

# Chiari I malformation in a Waardenburg phenotype with multiple malformations and 1q21.1 microdeletion

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## Abstract

The authors report on a patient with Chiari I malformation associated to Waardenburg phenotype, multiple malformations, osteochondrodysplasia and microdeletion of 1q21.1 chromosome, of which they underline the rarity. The pathogenesis connected to the features of neural crest cells-derived structures with mesodermal-derived tissues, mainly in the facial and boundary region of malformed posterior cranial *fossa*, is discussed. The authors hypothesize that chromosomal microdeletion, acting directly or on contiguous gene(s) or by long range control of gene expression, have modified the function of some developmental genes, causing consequently the association of symptoms observed in the patient.

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## Introduction

The association of Chiari I Malformation<sup>1</sup>(CMI) to other multiple malformations may express the complexity of the pathogenesis of these abnormalities.<sup>2</sup>Here we report a case presenting CMI in a context of multiple malformations, whose pathogenesis may be attributed to 1q21.1 chromosome microdeletion<sup>3-5</sup>as well as to Neural Crest (NC) and mesectodermal and mesodermal tissue-derived<sup>6-8</sup> derangement.

## Materials and Methods

Haematological tests were carried out by common laboratory methods. Blood samples were obtained from the patient and DNA was extracted from lymphocytes by using standard methods.

Array-CGH analysis was performed with reagents from the array manufacturer (Agilent 244B Technologies, SantaClara, CA, USA), with medial resolution of 75kb.

In Situ Hybridization (FISH) was performed by BAC RP11-698N18 and RP11-293J20 probe, mapping on chromosome 1, band 1q21.1.

Molecular analysis of genes Sonic Hedgehog (*SHH*), *SIX3*, *TGIF*, and *ZIC2* was performed from genomic DNA extracted from peripheral blood. The amplification and direct sequencing of the proband of exons 1, 2, 3, and of intronic sequences flanking *SHH* gene, of exons 1, 2, and intronic sequences flanking *SIX3* gene, of exons 1, 2, 3, 4, and intronic sequences flanking *TGIF* gene, and of exons 1, 2, 3, and intronic sequences flanking *ZIC2* gene were carried out.

Magnetic Resonance Images (MRI) and Computed Tomography (CT) images were acquired by standard methods.

## Results

The *proposita*, now 30 years old, was born at term by cesarean procedure from non-consanguineous, apparently healthy parents; at birth she presented weight (1850 g), length (36.5 cm), and head circumference (27.5 cm) all below 3<sup>rd</sup> percentile, cranial and facial asymmetry, right cranial fronto-parietal bossing, right hemifacial microsomia, brachyplagiocephaly, dystopia canthorum, epicanthal folds, broad nasal base with high tip, blue iris on left eye, blue iris

with some dark spots on right eye, left exotropia, hyperplastic medial part of eyebrow.

The patient showed red-blond hairs, pale skin, smooth *philtrum*, bilaterally grossly formed ears, hypotrophic and hypotonic muscle, small hands with clinodactyly and camptodactyly of fifth fingers. On ophthalmoscopy, retina showed aspecific pigmentation.

CT scan showed craniosynostosis of coronal and lambdoideal sutures and a fluid-filled cavity occupying the fronto-temporal-occipital supratentorial spaces: semilobar holoprosencephaly was suspected.

Ultrasonography of liver, spleen, kidneys, ureters, bladder was normal. Routine hematological and serological tests, cariotype, EEG, banded chromosomes were normal. No murmurs were present on heart auscultation. The development of the patient was defective for height, weight, Occipito Frontal Circumference (OFC), and psychological development. At 2 years of age, height was 77 cm, weight 6.70 Kg (all below 3<sup>rd</sup> percentile). X-rays showed skull asymmetry, thin long bones, small vertebral bodies, *coxa vara*, thin fingers with hypoplastic terminal phalanges and cone-shaped epiphyses of some fingers, hypoplasia of second phalanges of fifth fingers bilaterally; the patient presented difficulties in pronation and extension of wrists. Hypoacusia was present and speech was absent. Ophthalmology showed scarce corioretinal pigment.

At 5 years of age, MRI showed cranial asymmetry with right parietal bossing; partial agenesis of *corpus callosum*, with presence of only *genu* and small proximal part of the *corpus*. A large telencephalic cyst communicating with the third ventricle and very dysmorphic lateral ventricles, mainly on left side, were evident; the cyst, developing in lateral and posterior left side, pushed forward the cerebral hemisphere on the left side (Figure 1). Interhemispheric scissure, *adhaesio interthalamica*, separated basal nuclei: *falx cerebri* and sinusoideal structures were present.

