Diprosopus: Systematic Review and Report of Two Cases

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Background: Diprosopus is a subtype of symmetric conjoined twins with one head, facial duplication and a single trunk. Diprosopus is a very rare congenital anomaly. **Methods:** This is a systematic review of published cases and the presentation of two new cases born in Argentina. We estimated the prevalence of conjoined twins and diprosopus using data from the National Network of Congenital Anomalies of Argentina (RENAC). **Results:** The prevalence of conjoined twins in RENAC was 19 per 1,000,000 births (95% confidence interval, 12–29). Diprosopus prevalence was 2 per 1,000,000 births (95% confidence interval, 0.2–6.8). In the systematic review, we identified 31 diprosopus cases. The facial structures more frequently duplicated were nose and eyes. Most frequent associated anomalies were: anencephaly, duplication of cerebral hemispheres, craniorachischisis, oral clefts, spinal abnormalities, congenital heart defects, diaphragmatic hernia, thoracic and/or abdominal visceral laterality anomalies. One of the RENAC cases and three cases from the literature had another discordant nonmalformed twin. **Conclusion:** The conjoined twins prevalence was similar to other studies. The prevalence of diprosopus was higher. The etiology is still unknown. The presence of visceral laterality anomalies may indicate the link between diprosopus and the alteration or duplication of the primitive node in the perigastrulation period (12–15 days postfertilization). Pregnancies of more than two embryos may be a risk factor for diprosopus. Given the low prevalence of this defect, it would be useful to perform studies involving several surveillance systems and international consortiums.

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Key words: diprosopus-craniofacial duplication-conjoined twins-very rare congenital anomalies-neural tube defects-heterotaxy

Introduction

Multiple pregnancies (MP) carry a higher risk on maternal and perinatal health, and its prevalence has increased because of assisted reproduction techniques. In Argentina, MP represent 2% of total births (DEIS, 2014). MP can be classified according to their zygosity in dizygotic gestations (twins originating from two oocytes fertilized by two different spermatozoa) or monozygotic (twins originating from one oocyte fertilized by one spermatozoon). The latter represents approximately one-third of spontaneous MP. Depending on when monozygotic twinning occurs, the fetuses may or may not share the placenta and/or amniotic sac, thus giving three forms of presentation: dichorionic diamniotic, monochorionic diamniotic, and monochorionic monoamniotic. Conjoined twins share parts of the body and vital organs. This type of anomaly occurs in monoamniotic monochorionic twin pregnancies resulting from a disturbance during early embryonic development (approximately 12–15 days postconception). Conjoined twins can be classified according to different criteria (Spencer 2000a, 2000b): shared structure (i.e., cephalopagus, shared head; thoracopagus, shared thorax; etc.); joining area (dorsal, ventral, lateral) and if they have or not an equivalent body surface (symmetrical / asymmetrical). For an accurate description, the duplicated structures are numbered (i.e., di-tri-tetra). The International Clearinghouse for Birth Defects Surveillance and Research estimated the prevalence at birth of conjoined twins at 1.47 per 100,000 births (CI 95%: 1.32–1.62) (Mutchinick et al., 2011).

"Diprosopus" (from Greek: di-two; prosopon-face) is the duplication of facial structures in a single head. Diprosopus is considered a subtype of conjoined twins: symmetrical, monocephalic, and with a single trunk. This entity has a very low frequency. The pathogenesis of this anomaly is still unknown.

This study aims to delineate the features of diprosopus through a systematic review of published cases, to present two new cases, and to calculate the prevalence in Argentina.

Materials and Methods

SYSTEMATIC REVIEW OF CASE REPORTS

We carried out a systematic review of case reports published until June 30, 2015. We used as a methodological framework for this review: the "Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P)" checklist (Shamseer et al., 2015) (Fig. 1).

We searched in MEDLINE by means of PubMed; SciELO; LILACS; restricting the search to human cases. We considered scientific papers available in English and Spanish. We

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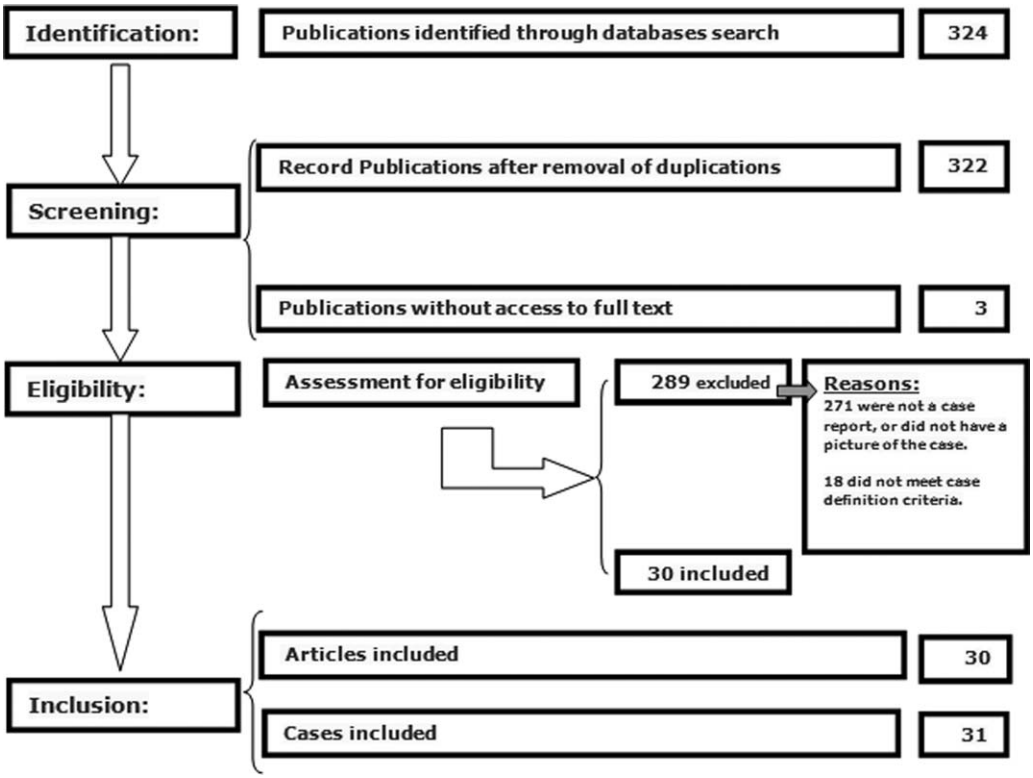


