVs) have been found in NB and recently used as prognostic markers to evaluate the patient's risk [11,12]. Chromosome 11q deletion (loss of heterozygosity of region 11q23) is one of the most frequent abnormalities observed in the tumor of patients with high-risk disease [13] and the presence of putative NB genes located within this region has been proposed [7,8,12,13]. Usually tumors with chromosome 11q deletion do not have *MYCN* amplification [11].

Germline deletion of 11q in NB is rare. Three patients with NB and constitutional chromosome 11q23 deletion have been observed until now. In 1995, Koiffmann et al. [14] described a patient presenting with multiple congenital anomalies, mental retardation, and NB: his constitutional karyotype unraveled an 11q23-qter deletion. More recently, Mosse et al. [15] observed a 3-month-old patient with *MYCN* not amplified NB that showed a 40.2 Mb wide deletion of chromosome 11q14.1-q23.3 (79,319,763–119,531,496) both in blood and tumor cells. The patient also showed facial dysmorphisms, including flat nasal bridge, bilateral parietal prominence and hypertelorism. Other findings were a coloboma of the right iris, haemangiomas of the neck and chest, and hypotonia. An echocardiogram showed a double outlet right ventricle, a subaortic large ventricular septal defect, and subpulmonic stenosis. Other imaging studies also showed an absent corpus callosum with cortical atrophy, a horseshoe kidney with hydronephrosis, and a left adrenal tumor (NB). Satgé et al. [16] reported on a boy with moderate general retardation of development, short stature, microcephaly and a slightly lower ear set with a constitutional karyotype 46,XY,del(11)(q14-q23), dn.. At the age of 6 years 9 months he developed an intra-abdominal $\sim 7 \times 8$ cm neuroblastoma, stage 3. Cytogenetic analysis of a tumor biopsy revealed no *MYCN* amplification but unbalanced gain of 17q. He developed a relapse in the mediastinum 15 months after the initial diagnosis and a further local relapse in the abdomen was observed 4 years after the initial diagnosis.

Hereby, we describe a 18 months old girl with germline 11q deletion, with clinical phenotype of trigonocephaly, facial dysmorphisms and MR, and with a stage 4 NB without *MYCN* amplification. The association between chromosome 11q deletion and the clinical phenotype is discussed and compared with literature data.

2. Methods of detection

2.1. Cytogenetics

Metaphase chromosomes were prepared from lymphocyte cultures of peripheral blood and tumor cells according to routine procedures using GTG-banding.

2.2. Array comparative genomic hybridization (aCGH)

For a refined analysis of the derivative chromosome, two microarray experiments were performed on patient's and her parents using the Human CytoSNP-12 Bead Chip (Illumina) for DNA of tumor cells and NimbleGen CGX-6 PKI (Roche NimbleGen, Inc., Madison, WI, USA) for DNA of peripheral blood. The microarrays were performed according to manufacturer's instructions. The SNP-array and Oligo Array data were analyzed with KaryoStudio software (Illumina) and Genoglyphix® software (Signature Genomics Spokane, WA), respectively. Chromosome positions were given using GRCh/hg19 (UCSC Genome Browser, http://genome.ucsc.edu, Feb. 2009 release) and Hg18 Genome Assembly (NCBI 36/hg 18), respectively.

2.3. Multiplex ligation-dependent probe amplification (MPLA)

DNA was extracted from tumor cells according to standard procedures. (MLPA analysis was performed using a probe-set for MYC gene (SALSA MLPA KIT P014-A1 Chromosome 8, MRC-Holland b.v., Amsterdam, The Netherlands). The analysis was performed with Coffalyser. Net software (MRC-Holland b.v., Amsterdam, The Netherlands) according to the manufacturer's recommendations.

2.4. Fluorescence in situ hybridization (FISH)

FISH was performed by using BAC probes RP11-796A5 (chr11: 88,548,676–88,696,036,Hg18-) and RP11-770J1 (chr11: 117,750,488–117,944,806,Hg18) on peripheral blood lymphocytes, following standard procedures. A FISH was performed on tumor cell using MYCN probe (Kreatech, Amsterdam, The Netherlands), according to the manufacturer's recommendations.

3. Results

Chromosome analysis at 550 bands resolution from the peripheral blood cells of the patient displayed a female karyotype with an interstitial deletion of chromosome 11q (46,XX, del(11)(q13-q21) (Fig. 1)). Maternal and paternal karyotypes were normal. The FISH study on peripheral blood lymphocytes revealed deletion of BAC RP11-796A5 (data not shown). A more detailed study of chromosome 11q region using aCGH was performed on patient's and her parents' DNAs.

The NimbleGen CGX-6 PKI analysis performed on DNA of peripheral blood revealed an approximately 21.41 Mb interstitial deletion at 11q14.1-q22.3 (81,564,363–102,970,568) and a 318.52 kb interstitial duplication at Xp22.33 (2,635,953–2,954,474), both *de novo*. Final result was: arr Xp22.33 (2,635,953–2,954,474)×3 dn, 11q14.1q22.3(81,564,363–102,970,568)×1 dn (Fig. 2).