Posterior *fossa* was small, dysmorphic and asymmetric; *clivus* was hypoplastic, with reduced angle at *clivus* and spinal junction;



**Figure 1.** *Proposita* at 5 years of age. MRI lateral view. A large dorsal cyst extending to the vertex and the posterior side is present. The *corpus callosum* is hypoplastic (only *genu* is present). More than 5 mm descent of cerebellar tissue beyond the *foramen magnum* is evident (Chiari I Malformation).

cerebellar tonsils and *vermis* were herniated more than 5 mm in the foramen magnum (CMI), hindbrain was adherent to *clivus*, with thin and abnormal morphology (Figure 1).

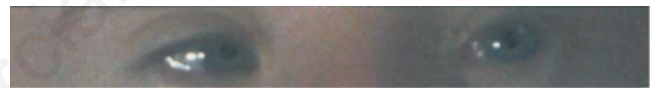
*Platybasia* with basilar invagination and *epistropheus* tooth scoliosis were present. The medullar cone ended at L1 level.

Subsequently, the development of *proposita* was always defective. At 10 years, the height was 113.0 cm (below 3<sup>rd</sup> centile), weight was 14.6 Kg (below 3<sup>rd</sup> centile); anyhow, bone age was that of 10 years; endocrine tests and starting of puberty were normal (Table 1).

Carpal X-rays, at 12 years, showed absent trapezoid and semi-lunar bones, ulnar *styloideusnucleus*, and deformed scaphoid.

Ear Nose and Throat (ENT) observation showed broad and high nasal root, *alae nasi* hypoplasia and nasal pyramid deviation, cupped external left ear with hypoplastic lobule. CT: on the left: hypoplastic *antrum*; middle cranial *fossa* more than normal developed; hypoplastic tympanic cavity, labyrinth and cochlea, stapes grossly formed; ENT observation showed grossly formed right external ear.

CT showed short and stenotic right external canal communicating with a cavity involving middle ear and *antrum*, without demonstrable ossicular chain; otitis media and cholesteatoma; hypoplastic labyrinth, semicircular canals, cochlea and internal ear *ductus*, hypoplasia of the right *caroticus* canal and of the third part of facial nerve. *Iridum* heterochromia was present (Figure 2). The strabismus was surgically corrected.



**Figure 2.** At twelve years of age. *Iridum* heterochromia with light blue left iris and dark blue right iris. Right strabismus.

**Table 1.** Hormonal and constitutional values of the patient at 10.5 years of age. Diagnostic conclusions: constitutional low height; normal pubertal start.

Height	113 cm (<3 <sup>rd</sup> centile; -4.3 SD/Tanner method)
Weight	14.4 Kg (<3 <sup>rd</sup> centile for height; -2.8 SD)
Bone age (years)	Carpal=9.5; Phalanges and metacarpal=10.5
Pubertal development	B (breast)=2; PH (pubis hair)=4; AH (axillary hair)=2
<b>Hormones in blood</b>	
ACTH	At 8 hrs=76 pg/mL. At 17 hrs=25 pg/mL
Cortisol	At 9 hrs=16 µg/dL. At 17 hrs=5.26µg/dL
Dehydroepiandrosterone (DHEAS)	89.8 µg/dL
Delta-4 androstenedione (D4 A)	163 ng/dL
FT3, FT4, TSH	3.88 pg/mL, 13.3 pg/mL, 1.31 µU/mL
IGF I	459.3 ng/mL
Prolactine	16.9 ng/mL
Basal LH	1.6 IU/LA. After LHRH, maximal rise=41.7 IU/L
Basal FSH	6.39 IU/LA. After LHRH, maximal rise=20.2 IU/L
Arginine and GHRH charge	Maximal rise of GH: 49 ng/mL
Pelvic sonography	Normal uterus with puberal morphology

Subsequently, the radiology showed slender bones of arms, bilateral ulnar deviation and low thumbs, camptodactyly of the five fingers, hypoplasia of the third phalanges of all fingers; irregular metaphyseal line of metacarpal bones; short and *valgi* halluces bilaterally, with gap between toes 1 and 2, short and in flexion the other toes, confirmed at 29 years (Figure 3); scoliosis with small and cuboid-shaped vertebrae. *Canthal* index  $W=1.961$  ( $NV<1.95$ )<sup>9</sup> mixed (transmissive and neurosensorial) hearing loss on right and transmissive on left side were demonstrated. After a right tympano-plastics, mild hypoacusia residuated.