FIGURE 1. *Diagram of the process of data collection and selection of the studies included in the systematic revision (using PRISMA flow chart; Shamseer et al., 2015; Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P: elaboration and explanation).*

used the keywords "Diprosopus", "Diprosopia", "Craniofacial duplication", "Duplicación craniofacial". Search was done on 1 July 2015. The publications were assessed independently by two authors of this study (M.P.B. and P.B.). In cases in which there was disagreement, a third author (B.G.) was consulted. The abstracted data were: (a) Publication information: first author, year. (b) Cases information: pregnancy outcome, sex, weight, gestational age; twinning maternal age, sonographic prenatal diagnosis, karyotype, consanguinity, facial structures affected, and associated anomalies (Appendices A and B).

For this study, we defined "Diprosopus" as the duplication of at least two full facial organs, or two structures from two different organs (eyes, ears, nose, oral cavity) in an individual with one head and one trunk (Fig. 2). We excluded cases of partial duplication, i.e., those who have only a duplication of a single organ or part of an organ from the face in an individual with one head and one trunk (i.e., duplication of nose).

Inclusion criteria were that the publication had at least one reported case with an image of the head and the observed phenotype met the case definition.

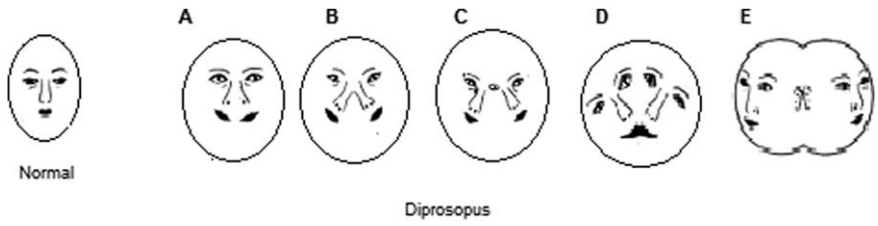


FIGURE 2. *Examples of different types of facial duplication in diprosopus cases. The pictures represent the duplication of at least two full facial organs, or two structures from two different organs (eyes, ears, nose, oral cavity) in an individual with one head.* **(A)** *Partial duplication of nose and complete mouth duplication.* **(B)** *Complete duplication of nose and mouth.* **(C)** *Complete duplication of nose, oral cavity and an extra orbit.* **(D)** *Tetraophthalmos, two noses; and partial mouth duplication.* **(E)** *Tetraophthalmos, two noses, two oral cavities and an extra ear in the middle of the facial region.*

PREVALENCE OF DIPROSOPUS IN ARGENTINA, AND THE REPORT OF TWO NEW CASES

We used data from the National Network of Congenital Anomalies of Argentina (in Spanish "Red Nacional de Anomalías Congénitas", RENAC) from November 1st, 2009 to January 31, 2015. The RENAC is a hospital based surveillance system of congenital anomalies working in 182 maternity hospitals distributed in the 24 jurisdictions of Argentina. It covers around 300,000 births per year, 42% of annual births in the country. The pregnancy outcomes included are live births and stillbirths weighing 500 grams or more, with major structural congenital anomalies detected from birth until hospital discharge. Neonatologists from each hospital send monthly reports to the RENAC coordination. The reports include a description of the affected cases and a core set of variables. The reporting neonatologists can send photographs and/or results of additional studies that contribute to the diagnostic accuracy. The reports are sent through an on-line forum. This methodology allows review and discussion of cases (Groisman et al., 2013). We analyzed cases reported and coded as conjoined twins (ICD-10 code: Q89.4), and specifically those fitting the case definition of diprosopus. The diagnosis of Diprosopus was made through clinical description and photographs of full body and face. Prevalence was calculated as the proportion between the number of cases and the total number of newborns, expressed per 1,000,000 births. The 95% confidence intervals were estimated according to the Poisson distribution for rare events.

Results

SYSTEMATIC REVIEW OF CASE REPORTS

The systematic review identified 324 publications, 33 publications met the inclusion criteria and 291 were excluded. Three full texts were not available, so 30 publications were included in our review, with 31 patients reported with Diprosopus (Fig. 1).

Fifteen cases were born alive, 5 were stillbirths and 9 had prenatal diagnosis followed by elective termination of pregnancy for fetal anomaly (ETOPFA). In two cases the pregnancy outcome was not described. The median maternal age was 24 years. The male to female ratio was 0.7. Excluding cases of ETOPFA, the median gestational age was 34.5 weeks. In 3 of 31 cases, there was an extra twin fetus without diprosopus (Appendix A). Two had a dichorionic placentation and one had diamniotic monochorionic placentation. Sex was discordant in one case (Rai et al., 1998); one had ambiguous genitalia (D'Armiento et al., 2010); in the third case, there was no information about the sex of the twin without diprosopus (al Muti Zaitoun et al., 1999). In the same report, coarctation of the aorta was found in the twin without diprosopus.