The Human CytoSNP-12 Bead Chip array performed on tumor DNA, revealed the identical 11q deletion to that found in constitutional DNA, and, in addition, monosomy of chromosomes 1p and 4p, gain of chromosome 7q and 17 (Fig. 3). FISH analysis performed on tumor cells revealed no MYCN amplification and 1p36 imbalance. MLPA confirmed FISH results (data not shown).

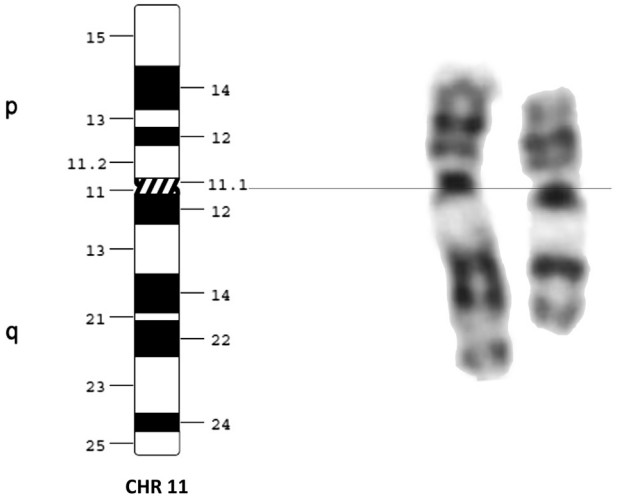

Fig. 1. Partial karyotype of the patient showing deletion 11q13-q21.

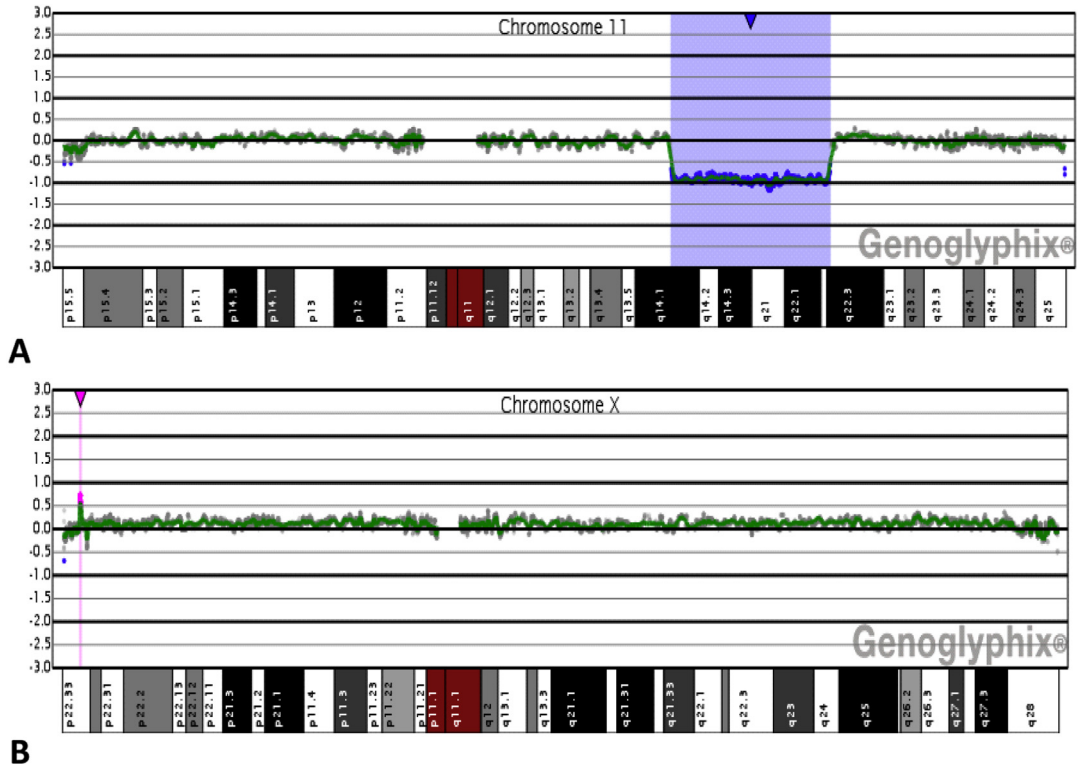

Fig. 2. Array-CGH analysis performed on patient DNA from peripheral blood lymphocytes. Array-CGH analysis showing an approximately 21.41 Mb interstitial deletion at 11q14.1-11q22.3 (81564363–102970568) (A) and a 318.52 kb interstitial duplication at Xp22.33 (2635953–2954474) (B).