At present, the patient is retarded (IQ=40), shows a normal language, works in a protective structure after completion (with assistance) of the second cycle of studies. Mutations of genes *SHH*, *SLX 3*, *TGIF*, *ZIC 2* were not shown.

Array – CGH: chr 1:142, 902, 432 ... 143, 236, 313x 1 microdeletion of chromosome 1:46 XX del (1) (q21.1) confirmed by FISH (these methods do not demonstrate balanced translocations). The *proposita* showed major features proposed by Waardenburg Consortium for the diagnosis of Waardenburg Syndrome (WS):<sup>9</sup> iris heterochromia/hypoplasia, hair depigmentation, sensory hearing loss, and minor features such as medial eyebrow flaring, broad and high nasal root, hypoplasia of *alae nasi*. In her family, the grandfather presented iris hypoplasia, the grandmother showed premature graying, an aunt (daughter of the grandmother) presented hypoplasia of *alae nasi* and neurosensorial hearing loss; another daughter showed depigmented skin and neurosensorial hearing loss. Parents are normal.

## Discussion

The pathogenetic mechanism of the complex malformation presented by our patient may be multiple.

The patient showed malformations affecting the neural tube (CMI, *corpus callosum* hypoplasia, telencephalic cyst), bones (face, axis and limbs), pigment defects (iris, skin, hairs and ear), and 1q21.1 chromosome microdeletion.

CMI is a syndrome characterized by descent of the cerebellar tonsils more than 5 mm, or at least 3-5 mm, beyond the foramen



**Figure 3.** Feet of the patient at 29 years of age. All toes are bilaterally short, with very short allux and 5<sup>th</sup> toe; proximal syndactyly of the 2<sup>nd</sup> and 3<sup>rd</sup> toes.

magnum,<sup>10</sup> connected to underdevelopment of the posterior *fossa*, and consequent to a defect of paraxial mesoderm and occipital somites, paraxial mesoderm-derived.<sup>10</sup>

In our patient, CMI is associated to mid-facial (fronto-nasal) abnormalities due to Neural Crest Cells (NCC) and NC-derived tissues,<sup>7</sup> and to occipital bone (mesoderm-derived) derangement.<sup>11</sup>

NCC originate shortly after gastrulation from the margins of neural tube,<sup>6,7</sup> having mesectoderm fate;<sup>7</sup> from these cells derive the osteocartilaginous structures of the face, membranous bones of the vault, anterior structures of skull base, metopic suture, melanoblasts of the skin, iris, hair, *stria vascularis cochlearis*, vascular structures, some neural and glial cells.<sup>6,7</sup> NCC possess integrin receptors for extracellular matrix molecules, and are associated to tenascin and some neurotrophic factors.<sup>7</sup>

The Central Nervous System (CNS) development is under NCC influence<sup>6</sup> which show a reciprocal relationship with FGF8.<sup>12</sup> NCC connected to prosencephalon<sup>8</sup> (prosencephalic NCC) give rise to fronto-nasal structures, induce prosencephalic ventral structures (*SHH*-dependent) and *corpus callosum*.<sup>13</sup> NCC are induced by the activity of many genes: dorsalizing genes, such as *BMP7* and *PAX3* expressed in embryonic NCC,<sup>8</sup> are the most important in the vertical axis of the neural tube.<sup>8</sup>

Ventralizing genes, such as *SHH* produced by notochord and floor plate, are expressed in the pre-chordal mesoderm, and inhibit NCC.<sup>8</sup> *PAX6*, *BMP4*, *BMP7*, *SHH* are primordial genes, present during gastrulation, which promote NCC;<sup>7,8</sup> they are diffuse in many embryo tissues.<sup>7</sup>

Subsequently, the prosencephalic NCC are regulated by *Otx* gene,<sup>8</sup> and the rhombencephalic NCC by *HOX* genes family.<sup>8</sup>

*SHH* induces the *PAX* family associated with prosencephalic structures. *PAX* gene family is expressed in early embryo, specifically in the developing CNS, and is critical for NCC migration and the consequent development of facial structures.<sup>8</sup>

For the development of structures derived from the first branchial arch (maxilla and mandible) is relevant the *SHH* pathway;<sup>12</sup> the Hedgehog (Hh) signal is necessary for growth of fronto-nasal structures (nasal cartilage, nasal bones, pre-maxilla, superior incisors),<sup>14</sup> but the formation of these structures is independent from Hh signal.<sup>14</sup> In frontal bone, NCC demarcate the position of the coronal suture and the boundary between NCC-derived and mesodermal-derived tissues.<sup>15</sup>

Alterations of prosencephalon development cause malformations of derived structures (ventral CNS structures, *corpus callosum*, midface).<sup>11,13</sup> In our case many features are referred to prosencephalic abnormalities in proliferating NCCs, and to migration or connection with other tissues of mesodermal or endodermal origin. In our patient and in her family the hereditary traits drive to the diagnosis of a genetic disease, associated with NCC derangement features, *i.e.* to a neurocristopathy.