Facial structures more frequently duplicated were nose and eyes; nine patients had oral clefts. Thirty cases had different central nervous system (CNS) anomalies such as anencephaly, duplicated hemispheres and rachischisis. At thoracic–abdominal level, reported cases presented diaphragmatic hernia and conotruncal heart defects; there were anomalies in the right–left laterality viscera, including situs inversus totalis, transposition of the great arteries, dextrocardia, anomalies in lung lobation, intestinal malrotation, asplenia or polysplenia, among others (Table 1 and Appendix B). Twelve patients had karyotype performed, with normal results in all of them. One patient also underwent CGH-microarray, showing an imbalance. In two cases, consanguinity was reported (Appendix A).

PREVALENCE OF DIPROSOPUS IN ARGENTINA, AND THE REPORT OF TWO NEW CASES

From November 1st, 2009 to January 31st, 2015, 20 cases of conjoined twins were reported from a total of 1,052,088 examined newborns in the RENAC, resulting in a prevalence of 19 per 1,000,000 births (95% confidence interval [CI], 11.6–29.4). Diprosopus was detected in two cases involving 10% of all conjoined twins. Prevalence of diprosopus was 1.9 per 1,000,000 births (95% CI, 0.2–6.8).

Case 1 was a female stillbirth. Maternal age was 29 years old and paternal age was 28 years old. The parents were a nonconsanguineous couple. They had a previous 5-year-old healthy son. Gestational age was 36 weeks; weight was 1600 grams. Clinical features were anencephaly, craniofacial duplication and diaphragmatic hernia. It was a dichorionic diamniotic twin pregnancy. The first twin was a healthy female live newborn weighing 2,200 grams. There were no exposures to teratogenic agents during pregnancy. Prenatal ultrasound at 17 weeks of gestation showed a fetus with exencephaly, left diaphragmatic hernia, heart with four chambers displaced by the intestinal loops, the presence of kidneys, undamaged spine, and normal amniotic fluid volume. The facial anomaly was detected prenatally. The other fetus was normal. Neither prenatal/postnatal karyotype nor necropsy was performed. The external examination showed anencephaly; tetraophthalmos (two very close eyeballs were placed at the midline, and the remaining two eyes were laterally located); two lateral noses; two lateral oral cavities and unilateral cleft lip and palate of the left oral cavity; two dysplastic lateralized ears; and a structure that seemed like a remnant of a pinna located in the midline of the cephalic pole. The structures of the trunk and upper and lower limbs were not affected. External genitalia were female (Figs. 3 and 4; Appendices A and B).

Case 2 was a female newborn. Gestational age was 28 weeks; weight was 740 grams. The patient had prenatal diagnosis of anencephaly. There were no exposures to teratogens during pregnancy. At birth, the patient had anencephaly, craniofacial duplication consisting of tetraophtalmos (four eyeballs in three orbits, with two eyeballs occupying a single orbit); two noses, two oral cavities and unilateral cleft lip. The structures of the trunk and upper and lower limbs showed no abnormalities. The external genitalia were

TABLE 1. *Specific Congenital Anomalies in the Systematic Diprosopus Review*

Specific congenital anomalies	No. of cases affected/total specified cases	% of affection*
CNS		
• Duplication of hemispheres	14/31	45.2%
• Craniorachischisis	10/31	32.3%
• Anencephaly	4/31	13.0%
• Rachischisis	2/31	6.5%
Oral Cleft	9/30	30.0%
Cardiovascular		
• CHD: Laterality defect (TGA/ Dextrocardia/Double right outlet)	9/17	53.0%
• CHD: Septal defect	7/17	41.2%
Diaphragmatic hernia	8/10	80.0%
Other localization		
• Other laterality defects (i.e.: in lung lobulation, gastrointestinal rotation, spleen defects)	7/19	37.0%
• Ambiguous genitalia	1/31	3.2%
• Gastroschisis	1/31	3.2%
• Omphalocele	1/31	3.2%
• Thumb duplication	1/31	3.2%

% of affection*, taking into account the total number of cases with the specification.
CHD, congenital heart disease; CNS, central nervous system; TGA, transposition of great arteries.

female. The patient died at birth. Neither karyotype nor necropsy could be performed. Maternal age was 34 with five previous healthy children. The parents were a nonconsanguineous couple (Figures 5 and 6; Appendices A and B).

Discussion

PREVALENCE

The prevalence of conjoined twins in RENAC was similar to that observed in other investigations. A multicenter study (Mutchinick et al., 2011) from 21 epidemiological surveillance programs with more than 26 million births showed a total conjoined twins prevalence of 1.5 per 100,000 births (95% CI, 1.3–1.6) and for the South-American region, the prevalence was 2.4 per 100,000 births (95% CI, 1.9–2.9). This study included 11 cases of diprosopus, involving 3% of conjoined twins. It is noteworthy that nine of them occurred in Latin American countries over 5.5 million births evaluated in the region. This may indicate a greater prevalence of diprosopus in Latin America. The overall frequency of diprosopus in this study was lower than that observed in the RENAC. However, given the low frequency of this anomaly, the prevalence of diprosopus estimated in our study could be artifactually elevated.

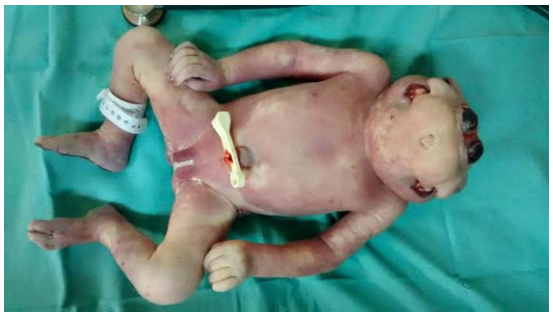


FIGURE 3. *Diprosopus: Case 1 RENAC. Anencephaly; facial duplication; female genitalia; normal trunk and limbs.*

Some authors have defined very rare defects as those with prevalence lower than 1 in 30,000 births (Castilla and Mastroiacovo, 2011). Knowledge of epidemiological features of very rare defects is important because an increase in frequency could indicate the introduction of new teratogens as a well-known example of this was the epidemic of phocomelia caused after the market introduction of thalidomide. In relation of conjoined twins, an investigation detected an increase of prevalence in Polissia, a region close to Chernobyl. The authors hypothesized a relationship between disruptions during early blastogenesis and exposure to ionizing radiation (Wertelecki, 2010).