4. Clinical description

The proband was the single daughter of apparently healthy parents. She was born at 35 week's gestational age. After birth, she was admitted to the Intensive Care Unit because of severe respiratory distress. Her birth weight was 2350 g, length 49 cm and head circumference within normal range. Clinical examination showed dysmorphic features including hypotelorism, broad depressed

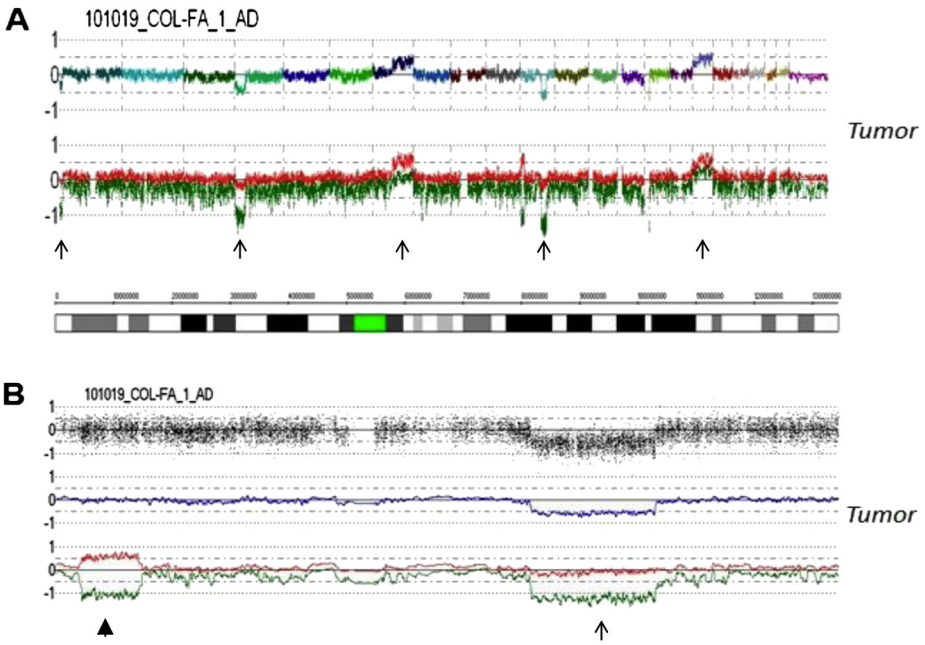

Fig. 3. SNPs analysis of tumor from the patient. SNPs array genomic profile for all chromosomal regions in tumor tissue is reported. A. The multicolored line shows the total copy number for the different chromosome regions and the red and green lines show the strongest and weakest allele intensity respectively. In the tumor the following alterations (indicated by arrows) have been observed: del 1p (pter-12.1 Mb); del 4p (4.1–33.2 Mb); dup 7q (76.8 Mb-qter); del 11q (84.4–103.3 Mb); dup 17. B. A copy neutral loss of heterozygosity of the region at 4.3–14.3 Mb of chromosome 11 is indicated by arrowhead; enlargement of chromosome 11 shows the same imbalances shared by the tumor and the normal tissue (arrow).

Fig. 4. Patient at 5 years. Note residual slight trigonocephalic form of the head, hypotelorism, prominent nasal bridge, mild prognathism.

nasal bridge, micrognathia, synophrys, epicanthal folds, and furthermore trigonocephaly that required surgical correction at 9 months of life. Furthermore, the patient showed postural and motor function disabilities with low-moderate psychomotor and speech development delay. At age of 14 months, an abdominal mass associated with leg pain was noted. Imaging studies showed a right suprarenal heterogeneous solid lesion with calcifications, $40 \times 59 \times 66$ mm in size, displacing the ipsilateral kidney. The aorta and inferior vena cava were displaced anteriorly. The portal vein, celiac axis, mesenteric and the right renal vessels were surrounded by the lesion. Furthermore radiologic imaging revealed liver, splenic and lymph nodal metastatic involvement. Laboratory results were as follows: white blood cell count 3130/mL, red blood cell count 3.25×10^6/mL, hemoglobin 8.8 g/dL, platelets 127×10^3/mL, lactate dehydrogenase 1125 IU/L, neuron-specific enolase 155.6 ng/mL, α-fetoprotein 5.4 UI/L, β-chorionic gonadotropin 7.75 ng/mL. Urinalysis revealed elevated levels of vanillyl mandelic acid (168.57 mg/mg creatinin) and homovanillic acid (248.95 mg/mg creatinin). A 123-iodine metaidobenzoguadinyl scintigrafy (^{123}MIBG) disclosed diffuse uptake in the area of primary tumor and the whole bone marrow. Histology of the primary tumor showed undifferentiated NB (Schwannian-stroma poor) with intermediate mitosis-karyorrhexis index. Two bone marrow aspirates were heavily infiltrated by tumor cells. A stage 4 NB without *MYCN* amplification according to International NB Staging System (INSS) [9] was diagnosed. The patient was treated according to 99.3 AIEOP NB Infant protocol (unresectable NB, stage 4, without *MYCN* amplification) consisting in several courses of chemotherapy (carboplatin, etoposide, cyclophosphamide, vincristin and doxorubicin) and high dose therapy with stem cell rescue. At the last follow-up at 3 years from the onset of the disease, the patient is in continuous complete remission. Her clinical examination at 5 years evidenced (Fig. 4): weight 11 Kg (below 5th centile), height 87.5 cm (below 5th centile), occipitofrontal circumference 48.3 cm (25th–50th centile); facies changed from first observation and was characterized by:asymmetric skull with slight trigonocephaly and prominence of right temporo-parietal suture, hypotelorism, faint synophrys, prominent nasal bridge, mild prognathism, and outstanding pinnae; hands and feet showed long fingers with a mild clinodactyly, palmar and plantar ridges were poorly represented. As to psychomotor development, she walked by herself, and she had an incomplete vocabulary, using a limited number of words structured