The lateral displacement of the medial *canthi* of the eyes (*dystopia canthorum*) and tubular nose may be consequent to a defect of the *intercanthal* ligament.<sup>8</sup> The hypopigmentation of the iris is due to a defective number of melanocytes; the iris *heterochromia* is attributed to the involvement of genes of bilateral symmetry.<sup>8</sup> Hair hypopigmentation is consequent to a lack of melanocytes in dermal follicles of the head, and skin hypopigmentation is due to a lack of melanocytes derived from the trunk NCCs,<sup>7,8</sup> which differentiate in melanoblast. Sensory hearing loss with consequent damage of the cochlea is due to an abnormality of melanocytes in the *stria vascularis*.<sup>9</sup>

In our case, these features occur in many relatives, and depose for a neurocristopathy<sup>7,16</sup> with hereditary dominant traits.

The symptomatology of our patient shows resemblance with that of some zebrafish mutants.<sup>17</sup>

In humans, the pigmentary defect with hearing loss is attributed to a defect of the melanogenesis in NCC-derived cells.<sup>9</sup> In zebrafish, the colorless mutant, which encodes a gene orthologue of the HMC-box-containing transcription factor Sox 10, presents a defect in NCC-derived pigmentary cells, which affects the survival and the specification of NCC-related neurons and *glia*, without constant cranio-facial bone abnormalities. This mutant remembers the Waardenburg IV syndrome.<sup>17</sup>

For members of *PAX* family was reported a *PAX6* long-range regulation and aniridia.<sup>18</sup> In our case, the pigmentation defect of iris, skin and hearing loss were referred to a melanocyte NCC-derived abnormality.<sup>6,7</sup>

In humans, the most important genes in NCC development of midline and ventral structures are ventralizing genes, such as *SHH*, which induce formation of prosencephalon.<sup>13</sup>

In zebrafish, the midline phenotypes (*yot/gli2*)<sup>17</sup> mutants resemble holoprosencephaly, with defects of ventral brain (defective prosencephalon, *corpus callosum*) and mid-face, associated to *Shh* mutation;<sup>17</sup> in this condition, the condensation and differentiation of chondrocytes directly respond to *SHH* signaling; notably, the genes regulate mesenchymal cells condensation and differentiation.<sup>17</sup>

In our case we hypothesize that altered functional activity of *SHH*<sup>18</sup> may have been responsible of ventral CNS and midface abnormalities through an abnormal/enhancer transcriptional mechanism.<sup>18</sup>

The hand malformations of our patient could be also attributed to a long-range abnormal regulatory domain of *SHH*.<sup>18</sup>

In humans, some bone abnormalities conditions, due to abnormal cells proliferation and differentiation by Extracellular Matrix Component (ECM) mutation, are characterized by reduced limb length and premature fusion of cranial sutures (Shaetre-Chatzen, Crouzon, Muenke syndromes); these syndromes present *FGFR* mutations.<sup>17</sup>

In zebrafish, mutations such as hammerhead which induces lack of tissues rostral to eyes and lateral position of eyes, present endochondral bone abnormalities and reduced bone length, without bone shape abnormality, associated to premature fusion of cranial sutures.<sup>17</sup> The skull bone abnormalities of our patient may be due to mesenchymal abnormal genes, mainly dorsalizing, as *FGF* and others, which cause reduced bone length and premature fusion of cranial sutures.

On the basis of these reports, we could attribute, at least in part, the pigmentary cutaneous, hair and iris defect observed in our case to melanocyte NCC-derived defect, the midline face and CNS abnormalities to *SHH* pathway abnormalities, the small posterior *fossa* and the consequent CMI to mesodermal-derived bone defect, and the premature fusion of sutures to *FGFR* abnormalities.