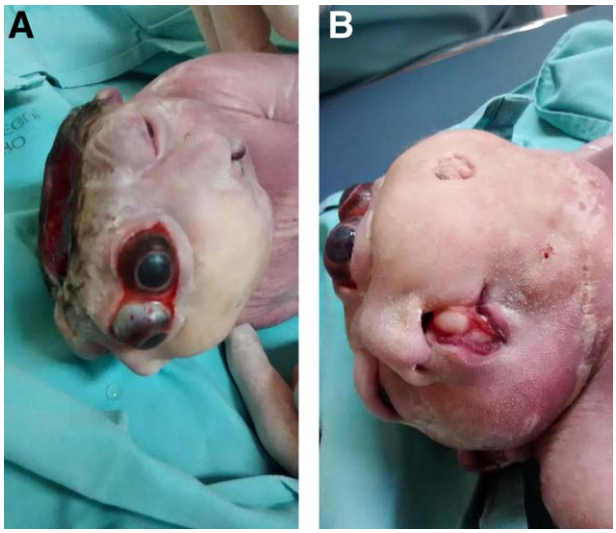


FIGURE 4. *Diprosopus: Case 1 RENAC. Cephalic pole. Anencephaly, tetraophthalmos, rudimentary and dysplastic ear in the midline, unilateral cleft lip and palate in the two oral cavities. A: left profile B: right profile.*

MAIN FEATURES AND POSSIBLE RISK FACTORS

In this systematic review of case reports, we excluded cases with partial facial duplication, because it is not clear whether it is a subtype of conjoined twins, a focal duplication or the result of interference with other structure, such as a teratoma (Baar, 1982; Costa et al., 2014). Cases reported to RENAC were female, which is consistent with the observed female predominance in Diprosopus and conjoined twins (Mutchinik et al., 2011). This female predominance could be related to the presence of a conditioning gene on the X chromosome still unknown. Gene expression could result in a high lethality in male embryos following an X-linked dominant pattern, such as incontinentia pigmenti (Le Roux et al., 1996). RENAC cases had maternal age under 35 years. In reviewed cases, maternal age did not show a tendency to extreme ages. Diprosopus is associated with a high neonatal lethality, mostly due to severe neural tube defects (anencephaly, rachischisis). In the reviewed literature, there were only two cases that survived the neonatal period. Both patients showed duplication of cerebral lobes (Kudo and Toda, 1970; Hahnel et al., 2003).

Prenatal sonographic findings during the first and second trimesters included heterogeneous features such as: variable range of duplication of craniofacial structures; CNS and spine anomalies, diaphragmatic hernia, heart disease and/or polyhydramnios. In some prenatally detected cases, tomography and / or nuclear magnetic resonance (NMR) helped define facial, CNS and other internal anomalies (Dhaifalah et al., 2008; Maruotti et al., 2009; Laor et al., 2012; Thornton, 2014). In RENAC case 1 (Figs. 3 and 4), exencephaly was detected prenatally, and anencephaly was diagnosed postnatally. This situation could be explained by the sequence of conversion of exencephaly in anencephaly (Wood and Smith, 1984). Two cases reviewed had parental consanguinity (Amr and Hammouri, 1995; Suryawanshi et al., 2013). That might suggest a genetic factor with an autosomal recessive pattern of expression. However, both patients were from India and Saudi Arabia, countries with a high proportion of consanguineous couples. Recurrence among siblings was not reported.

In the cases reviewed, no chromosomal abnormalities were identified in the karyotype. In one case, a CGH microarray study showed a complex genomic imbalance (Thornton et al., 2014). In this report, the study showed two duplicated areas: Xp22.31p22.2 and 13q12.11; and a deletion of 4q34.3. The duplications were also observed in the patient's mother; therefore, the 4q34.3 deletion may be associated with diprosopus. However, the study was not performed in the patient's father and it is not possible to determine if the deletion was pathogenic.

Diprosopus includes a monozygotic twinning event (it is a subtype of conjoined twins); a third twin without disopropus could be a risk factor. It is important to consider that there is a 48% of monozygotic twinning in triplets, and a higher frequency of conjoined twins in triplets (Baldwin, 1999; Guilherme et al., 2009). In this study 4 of the 33 cases had another twin without this defect; this happened in case 1 from RENAC and cases 8, 18, and 20 from the review (Rai et al., 1998; al Mutti Zaitoun, 1999; Maruotti, 2009). In these reports, placentation and sex concordance between twins was described, but without an analysis of polymorphic genetic markers for the accurate identification of the twinning type. Discordant sex was observed in case 20, which is the only one that could be interpreted as dizygotic (Rai et al., 1998). The case 18 had biamniotic monochorionic placentation, which could be an indicator of monozygotic twinning. However, in that case the thinning of the chorionic plate and some mild vascular changes were also observed, without being able to rule out an etiological mechanism related to oxygen deficiency (al Muti Zaitoun, 1999).