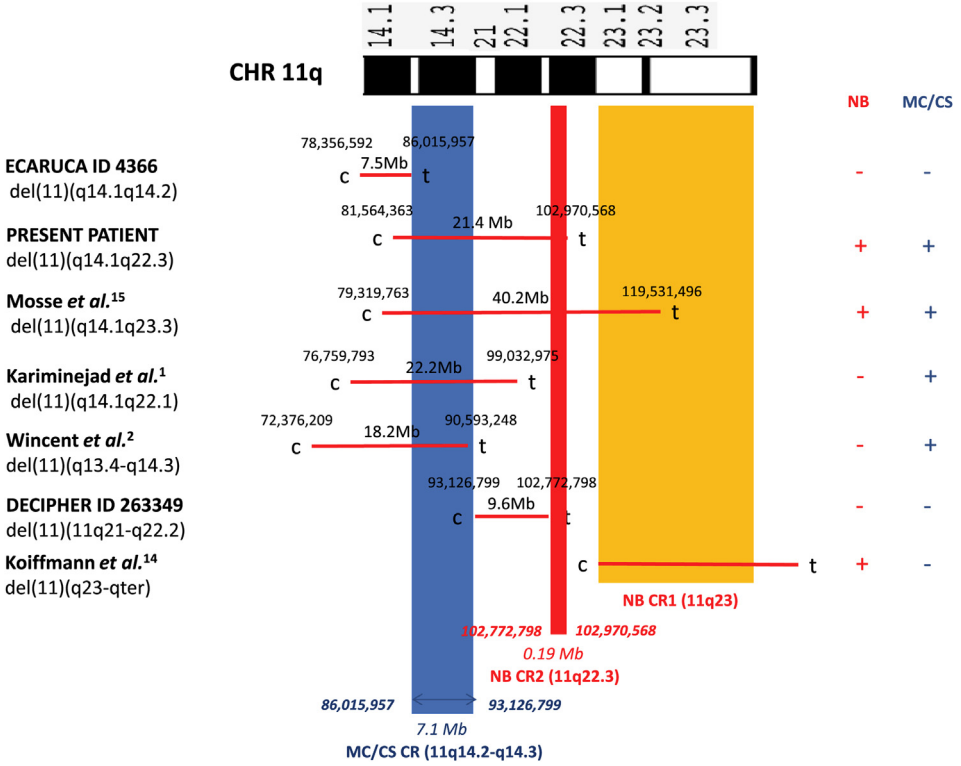

Fig. 5. Minimal critical regions for cranial development and neuroblastoma on 11q. Cytogenetic findings of most significant 11q deleted patients are correlated to the presence or absence of microcephaly (MC)/craniostenosis (CS) and neuroblastoma (NB). A supposed minimal critical region (CR) for MC/CS is narrowed between 86,015,957 bp and 93,126,799 bp positions, at chromosome region 11q14.2-q21; on the other hand, a hypothetical minimal critical region 2 for NB (NB CR2) is narrowed between 102,772,798 bp and 102,970,568 bp position, at chromosome region 11q22.3. More distally, at chromosome region 11q23, the well-known NB critical region (here indicated as NB CR1). Red lines indicate deleted regions, with superscripted extension of deletion and genomic boundaries; t, telomere, c, centromere.

Table 1
Patients with segmental deletion 11q14.1 → q23.3 and their clinical phenotype. Patients from literature and present case are listed on the base of chromosome 11q deleted region. Method of detection of chromosome anomaly, inheritance, extension and position of deletion, and patients' clinical phenotype are indicated.