These features of our patient drove the diagnosis of phenotype to an auditory-pigmentary neurocristopathy;<sup>7</sup> the presence of some of these symptoms in relatives in three successive generations of her family were consistent with the pattern of autosomal dominant inheritance of the syndrome with reduced penetrance (*i.e.* transmission through apparently unaffected relatives).<sup>10</sup> The familial data and the association of neurosensory hearing loss with hypopigmentation of skin, hair, iris and *dystopia canthorum* with tubular nose were typical features of the Waardenburg syndrome type 1-3<sup>9</sup> due, in most cases, to haploinsufficiency of *PAX3* gene mutation.<sup>16</sup>

WS is a typical example of patterning defect by disturbed NCC migration, rarely associated to CMI or spina bifida.<sup>16,19</sup>

CMI development may be primitive or secondary to other conditions<sup>2,10</sup> isolated or associated to myelomeningocele.<sup>10</sup>

Primitive CMI is strictly connected to posterior fossa volume<sup>10,20</sup> and to occipital bone development, a mesodermal-derived structure prone to malformations because the complexity of region development.<sup>21</sup>

Studies in avians demonstrated that occipital bone may be considered a transformed giant vertebra, in which the body is formed by basi-occipital bone, the arches by exoccipital (derived from the first somite) and supraoccipital (derived from paraxial mesoderm). The basioccipital originates from the fusion of 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> somites and minimally from the 1<sup>st</sup> somite.<sup>11</sup> Basioccipital, supraoccipital and exoccipital form a structure (the posterior fossa) in which the *cerebellum* is placed, up limited by *tentorium*, and limiting down the foramen magnum.<sup>11</sup>

In most cases of CMI the posterior *fossa* is small<sup>10</sup> if associated to basilar invagination characterized by shortening of the basioccipital bone.<sup>10</sup> In our case it was small, deformed and associated with basilar invagination. In CMI, a disproportion between the volume of the *fossa* and *cerebellum* occurs, with overcrowding and consequent uni- or bi-lateral tonsillar descent beyond the foramen magnum;<sup>10,20</sup> when the tonsillar descent is 3 mm, on radiology, diagnosis is indetermined; when it is at least 4 mm or 5mm;<sup>10</sup> the clinical diagnosis of CMI is given.<sup>10</sup>

Some authors<sup>20</sup> think that CMI results only from raised pressure in CNS, with tonsils descent beyond the foramen magnum due to the hydrostatic pressure,<sup>20</sup> and that the differentiation between Chiari I and Chiari II Malformation (CMII) on anatomical ground should be arbitrary, because many intermediate cases are reported.<sup>20</sup> Marín-Padilla<sup>22</sup> considers the CMI a cephalic axial skeletal-neural dysraphic disorder consequent to a paraxial mesoderm cellular-neural insufficiency or to a primary lesion of the somites. For other authors CMI is a condition frequently associated to *status dysraphicus* (spina bifida, myelomeningocele)<sup>13</sup> due to a stop in raphe formation and to abnormalities in the primitive central canal (neurocele), frequently associated to *cheiloschisis* and facial abnormalities.<sup>13</sup>

CMI should be considered the expression of insufficient mesoderm cells proliferation and connected to basilar invagination and platybasia,<sup>10,13</sup> conditions in which the three parts forming the basilar enchondrion of the posterior fossa (basioccipital, supraoccipital, exoccipital) are more severely underdeveloped and occipital bone is hypoplastic.<sup>11</sup> The association of CMI with basilar invagination and platybasia shows a similar origin with epistropheus tooth scoliosis,<sup>11,13</sup> present also in our case. Therefore, CMI formation is characterized by a complex mechanism in humans.

In human embryo of 24-28 days the thoracic spinal neurocele occludes,<sup>2</sup> contemporaneously to posterior neuropore closure, and the cerebral ventricles enlarges,<sup>2</sup> due to the high pressure of Central Neural Fluid (CNF) induced in the superior neurocele. If the posterior neuropore does not occlude, as was observed in homozygous delayed *Splotch* mice<sup>9,16</sup> having gene mutation homologue to that of Waardenburg syndrome, a dispersion of the central nervous fluid in the surrounding mesenchymal tissues occurs, with lack of central pressure, and collapse of cerebral ventricles. The IV ventricle does not exert a sufficient pressure on the surrounding mesenchymal tissues, with consequent formation of a small posterior *fossa*;<sup>2</sup> the overcrowding nervous tissue in the posterior fossa induces the descent of cerebellar tonsils and hindbrain in the foramen magnum (CMII), but if the overcrowding is minor, minor malformation (tonsillar herniation, *i.e.* CMI) occurs.<sup>2</sup> CMI may be associated to the inclination of odontoid process and syringomyelia.<sup>23</sup>