PATHOGENESIS AND PROPOSED HYPOTHESIS

Conjoined twinning is a primary congenital anomaly, followed by other secondary anomalies (malformations and /or disruptions). These disorders occur most likely in epiblast cells during the perigastrular period (12–15 days postfertilization in humans). There are two alternative models to explain conjoined twins pathogenesis: the fusion of two monozygotic embryos that were separated in previous stages; and the fission (splitting) of a single embryo (Spencer 2000a, 2000b; Kaufman, 2004). Given the impossibility of using an experimental model in humans, some authors have postulated different pathogenic mechanisms of diprosopus based on the analysis of the phenotype and associated anomalies of the affected and its relationship with concepts

FIGURE 5. *Diprosopus: Case 2 RENAC. Anencephaly; facial duplication; female genitalia; normal trunk and limbs.*

of developmental biology emerged from experimental models.

First, Spencer (2000a, 2000b) classified and analyzed 1200 cases of conjoined twins. In the case of Diprosopus the author interpreted the initial abnormality as the early fusion of two monozygotic embryonic discs at the ventral and lateral levels. Secondary to this fusion, there might be an aplasia and divergence of tissues from the midline. This would explain the presence of CNS anomalies arising from the forebrain, the duplication of facial structures and cervical and thoracic vertebrae duplication as well as cardiac and diaphragmatic anomalies (Table 2).

Second, Carles and colleagues (1995) considered histopathological findings of two cases with Diprosopus and defined the condition as a neurocristopathy with excess of neural crest cells formation in a single embryonic disc. These authors postulated that this excess would be secondary to the formation of two nothocords in a single embryo, which produces two neural induction plates, and an extra edge-neural crest medially located between the two plates. These altered structures could explain the presence of anencephaly, duplication of facial structures, conotroncal heart disease and spinal disorders (Table 2).

Third, we propose that diprosopus occurs in a single embryo but before the formation of the notochord, by the presence of two early primitive nodes instead of one. This hypothesis arises from the presence in many cases of anomalies of visceral laterality-heterotaxy. The sequence of events could be the following: The initial alteration would be a doubling of the early primitive node and secondarily, a group of specific congenital anomalies occurs in response to the nodal alteration (Table 2). In the human epiblast, the early primitive node begins to develop at the end of the second week of gestation. It has multiple roles during the gastrulation process such as being a signal emission center that induces the neural plate. It participates in the left–right axis establishment through the node ciliary cells, which have a nonrandom preferential movement that generates different cell behavior in the left and in the right embryo position. The node derivated structures also are involved in the specification of various structures of the head (Foley and Stern, 2001; Boettger et al., 2001).

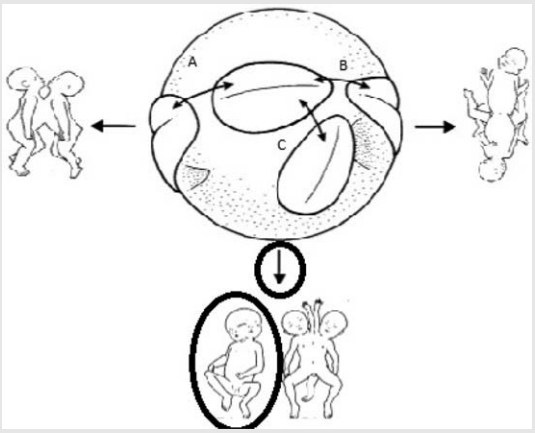
In diprosopus cases the craniofacial duplications and the brain alteration (anencephaly or brain hemispheres duplication) are related with alterations in the node and its derivated signals during anterior neural induction process and the craniofacial development. If there is a node duplication, there will be a duplicated ciliary flow. This causes the reception of signals (from both right and left identity) in the midline area of the embryo. Consequently, it generates a random assignation of laterality in the trunk causing the heterotaxia event (Burn and Hill, 2009; Hirokawa et al., 2009). We agree with Carles et al., in that the alteration of the neural induction could generate extra neural crest cells, which can explain the presence of facial anomalies and cono-truncal heart defects. We add to this hypothesis that the alteration of neural induction could be the result of an initial event in the early primitive node.

STRENGTH AND WEAKNESSES

RENAC is a surveillance system with high coverage which makes it possible to detect this rare birth defect. RENAC operation allows the attachment of photos, which are essential for diagnosing this condition. One weakness is that in RENAC cases, karyotype and necropsy were not performed. Therefore, we do not have any data on the presence of

FIGURE 6. *Diprosopus: Case 2 RENAC. Cephalic pole. Anencephaly, tetraophthalmos: four eyeballs in three orbits, two noses, and two oral cavities with unilateral cleft lip.*

TABLE 2. *Diprosopus: Mechanisms, Proposed Sequence of Events, and Its Link with the Associated Congenital Anomalies*

	Spencer's Hypothesis
Congenital anomalies	- Duplication of organs (brain hemispheres, facial structures, cervical spine) - Malformations without duplications (congenital heart defects, diaphragmatic hernia; respiratory and digestive tract anomalies)
Embryological mechanisms and defects	Early ventrolateral fusion of monozygotic twins and reorganization of merged tissues (structures in the midline) Structures with aplasia and structures that diverge from the midline.
Schemes	 Types of fusion of monozygotic twins: A) Rostral fusion B) Caudal fusion c) Ventrolateral fusion (the Diprosopus case is indicated). Picture adapted from Figure 3 in Spencer R, 2000. Theoretical and analytical embryology of conjoined twins: part I: embryogenesis. Clin Anat.; 13(1): page 43.