Patient gender age at 1st observation	Deleted 11q region	Method of detection	Inheritance	Extension and position of deletion	Clinical phenotype							
					Microcephaly/ craniostenosis	Facial dysmorphisms	Cardiac anomalies	Uro-genital anomalies	Hand and foot abnormalities	MR/DD/ Hypotonia	Other anomalies	NB
DECIPHER ID 267087 Male 3 years	del(11) (q14.1)	aCGH	Inherited from normal parent	0.13 Mb 82,987,709 → 83,119,360	−					±		−
ECARUCA ID 4366 Male 6 years	del(11) (q14.1-q14.2)	aCGH	*De novo*	7.5 Mb 78,356,592 → 86,015,957	−	Small ears, ptosis of eyelids, palpebral fissures slant up, epicanthic folds, large nose with wide nasal bridge, micrognathia, simple philtrum,		Overriding scrotum, cryptorchidism	Small hands with brachydactyly, clinodactyly and short phalanges, dysplastic hip, flat arches of feet	±		−
ECARUCA ID 3945 Male 2.5 years	del(11) (q14.1-q14.2)	Standard cytogenetics	NS	NA	−	Broad face, coarse hair, wide forehead, large and uplift ear lobule, palpebral fissures slant up, fullness of peri-orbital region, flared nares, full cheeks, thin upper lip			Abnormal dermatoglyphic patterns	±		−
Wincent et al. [2] Male 3.5 years	del(11) (q13.4-q14.3)	aCGH	*De novo*	18.2 Mb 72,376,209 → 90,593,248	±	Round face with a short middle face and bilateral ptosis, epicanthus mild strabismus, broad nasal base, thin lips, large ears, submucous cleft palate		Cryptorchidism and bilateral inguinal hernia		±	Generalized seizures, hyperactive behavior sleeping disorder	−
Kariminejad et al. [1] Male 1.5 years	del(11) (q14.1-q22.1)	aCGH	*De novo*	22.2 Mb 76,759,793 → 99,032,975	±	Round face, broad forehead, hypotelorism, short palpebral fissures, short nose, small mouth, short neck, salt and pepper pigmentary alteration at fundus oculi examination			Broad thumbs, and clinodactyly of fifth fingers	±	Infantile spasms	−
Wakazono et al. [17] Female 12 months	del(11) (q14.1-q22.3)	FISH	*De novo*	NS	+/−	Dolichocephaly, telecanthus, bilateral blepharoptosis, flat nasal bridge, anteverted nares, high-arched palate, carp-shaped mouth, micro-retrognathia, and low-set and posteriorly rotated ears				±	Transient neonatal hypocalcemia	−
Present case Female 1 month	del(11) (q14.1-q22.3)	aCGH	*De novo*	21.41 Mb 81,564,363 → 102,970,568	**±**	Asymmetric skull with trigonocephaly and prominence of right temporo-parietal suture, hypotelorism, synophrys, prominent nasal bridge, mild prognatism, outstanding pinnae			Hands and feet with long fingers with a mild clinodactyly, palmar and plantar ridges poorly represented	±		**±**

Mosse et al. [15] Male 3 months	del(11) (q14.1-q23.3)	aCGH	Not inherited from mother (father's DNA not available)	40.2 Mb 79,319,763 → 119,531,496	±	Broad forehead, flat nasal-bridge, bilateral parietal prominence, and hypertelorism, coloboma of the right, iris, haemangiomas of the neck and chest	Double outlet right ventricle, VSD, subpulmonic stenosis	Horseshoe kidney with hydronephrosis	NS	Absent corpus callosum with cortical atrophy	±
Koiffmann et al. [14] Male NS	del(11) (q23-qter)	Standard cytogenetics	*De novo*	NA	-				±		±
Li et al. [4] Male NS	del(11) (q14.3-q21.1)	FISH	Inherited from normal parent	3.6 Mb	–	–	–	–	–	–	–
Goumy et al. [3] Female 25 years	del(11) (q14.3-q22.1)	FISH and CGH	Inherited from normal mother	8.5–16 Mb	–	–	–	–	–	–	–
ECARUCA ID 4129 Male 5 years	del(11) (q14.3-q22.1)	Standard cytogenetics	*De novo*	NA	–	High frontal hairline, micrognathia, thin upper lip			±	Ig deficiency	–
DECIPHER ID 263349 Male 2 years	del(11) (11q21-q22.2)	aCGH	*De novo*	9.65 Mb 93,126,799 → 102,772,798	–	Epicanthus		Joint laxity	±	Feeding problems in infants	–

MC, microcephaly, PCS, premature closure of sutures, CS, craniostenosis, NB, neuroblastoma.

in simple phrases, but when asked, she could perform simple and more complex orders, thus demonstrating a sufficient level of comprehension.

5. Discussion

Germline 11q deletion is variously associated with different clinical phenotypes, according to position and extension of the chromosome deletion [1–4].

Acquired chromosome 11q loss has been described in about 40–50% of *MYCN* single copy stage 4 NB [10]. The patient observed by us showed a germline 11q deletion (11q14.1-q22.3), outside from Jacobsen syndrome (11q23.3-qter) and from neuroblastoma 11q critical regions (11q23) (see Fig. 5). This breakpoint was coincident with the interstitial deletion encompassing the 11q14.1-q22.3 region in the patient reported in 1992 by Wakazono et al. [17]. They reported on a 12-month-old female infant with developmental delay, growth retardation, and dysmorphic features including dolichocephaly, telecanthus, ptosis, flat nasal bridge, anteverted nares, high-arched palate, carp-shaped mouth, micro-retrognathia, and low-set and posteriorly rotated ears. Her karyotype was: 46,XX,del(11)(q14.1-q22.3). The deletion was defined first by traditional cytogenetics and subsequently by FISH. Both our and this patients shared many clinical findings, including mental and growth retardation, hypotonia, some facial features (namely flat nasal bridge, micrognathia, malformed ears) whereas no presence of neoplasm was noticed in the patient reported by Wakazono et al. [17] (even if she was only 12 month-old at moment of description). However, alert on neoplasm onset was suggested by authors, because a tumor suppressor gene had been assigned to 11q13-q23 region. Some other cases are reported in the literature with 11q deletion overlapping the present case that is summarized in Table 1. Analyzing those cases, it is supposable that deletion of segment 11q14.3-q22.1 has probably no clinical effect, whereas proximal deletions at 11q14.1-q14.3 are possibly associated with developmental delay and/or mental retardation and with minor dysmorphic features, as already suggested by Kariminejad et al. [1] (see Table 1). On the other hand, they also outlined that probably other factors within the genome (including other cytogenetic imbalances) could influence the function of the genes in this region, thus determining the variability in phenotypes of patients with the same deletions, as also reported by Sparkes et al. [18]; they eventually encouraged description of further molecularly characterized patients.