If the posterior fossa defect is minor<sup>2</sup> or occurs later, *e.g.* after 28 days of gestation, when the physiological connection of the neurocele with the amniotic cavity is closed and the ventricles are moderately decompressed, dysgenesis with disruption of the cerebellar-frontal connection<sup>2</sup> and agenesis of corpus callosum develops.<sup>2</sup>

In our patient, CMI was associated to *corpus callosum* hypoplasia, facial abnormalities and a voluminous cyst, a condition frequently present in holoprosencephaly due to *SHH* mutation.<sup>13</sup>

Naso-fronto-facial dysplasia associated to corpus callosum agenesis was reported in 7/7 patients:<sup>24</sup> naso-fronto-facial dysplasia, *corpus callosum* agenesis and prosencephalic cyst were observed and hypothesized to be expression of an embryonic prosencephalic-derived unity. Naso-fronto-premaxillary abnormalities and corpus callosum hypoplasia are expression of a disorder of the development of the induction of ventral mid-line structures,<sup>13</sup> (for which *SHH* gene is very important),<sup>13</sup> as observed also in mice.<sup>8</sup>

In our case, the voluminous prosencephalon-derived telencephalic cyst, demonstrated on MRI, seems to be an interhemispheric dorsal and ventricular cyst, rather than an ependymal or leptomeningeal cyst with lateral extrinsecation, sometimes associated to CMI in which *ZIC2* mutation is present.<sup>25</sup>

In our case, mid-line facial abnormalities, *corpus callosum* hypoplasia and the cyst may be expression of a malformative mid-line ventral pathological process, connected to NC-linked prosencephalic abnormal development.<sup>8</sup>

CMI is expression of a dorsal mid-line pathology. In some cases, the agenesis of the *corpus callosum* was reported to be associated to CMI;<sup>22</sup> furthermore, *corpus callosum* disgenesis, mid-face hypoplasia and Chiari I-II (intermediate) malformation were reported in a patient with 2-5 balanced chromosomes translocation.<sup>20</sup> On the basis of these reports, CMI in our patient could be considered a mid-line-connected developmental abnormality. CMI is known to be present in other neurocristopathies, such as in neurofibromatosis I in 6.8% of cases,<sup>8</sup> in oculo-auriculo-vertebral *spectrum*,<sup>2</sup> but it was observed that it is difficult to relate CMI to NC.<sup>8</sup> We hypothesize that in our case altered boundary relationships between NCC-derived tissues and mesodermal-derived structures in cranial base may have caused mesodermal posterior *fossa* abnormality and CMI. Analogue mechanism was advocated for cranio-synostosis of coronal suture between frontal bone (NC-derived) and parietal bone (mesodermal derived);<sup>15</sup> it may be considered also in our patient, as due to involvement of gene mutation (*FGFR*, *TWIST*, *MSX*) as previously reported.<sup>15</sup>

In the formation of dorsal structures other genes than *ZIC2* intervene. For example, FGF signaling is a general mesoderm inducer,<sup>7</sup> and *SHH*<sup>8</sup> induces *PAX* family. *PAX3* is a relevant gene, dorsalizing in the vertical axis;<sup>8</sup> it is a primordial gene essential for the formation of NC and cranio-facial structures associated with prosencephalic and mesencephalic NCC migration.<sup>8</sup> *PAX3* regulates the development of NCC and its derivatives.<sup>26</sup> This gene meets the neural tube, inducing the normal vertebrae,<sup>26</sup> for which *SHH* is essential. It was observed experimentally that the mouse without *SHH* signal does not develop the spinal cord, but *Pax3* spreads toward the midline and interferes with the sclerotomes (structures from which the vertebrae would give rise), expands in dermomyotomes (necessary for the migration of precursors cells of limbs muscles), and plays a key role in abnormal skeletal myogenesis.<sup>26</sup> *Pax3* is expressed in unsegmented paraxial mesoderm, in epithelial somites,<sup>26</sup> and later in mutated dermomyotome.<sup>26</sup>

*PAX3* is a gene responsible of WS1-3 in humans,<sup>16</sup> and it is analog to the *Splotch* gene in mice, which when mutated induces

facial defects, pigmentary disturbances and hearing loss strictly resembling WS 1-3 in heterozygous mice.<sup>7,16</sup>

It is known that in humans WS is a neurocristopathy showing symptoms present in other neurocristopathies, and is possibly associated to other neurocristopathies;<sup>16</sup> also in our patient, additional features to WS phenotype occurring also in other neurocristopathies were observed, such as ocular strabismus, external ears malformations, vertebral abnormalities which are major components of oculo-auriculo-vertebral *spectrum*, a condition reported as a possible separate entity.<sup>7</sup>