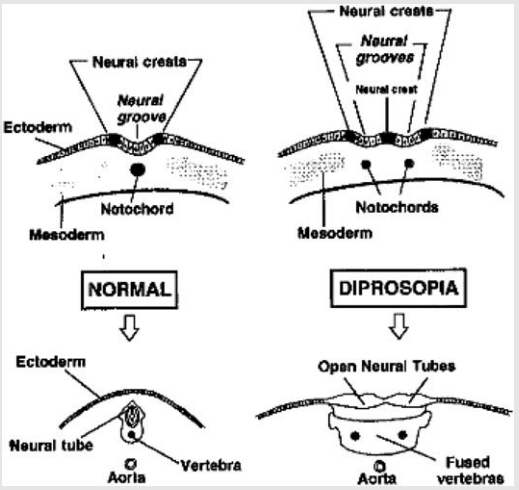
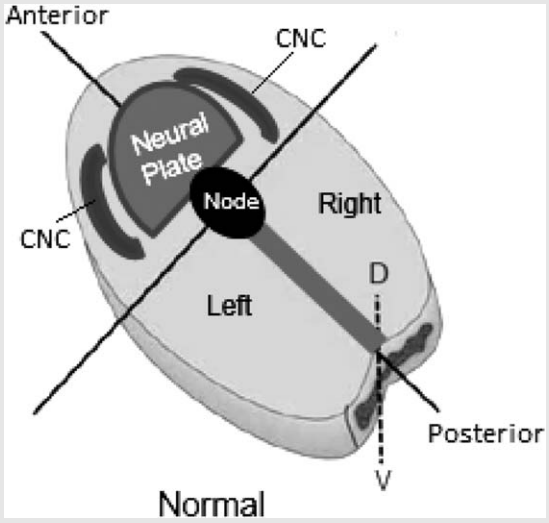
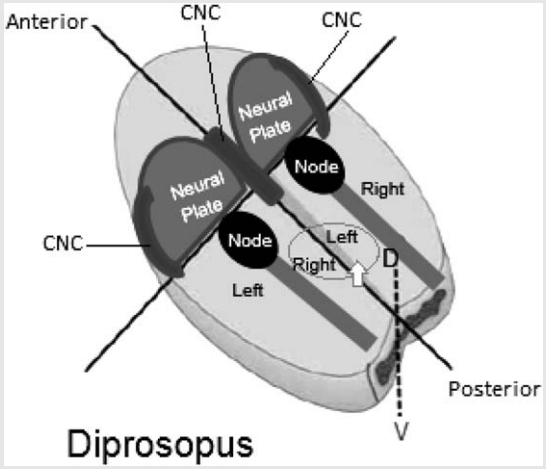
	Carles' Hypothesis
Congenital anomalies	- Anencephaly - Facial anomalies; conotroncal cardiac defects - Anomalies in vertebrae - Diaphragmatic defects
Embryological mechanisms and defects	Fission in a monozygotic gestation that produces the presence of two notochords. The two notochords in consequence generate the duplication of the cephalic neural plate, the presence of an extra medial cranial neural crest, and alterations in paraxial mesoderm
Schemes	 Diprosopia as a result of the duplication of the notocohord, neural plate and excessive component of neural crest. Picture adapted from Figure 10 in Carles D., et al.; 1995. Diprosopia revisited in light of the recognized role of the neural crest cell in facial development. J Craniofacial Genet Dev Biol; 15:page 95

TABLE 2. *Continued*

	Our Hypothesis
Congenital anomalies	- Neural tube defects (anencephaly, hemispheres duplication, rachischisis) - Facial anomalies (duplication in different structures with or without oral clefts) - Conotroncal heart defects - Vertebral anomalies - Laterality defects (Dextrocardia, TGV; intestinal malrotation, spleen defect, alteration in lung lobulation)
Embryological mechanisms and defects	Duplication of the early primitive node and secondarily, a group of specific congenital anomalies occurs in response to the nodal alteration: a) Alteration in node signaling for anterior neural induction correlated with: Duplication and / or alteration in the formation of the neural plate; presence of an extra cephalic neural crest (facial and conotroncal alterations) b) Alteration of node derivate structures (floor plate; precordal mesoderm; notochord and pharyngeal endoderm) correlated with: Duplication and / or alteration in the formation of the neural plate; facial anomalies; vertebral anomalies and diaphragmatic defects. c) Alteration in ciliary node flow correlated with: Laterality anomalies in the visceral thoracic and abdominal organs.
Schemes	 **Epiblast structures in a normal human embryo** CNC: Cephalic neural crest. D: dorsal. V: Ventral  **Epiblast structures in a Diprosopus human embryo** CNC: Cephalic neural crest. D: dorsal. V: Ventral Note: The white arrow indicates the area in the midline of the embryo that receives signals from both right and left identity. This causes random determination of laterality at the level of the trunk (heterotaxia).

additional internal congenital anomalies. Most of the cases from the literature did not have cytogenetic studies.

CONCLUSIONS

To our knowledge patients with diprosopus reported to RENAC are the first cases described in Argentina. In the systematic review we included 31 cases with diprosopus. Diprosopus prevalence in our study was 1.9 per 1,000,000 births and they represented 10% of conjoined twins. This proportion is higher than observed in other epidemiological studies. However, because only two cases were detected, we need larger studies to confirm these results. It seems that diprosopus may be associated with triple twin pregnancies. Given the low prevalence of the defect, it is recommended to have a monitoring system for congenital anomalies with high coverage and /or to perform studies involving several surveillance systems and international consortiums.

The etiology of diprosopus is still unknown, but associated congenital anomalies support some of the mechanisms that may be involved. The laterality anomalies were not previously described as a main associated anomaly in this entity; it is one of the reasons to raise the hypothesis of the early primitive node duplication. This hypothesis could provide an initial step for further research as it establishes a period of time (perigastrulation) for the occurrence and the structures involved (node and its derivates).

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Authors declare no conflict of interest.