Analyzing data from different reports, it seems that a hypothetic region for microcephaly/craniostenosis could be mapped at 11q14.2-q14.3. In fact, it appears that cases with a more proximal deletion, at 11q14.1, lack this finding, whereas cases with deletion involving band 11q14.2 present with microcephaly/craniostenosis: the present case, with deletion 11q14.1-q22.3 (81,564,363–102,970,568), the case from Mosse et al. [15], with deletion 11q14.1-q23.3 (79,319,763–119,531,496), the case from Kariminejad et al. [1], with deletion 11q14.1-q22.1 (76,759,793–99,032,975), and other cases from Table 1 of the same report with deletion of segment 11q14.2, all present with microcephaly/craniostenosis. Also other cases seem to support this hypothesis. In 2010, Wincent et al. [2] reported a boy with a deletion 11q13.4-q14.3, fine mapped by aCGH, revealing a 18.2 Mb deletion (72,376,209–90,593,248). His main clinical features included microcephaly. A ECARUCA case (ID 4366) reported with deletion 11q14.1-q14.2 (see also Table 1 from Kariminejad et al. [1]) was characterized by aCGH, and a 7.5 Mb loss was found (46,XY.arr cgh 11q14.1q14.2[RP11-7H7 → RP11-157B22]×1, deletion extending at least from 78,356,592 to 86,015,957, according to UCSC Genome Bioinformatics Site data). He lacked microcephaly/craniostenosis. A further

case with isolated deletion 11q14.1 (82,987,709–83,119,360) described in Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER) (ID 267087) showed mental retardation/developmental delay, lacking microcephaly; a second DECIPHER patient (ID 263349) with a larger more distal deletion at 11q21-q22.2 (93,126,799–102,772,798), *de novo*, also lacked microcephaly.

In conclusion, a possible role of a narrowed critical region at 11q14.2-q14.3 (86,015,957–93,126,799) influencing cranial development is supposable (see Table 1 and Fig. 5), even if other factors certainly influence the development of this non-specific clinical finding.

Since chromosome 11q region is candidate to contain NB suppressor genes, tumor bearing constitutional 11q deletions may give information about genes involved in NB development. To our knowledge, present patient is the first case of NB in which the germline 11q deletion is more proximal than usually found deleted in NB, at 11q23 (indicated as neuroblastoma critical region 1, NB CR1, in Fig. 6). In 1995, Koiffmann et al. [14] described a patient presenting with multiple congenital anomalies, mental retardation, and NB: his constitutional karyotype unraveled an 11q23-qter deletion, just overlapping the distal part of 11q deletion of present patient, and including NB CR1 at 11q23. More recently, Mosse et al. [15] observed a 3-months old patient with MYCN not amplified NB that showed loss of chromosome 11q14.1-q23.3 (79,319,763–119,531,496) both in blood and tumor cells. The patient also showed facial dysmorphism, flat nasal bridge, bilateral parietal prominence and hypertelorism. This patient partially overlaps clinical features of our patient and probably his dysmorphic features are due to the chromosome imbalance; also cytogenetic findings overlap that found in our patient, but his deletion extends more distally, including band 11q23.3 (segment 102,970,568–119,531,496), which includes the segment probably responsible for NB within chromosome region 11q23.3 (position 118,469,799–118,471,369), where a candidate gene (*H2AFX*) resides [12]. Searching DECIPHER database, we were able to find a 2 year-old male patient (ID 263349) presenting with hypotonia, developmental delay, feeding problems, joint laxity, epicanthic folds, with deletion 11q21-q22.2 (93,126,799–102,772,798), *de novo*, totally included within deletion of present patient, but lacking NB. Also the patient described by Kariminejad et al. [1], a 1.5 year-old boy, affected by multiple facial and hand dysmorphisms, mental retardation and infantile spasms, presented a 22.2. Mb deletion at 11q14.1-q22.1 (76,759,793–99,032,975) partially overlapping that here described, and he also lacked NB. However, both these 2 last cases had less than 2 years of age when described, so a possible onset of NB later in time cannot be excluded. Taking into account these observations, if further cases presenting with NB and a deletion including 11q22.3 region (and lacking deletion of 11q23) will be described, a second minimal critical region for NB could be considered at 11q22.3 (indicated as NB CR2 in Fig. 6), possibly responsible for NB in present patient. This region would contain genes regulating expression of other NB genes, responsible, when deleted, for NB development. On the other hand, it should be taken into account also that other factors other than 11q deletion could contribute in developing NB in our patient.