Our patient presented with cranial (posterior fossa) and axial (vertebrae) abnormalities for which the *HOX* genes may have had some relevance. The *HOX* genes, present at gastrulation in the three sheets,<sup>21</sup> induce the rostral-caudal gradient. The family of *HOX* genes, sometimes effectors of *SHH*, plays a critical role in global axial skeleton patterning;<sup>21</sup> they are segmentation genes which program the neuromeres formation at CNS level, encoding for posterior brain, rhombencephalic NC, axial skeleton,<sup>21</sup> antero-posterior axis, NC with dorsaling effect,<sup>8</sup> and pharyngeal arches.<sup>8</sup>

Hox proteins act by regulating the transcription of specific groups of target genes.<sup>8,21</sup> *HOX* genes are necessary for proper development of vertebral segments;<sup>21</sup> in mouse they are expressed in axial skeleton up to a level of 5<sup>th</sup> somite, which participates to atlas formation.<sup>21</sup>

The occipital bone in humans is frequently short in CMI<sup>21</sup> and exoccipital bone is altered in CMI; *HOX* genes could have had a role in CMI pathogenesis of our patient.

In humans *HOXD4* is important in the hindbrain development; scoliosis, which is associated frequently to CMI, may be due to abnormal development of *HOX* genes as in our case. In mice, members of all four cluster of *HOX* genes are implicated in neural tube closure,<sup>21</sup> but in humans the association of *HOX* genes and neural tube closure abnormalities resulted not significant.<sup>21</sup>

The association of CMII and myelomeningocele was repeatedly reported.<sup>10</sup> Notably, this association was observed in the presence of mutation of *VANGL2* gene, responsible of cell polarity and neural tube closure;<sup>19</sup> other genes candidates for familiar CM (*DKK*, *EP300*, *MAT3*, *ITGA*) were proposed.<sup>27</sup>

In our patient, *HOX* genes may have acted at various levels, mainly in association with *SHH*; it should be considered that many alterations of CNS and vertebral/occipital bones may depend on *SHH* abnormalities.

At the limbs level, modestly interested in our patient, *HOX* genes are target of *SHH* and play an important role by the transcription of specific target genes.<sup>28</sup> Most of *abd B* related genes are expressed with overlapping domains in the developing forelimbs and hindlimbs with the fate of specification of the digit patterning;<sup>28</sup> they control, during a first phase, the pre-chondrogenic condensation, allocation and growth of cells and, in a second phase, the cartilage models of ossification.<sup>28</sup>

*HOXD13* is specifically expressed in the future digit territory;<sup>21</sup> the disruption results in smaller metacarpal and metatarsal bones and short phalanges,<sup>21</sup> and in sympolydactily.<sup>28</sup>

In humans *HOXA13* mutation, responsible of hand-foot-genital syndrome, induces very short primary first digits (thumbs and alluces) and short first metacarpal, metatarsal and phalanges, with clinodactyly of 5<sup>th</sup> fingers.<sup>28</sup>

In our patient, the abnormalities of *HOX* genes may have had some relevance in hands (low thumbs, clinodactyly of the 5<sup>th</sup> digit) and feet (short halluces) abnormalities, in association with other genes (Figure 3).

We emphasize that in our patient 1q21.1 microdeletion falls

into a gene-free zone and this removes a gene region playing a regulatory role on a set of downstream genes which are involved in critical development processes, potentially contributing to phenotype differences.

We hypothesize that the microdeletion of a gene-free zone could play an abnormal regulatory role on a set of downstream genes, mainly *RBM8A*, *ITGA10*, and *HYDIN*, candidates for the development of the new phenotype in association with the other reported genes.

On the basis of the medical literature, the subjects with 1q21.1 microdeletion<sup>3</sup> show frequently low weight and height, as well as bone defects,<sup>4,5</sup> microcephaly with consequent defect of physical and mental development,<sup>4</sup> and psychic disturbances<sup>4,5</sup> (all features being present in our patient), possible heart malformations,<sup>4,5</sup> and loss of joints and cataract.<sup>4</sup> This may be caused in our patient by 1q21.1 deletion, mainly at proximal side, containing the gene *RBM8A* for Thrombocytopenia-Absent Radius syndrome (TAR),<sup>3</sup> but other possible candidate genes are in the proximal and distal regions.