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Appendix A. *Diprosopus Cases (RENAC and Systematic Review)*

	ID –author – year- country			Pregnancy outcome	Sex	Weight (g)	Gestational age (weeks)	Twinning	Maternal age	Sonographic Prenatal diagnosis	Karyotype	Consanguinity
1	RENAC	2015	ARGENTINA	SB	F	1600	36	Yes	29	YES	NO	NO
2	RENAC	2015	ARGENTINA	LB	F	740	28	NO	34	YES	NO	NO
3	THORNTON	2014	USA	LB	M	2440	36	NO	29	YES	46,XY[a]	NO
4	PACHAJOA	2014	BRAZIL	Elective TOP	F	NA	28	NO	37	YES	46,XX	NA
5	SURYAWANSHI	2013	INDIA	LB	M	NA	NA	NO	NA	NA	NA	YES
6	LAOR	2012	USA	LB	F	3400	32	NO	24	YES	NA	NO
7	ULKER	2012	ITALY	Elective TOP	F	NA	NA	NO	22	YES	NA	NO
8	D'ARMIENTO[b]	2010	ITALY	LB	I	1800	37	YES	26	YES	NA	NO
	MARUOTTI	2009										
9	CHOH	2010	INDIA	LB	M	3200	NA	NO	NA	NO	NA	NO
10	FERNANDES	2010	BRAZIL	LB	F	1700	30	NO	13	YES	46,XX	NO
11	KASTEMBAUM	2009	USA	LB	M	3524	38	NO	21	YES	46,XY	NO
12	DHAIFALAH	2008	CZECH	Elective TOP	M	450	23	NO	33	YES	NA	NA
13	EKINCI	2005	TURKEY	SB	F	130	28	NO	18	NA	46,XX	NO
14	BUBUL	2004	FRANCE	Elective TOP	F	NA	15	NO	34	YES	46,XX	NO
15	KOSEOGLU	2003	TURKEY	LB	F	NA	NA	NO	NA	NA	NA	NA
16	HAHNEL	2003	GERMANY	LB	NA	NA	NA	NO	NA	NA	NA	NA
17	RODRIGUEZ MORALES	2002	PUERTO RICO	Elective TOP	M	1850	39	NO	23	YES	NA	NA
18	AL MUTI ZAITOUN	1999	UK	SB	M	1260	33	YES	16	NA	46,XY	NO
19	ANGTUACO	1999	USA	LB	M	NA	30	NO	20	YES	NO	NA
20	RAI	1998	UK	LB	M	1852	28	YES	NA	YES	46,XY	NO
21	OOSTRA	1998	HOLLAND	NA	F	NA	NA	NO	NA	NO	NO	NA
22	OOSTRA	1998	HOLLAND	NA	F	NA	NA	NO	NA	NO	NO	NA
23	AMR	1995	SAUDI A.	SB	M	1260	30	NO	28	NA	NO	YES
24	FONTANAROSA	1992	ITALY	Elective TOP	M	NA	14	NO	30	YES	NO	NO
25	PAVONE	1987	ITALY	LB	F	1210	28	NO	24	NA	46,XX	NO

Appendix A. *Continued*

	ID –author – year- country			Pregnancy outcome	Sex	Weight (g)	Gestational age (weeks)	Twinning	Maternal age	Sonographic Prenatal diagnosis	Karyotype	Consanguinity
26	PAVONE	1987	ITALY	LB	F	2450	NA	NO	24	NA	46,XX	NO
27	OKAZAKI	1987	PHILIPPINES	SB	F	NA	31	NO	31	YES	NO	NA
28	STRAUSS	1987	ISRAEL	Elective TOP	F	NA	NA	NO	27	YES	NA	NA
29	RYDNERT	1985	SWEDEN	Elective TOP	NA	NA	20	NO	NA	YES	NO	NO
30	MOERMAN	1983	BELGIUM	Elective TOP	F	NA	29	NO	21	YES	46,XX	NO
31	JASCHEVATZKY	1980	ISRAEL	SB	NA	4200	38	NO	24	NO	NO	NO
32	KUDO	1970	JAPAN	LB	F	2980	NA	NO	33	NO	46,XX	NO
33	BRODER	1935	USA	LB	F	2500	NA	NO	NA	NO	NO	NA

Pregnancy outcome: LB, livebirth; SB, stillbirth; Elective TOP, termination of pregnancy. SEX: M, Male; F, female; I, Indeterminate.

[a]CGH array, Del4q34;DupXp22.31p22.2;Dup13q12.11.

[b]Maruotti 2009 and D'Armiento 2010 are two different publications of the same case.

NA, not available data.

Appendix B. *Diprosopus cases (RENAC and systematic review)*

			Facial structures						Other malformations		
	ID –author - year		Ears	Eyes	Nose	Mouth	Oral clefts	CNS/NTD	DH	Heart	Others
1	RENAC	2015	2 + 1 PIT	4; 3 ORBITS	2	2	BILATERAL Y UNILATERAL	ANENCEPHALY	YES	NA	NA
2	RENAC	2015	2	4; 3 ORBITS	2	2	UNILATERAL	ANENCEPHALY	NA	NA	NA
3	THORNTON	2014	2 + 1 PIT	4	2	2	NO	DUPLICATION OF HEMISPHERES Y RAQUISQUISIS	YES	TF	ACCESORY SPLEEN, ECTOPIC KIDNEYS, INTESTINAL MALROTATION
4	PACHAJOA	2014	4; 2 MEDIAL LOCATION	5; 4 ORBITS	2	2	BILATERAL	RACHISCHISIS	NA	ASD	NA
5	SURYAWANSHI	2013	2	3	2	2	NO	DUPLICATION OF HEMISPHERES	NA	NA	NA