Analyzing genes contained within region 11q22.2-q22.3, at least 20 OMIM characterized genes are scored. Narrowing region between position 102,772,798 and 102,970,568, 2 known genes are included, namely matrix metalloproteinase 13 (*MMP13*), and defective in cullin neddylation 1 (*DCN*1), domain containing 5 (*DCUN1D5*). The first gene encodes a matrix metalloproteinase genes (*MMPs*) member, responsible, when mutated, of some forms of chondrodysplasias (namely Metaphyseal anadysplasia type 1, and Spondyloepimetaphyseal dysplasia, Missouri type, OMIM 602111), with autosomal dominant pattern, due to a gain of function mechanism. Effect of heterozygote deletion is not known (homozygous deletion determines a severe form of the disease), but it is not probable an involvement in tumor development. The second gene, *DCUN1D5*, has been recently involved in squamous cell carcinomas of mucosal origin, including those of the lung, head and neck, esophagus, and cervix, found up-regulated in these tumor types [19,20]. A possible role in repair of damaged DNA has been also hypothesized, but the exact mechanism by which it could regulate tumor development is not known. Moreover, effect of its deletion is still to demonstrate. In conclusion, other mechanisms involving above mentioned genes or other unknown genes within this region maybe will explain mechanisms in predisposing to NB development in 11q22.3 deleted patients.

A second possibility to explain such a variability among patients sharing a very similar 11q deletion is to search for other factors that possibly determine this difference. By aCGH analysis, in addition to 11q deletion, we unraveled a *de novo* 318.52 kb interstitial duplication at Xp22.33 (2,635,953–2,954,474) in our patient. The duplication partially overlaps the pseudo autosomal region 1 (PAR1), and includes arylsulphatases genes (*ARSH*, *ARSE*, *ARSD*) and glycogenin 2 (*GYG2*) gene, outside from PAR1 region, Xg blood group gene, spanning PAR1 boundary, and centromeric portion of CD99 molecule gene (also known as *MIC2*), within PAR1 region.

The role of microduplication of Xp22.33 region, within PAR1 region, in determining a clinical phenotype is still to establish. First, we searched for online databases for overlapping Xp22.33 cytogenetic imbalances, namely DECIPHER database, Database of Genomic Variants (DGV), Copy Number Variation database, ISCA Consortium database. We were able to unravel 3 male patients in DECIPHER database: first patient (ID 250733) presented with a complex rearrangement showing a 2.62 Mb wide duplication of

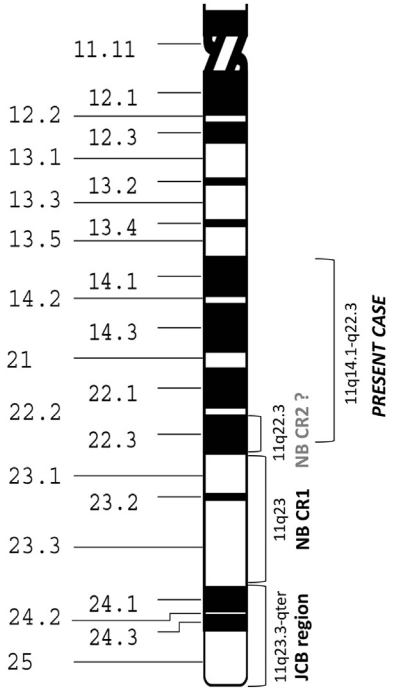

Fig. 6. Ideogram of chromosome 11 showing the deletion of present patient and Jacobsen syndrome and neuroblastoma critical regions. In the ideogram the chromosome deleted region observed both in constitutional and tumor cells of the patient is compared with the classical deletions described in Jacobsen syndrome (JCB) at 11q23.3-qter and in neuroblastoma critical region (NB CR1) at 11q23. The deletion observed in the patient is not overlapping neither JBS nor NB CR1, but include a hypothetical second neuroblastoma critical region (NB CR2) at 11q22.3.

Xp22.33 (80,335–2,703,633), a 3.28 Mb duplication of 4q25 region, and a 170 Kb deletion of chromosome 11q25, distal to deleted region of our patient; his phenotype was of mental retardation, oral clefting and syndactyly of fingers and toes; second patient (ID 808) had a 333 kb Xp22.33 interstitial duplication (2,779,811–3,112,894), overlapping present patient's duplication for about 174 kb; reported clinical phenotype is of mental retardation and ears anomaly, without further details; third patient (ID 256121) has a 460 kb interstitial Xp22.33 duplication (2,476,909–2,933,475) overlapping our patient's duplication for 297 kb, and presented with cognitive and behavioral problems, and teeth and skin pigment abnormalities; he inherited duplication by a normal parent.