Some patients with 1q21.1 deletion do not present the complete TAR syndrome, but only some features specific of TAR syndrome (mainly radial defect), and it was supposed that other modifier genes, besides *RBM8A* gene, acting as neural gene regulators are involved in the complete development of TAR syndrome.<sup>3</sup> The *RBM8A* mutation is associated to neurologic and psychic symptoms, due to CNS abnormalities,<sup>5,29</sup> in about 7% of cases; in mice with this mutation, a reduction of cerebral volume and size, due to scarce proliferation and raised apoptosis of neural and glial cells, was reported.<sup>29</sup> In some patients cerebral cysts and subtle *corpus callosum*, possibly attributed to abnormal CNS development, were reported.<sup>29</sup> *RBM8A* associated to modifier *locus* is a candidate gene for the abnormal psychic and somatic development.

In our patient, the 1q21.1 microdeletion and modified loci may have had relevance in the development of microcephaly, defective somatic and mental development. The association of cerebral cysts and corpus callosum hypoplasia, observed in our case associated with ventral cerebral structures underdevelopment,<sup>13</sup> possibly due to *SHH* functional defect,<sup>13</sup> may express the interference of 1q21.1 microdeletion with the Hh pathway.

The 1q21.1 microdeletion was repeatedly reported to be associated with facial abnormalities without specificity.<sup>29</sup> *ITGA 10* was reported to be a candidate gene for these facial abnormalities.<sup>29</sup>

Gamba *et al.*<sup>29</sup> reported on a patient with microcephaly, facial dysmorphism, delay of the neuropsychological development, scoliosis, hypoplasia of distal phalanges of fingers 2-3, *halluces valgi*, reminiscent of the osteochondrodysplasia of the Elkhound dog, due to *ITGA 10* (located in the proximal 1q21.1 zone) mutation.<sup>30</sup>

This condition in dogs induces bowing of paws and, on radiology, decreases the width of growth plates of radius, ulna, metacarpal and phalanx bones, by endochondral pathological ossification of metaphyseal zone.<sup>30</sup> Recently, it was demonstrated that *Itga 10* gene mutation induces an abnormal integrin receptor subunit  $\alpha 1$ , with consequent bone disease.<sup>30</sup> Integrins receptor interacting with molecules of extracellular matrix are normally present in NC,<sup>7</sup> originating the osteo-chondral skull bones.<sup>11</sup> In our case, the mechanism of NCC-derived abnormalities of bones seems to be partially related to the defect of integrin receptor, yielding *ITGA 10*, an obvious candidate gene for the osteochondral dysplasia.

*HYDIN* gene<sup>4,5</sup> activity is connected with cilia development in the ependyme of III-IV ventricles; a mutation of this gene in mice causes a defect of ciliary function with consequent hydrocephalus, as well as head size and facial dysmorphism.<sup>4,5</sup>

In our patient *HYDIN* gene may be involved, by contiguity to

other genes, for the microcephaly, CNS cyst formation and consequent neuropsychological abnormalities.

It has been speculated that complex traits result frequently from non-coding regulatory variants and pose special problems in coding regions, which induce the researcher to not easily tackle the regulatory regions problems.<sup>29</sup>

On these bases, the present work is addressed to biological-clinical problems of the pathogenesis of a malformative syndrome; a subsequent work will deal with the more complex genetic problems, such as the analysis of all candidate genes and the whole exome sequencing of patient.

Furthermore, in our patient the 1q21.1 microdeletion could have acted on genes or pathways active during or immediately after gastrulation (ventralizing *SHH*, dorsalizing *FGF-FGFR*, effector genes such as *HOX*), inducing a variation of Waardenburg gene activity and modification of gene cascade in the consequent pathways. A consequence may be the complex phenotype we observed, interesting mainly CNS mid-line, axial (vertebral) and occipital bones of the posterior fossa, with consequent CMI, arms, bones and somato-mental abnormal development.

This study demonstrates the individual susceptibility to a 1q21.1 microdeletion, which poses particular challenge for genetic analysis, due to the possible gene-gene, genetic pathways modifications, and gene-environment interactions. In our case, the developmental genes study and the interactions with NCC-derived and mesenchymal-derived structures are underlined; on the basis of clinical features, they induced the hypothesis about the possible role of multiple genes, ventralizing and dorsalizing, which can be at the basis of the complex symptoms and of CMI. But in this case, as in complex traits of diseases, to demonstrate the single gene(s) causing every malformation is difficult.

Only linkage studies, simple mapping, sequence analysis, whole exome sequencing, and functional tests for candidate genes could establish the discovery of responsible gene(s),<sup>18</sup> as reported in other complex diseases as diabetes and Crohn disease.<sup>18</sup>

Future studies could demonstrate the relevance of single gene and pathways on the phenotype building.<sup>18</sup>

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