Appendix B. *Continued*

			Facial structures						Other malformations		
	ID –author - year		Ears	Eyes	Nose	Mouth	Oral clefts	CNS/NTD	DH	Heart	Others
6	LAOR	2012	NA	4; 3 ORBITS	2	2	BILATERAL	DUPLICATION OF HEMISPHERES	NA	TF	TRACHEOESOPHAGEAL FISTULA AND ABSENT SPLEEN
7	ULKER	2012	4; 2 MEDIAL LOCATION	4	2	2	NO	CRANEORACHISCHISIS	NA	TGA, VSD	NA
8	MARUOTTI[b]	2009	2	4; 4 ORBITS	2	2	NO	CRANEORACHISCHISIS	NA	TGA	AMBIGUOUS GENITALIA, ABSENT PANCREAS, TALIPES
9	CHOH	2010	2	4; 3 ORBITS	2 + 1PIT	2	NO	DUPLICATION OF HEMISPHERES	NA	NA	NA
10	FERNANDES	2010	2	4	2 + 1 PIT	2	NO	OTRO	YES	DEXTROCARDIA, HYPO-PLASTIC LEFT VENTRICLE	NO
11	KASTEMBAUM	2009	2	2 + 1PIT	1 + 1PROBOSCIS	2	UNILATERAL	DUPLICATION OF HEMI-SPHERES AND ALTERATIONS OF NEURONAL MIGRATION	NO	TGA and DUPLICTION OF CARDIAC CHAMBERS	INTESTINAL MALROTATION
12	DHAIFALAH	2008	2	3	2	2	NO	NO	NA	NA	DUPLICATED THUMB, PULMONARY HYPOPLASIA
13	EKINCI	2005	2	4	2	2	NO	CRANEORACHISCHISIS S	YES	DEXTROCARDIA	SITUS INVERSUS TOTALIS
14	BUBUL	2004	NA	4	2	2	BILATERAL	DUPLICATION OF HEMISPHERES Y RACHISCHISIS	NA	NA	VERTEBRAL DUPLICATION
15	KOSEOGLU	2003	NA	4	2	2	NA	DUPLICATION OF HEMISPHERES	NA	NA	NA
16	HAHNEL	2003	2	2 + 1PIT	2	1	NO	DUPLICATION OF HEMISPHERES	NA	VSD	NA

Appendix B. *Continued*

	ID –author - year		Facial structures					CNS/NTD	Other malformations		
			Ears	Eyes	Nose	Mouth	Oral clefts		DH	Heart	Others
17	RODRIGUEZ MORALES	2002	NA	4; 3 ORBITS	2	2	NO	CRANEORACHISCHISIS	NA	TGA	TRACHEOLARINGEAL AND PULMONARY DUPLICATION
18	AL MUTI ZAITOUN	1999	2	2 + 1PIT	2	2	BILATERAL	CRANEORACHISCHISIS	YES	VSD	UNILATERAL RENAL AGENESIS; VERTEBRAL DUPLICATION
19	ANGTUACO	1999	2	4; 3 ORBITS	2 + 1 PIT	2	NO	DUPLICATION OF HEMISPHERES	YES	NA	NA
20	RAI	1998	NA	4	2	2	NO	DUPLICATION OF HEMISPHERES	NA	NA	NA
21	OOSTRA	1998	2	4; 3 ORBITS	2	2	UNILATERAL	CRANEORACHISCHISIS	NA	NA	NA
22	OOSTRA	1998	2	4; 3 ORBITS	2	2	NO	DUPLICATION OF HEMISPHERES	NA	NA	NA
23	AMR	1995	3	4	2	2	BILATERAL	CRANEORACHISCHISIS	NA	NA	VERTEBRAL DUPLICATION, OMPHALOCELE
24	FONTANAROSA	1992	2	4	2	2	NO	CRANEORACHISCHISIS	NA	NA	GASTROSCHISIS
25	PAVONE	1987	2 + 1 PIT	4; 3 ORBITS	2	2	NO	CRANEORACHISCHISIS	YES	VSD	NO
26	PAVONE	1987	2 + 1 PIT	3	2	2	BILATERAL	ANENCEPHALY	NA	NA	ANGIOMA IN LOWER LIMB
27	OKAZAKI	1987	2	4; 3 ORBITS	2	2	NO	DUPLICATION OF HEMISPHERES	NA	DEXTROCARDIA	NO
28	STRAUSS	1987	2 + 1 PIT	4	2	2	NO	DUPLICATION OF HEMISPHERES Y RACHISCHISIS	YES	NA	NA
29	RYDNERT	1985	2	4	2	2	NO	CRANEORACHISCHISIS	NO	NO	NO
30	MOERMAN	1983	2	4 + 1 PIT	2	2	NO	ANENCENPHALY	NA	TGA , AVC, DUCTUS	DUPLCATED SPLEEN AND PANCREAS; INTESTINAL MALROTATION
31	JASCHEVATZKY	1980	NA	NA	2 + 1 PIT	2	BILATERAL	ANENCEPHALY	NA	NA	NA

Appendix B. *Continued*

			Facial structures						Other malformations		
	ID –author - year		Ears	Eyes	Nose	Mouth	Oral clefts	CNS/NTD	DH	Heart	Others
32	KUDO	1970	2	4; 3 ORBITS	2 + 1 PIT	2	NO	DUPLICATION OF HEMISPHERES	NA	DOUBLE OUTLET OF RIGHT VENTRICLE; VSD; ASD; COA	DUPLICATED MESENTERY
33	BRODER	1935	2	2	2	2	NO	ANENCEPHALY + LUMBOSACRAL MYELOMENINGOCELE	YES	NO	DUPLICATED LOBES OF RIGHT LUNG

NR, non reported; NTD, neural tube defects; DH, diaphragmatic hernia; TF, tetralogy of Fallot; ASD, atrial septal defect. TGA. transposition of great arteries; VSD, ventricular septal defect; AVC, atrio-ventricular canal; COA; coarctation de aorta. (b): Maruotti 2009 and D'Armiento 2010 are two different publications of the same case.