On the other hand, analysis of DGV database showed that duplications partially overlapping the one found in our patient (2,635,953–2,954,474) often represents normal variants. In particular, variation 8293 is a duplication without clinical effect that quite completely overlaps that of our patient, extending from 2,551,655 to 3,186,492. Also some cases from ISCA Consortium database (namely nssv581395, nssv581396, nssv581397, nssv582070, nssv582150, nssv1415393, nssv1495630, nssv584835, nssv584989) showed duplication in Xp overlapping or included within that found in our patient. All these cases are reported with "developmental delay and additional significant developmental and morphological phenotypes", but most of them are considered of "uncertain value" or "benign rearrangements". However, some considerations are possible. First, Xp duplication found in the present patient appears to be *de novo* and its size is >200 kb: this is a threshold considered acceptable for detecting clinically relevant microduplications in routine diagnostics, and considered suggestive for clinical relevance [20]. Second, 6 genes are located within the duplicated region found in our patient, namely *ARSH*, *ARSE*, *ARSD*, *GYG2*, *XG*, and *CD99*. Some of these genes (namely *ARSH*, *ARSE*, *ARSD*, *GYG2*, *XG*) are located outsides PAR1 region and are consequently under X-inactivation mechanism; thus a possible effect of their triplication on clinical phenotype is to be considered not probable in a female patient. Furthermore, to the best of our knowledge, no phenotypical effects of *ARS* and/or *GYG2* and/or *Xg* genes duplication are known (particularly, for genes *ARSD*, *GYG2* and *XG*, ISCA triplosensitivity score is classified as "under review"). On the other hand, *CD99* (OMIM no. 313470) (mapping at Xp22.33; 2,609,228–2,659,350, within PAR1 region) was found duplicated in some human tumors. *CD99* gene belongs to XG blood group system and encodes a 32-kd transmembrane protein expressed on all human tissues tested so far, with particularly high expression in cortical thymocytes and T cells and is involved in adhesion processes and apoptosis of T cells. High levels of the *CD99* have been found associated with Ewing's tumor and primitive neuroectodermal tumors, and it constitutes a useful positive marker for the Ewing's sarcoma family of tumors [21]. Even if many studies suggest that *CD99* is negative in NB and the presence of *CD99* positivity virtually excludes the diagnosis of NB, recently Kaur et al. [22] described the case of a 4-year-old boy presenting with a retroperitoneal mass identified as a small round cell tumor by fine needle aspiration and a final diagnosis of NB. Immunostaining for *CD99* done on the surgical biopsy specimen resulted strongly positive, and they conclude that *CD99* positivity in a small round cell tumor in appropriate clinical setting should not exclude the possibility of a NB. On the other hand, Chagnon et al. [23] described a triple copy and triplicated expression of *CD99* gene in a 19-year-old XX male with severe Systemic Lupus Erythematosus (SLE). On this base, a possible relationship between the partial triplication of *CD99* and of other genes within PAR1 region, and the development of SLE was hypothesized. Patient's phenotype was of primary hypogonadism, but neither developmental delay nor neoplasms are reported by authors. In this respect, a possible role of triple *CD99* and possibly other unknown triplicated genes within Xp22.33 region, in combination with 11q deletion, in developing NB cannot be ruled out in present patient.

Recently a "two-hit", or "second site", model has been proposed by Girirajan et al. [24], stating that the presence of a supplementary large copy-number variant in addition to the primary genetic lesion could determine clinical significance, even when inherited. In the paper, more significant CNV length is defined as >500 Kb and effects of X-chromosome CNVs are not analyzed. However, even if authors considered stronger effect of larger duplication (>500 Kb), the effect of shorter CNVs should be also taken into account. In this respect, it is conceivable that the 318.52 kb interstitial duplication at Xp22.33 (partially overlapping PAR1 region) found in present patient could have some minor influence on the clinical phenotype, thus differing our patient from other described cases with same 11q deletion.

In conclusion, we report on a 18 months old girl with a 21.41 Mb wide germline 11q14.1-q22.3 interstitial deletion and a 318.52 kb wide interstitial duplication at Xp22.33, with a clinical phenotype of developmental delay and dysmorphic features, associated with a stage 4 NB without *MYCN* amplification. We outline the possibility that some clinical features (i.e. craniostenosis) found in the patient described here could be narrowed to a specific chromosome region at 11q14. Furthermore, we hypothesize that NB development in the patient could be due to disruption of another NB critical region (that we called NB CR2) at 11q22.3, different from the well-known NB critical region at 11q23 (that we called NB CR1), containing some genes possibly deregulating expression of other NB genes. The duplication of a small segment on Xp22.33, in combination with 11q14.1-q22.3 deletion could also contribute to clinical phenotype and tumor development in the patient. Further clinical reports sharing chromosomal imbalances detected in our patient could contribute to unravel the exact role of these chromosomal rearrangements in developing NB and the other clinical findings of our patient.

Conflicting of interest

The authors have no conflicting financial interests.

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Web resources

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[27] The Database of Genomic Variants, http://projects.tcag.ca/variation.
[28] The Copy Number Variation database, http://cnv.chop.edu.
[29] ISCA Consortium database, www.iscaconsortium.org.