RESEARCH ARTICLE

THE DUMBO RAT MUTANT: A CYTOGENETIC, MORPHOLOGIC AND SAGITTAL MORPHOMETRIC ANALYZIS.

S. Katerji, N. Vanmuylder, M. Rooze, S. Louryan

Laboratory of Anatomy and Embryology, Université Libre de Bruxelles, Brussels, Belgium

Abstract

Objective
To study the possibility of use Dumbo rat as, experimental model for understanding abnormal craniofacial development.

Design
We investigated craniofacial morphogenesis in the Dumbo rat by morphologic and morphometric technics. We also performed a cytogenetic study of this rat. Wistar strain was considered as control. For morphologic and morphometric studies, we used Dumbo and Wistar embryos at E15 to 21. We stained these embryos in toto with alcian blue and alizarin red. The skeletons of the embryos were examined and drawn under a Lucida camera, and the following sagittal measurements were taken: zygomatic length and thickness, length of the mandible and its anterior and posterior thicknesses, length of the maxillary, and petrous bone height. Statistical analyses were realized using Mann Whitney test in SPSS. For cytogenetic study, chromosome spreads were prepared from lymphocyte cultures obtained from the blood of adult rats of both strains.

Results
The Dumbo embryos exhibed hypoplasia of the zygomatic, maxillar and mandibular bones, and micrognathia, evoking some human dysmorphogenesis . Moreover, the position of the preliminary ear was abnormally low. The differences in the measurements of the craniofacial structures between the two groups of rats are significant. However, the cytogenetic study did not reveal any differences between the two strains.

Conclusion
Our data indicate that the considerable morphometric differences between the craniofacial structures of Dumbo and Wistar rats might be due to genetic mutations that are undetectable by chromosome mapping. Further histologic and genetic analyses might contribute to elucidate the early determinism of the Dumbo phenotype.

Key words
Dumbo rat, cytogenetic analysis, morphometry, craniofacial.

Résumé

Objectif
Valider l'intérêt du rat Dumbo comme modèle expérimental du développement crâniofacial humain anormal.
Matériel et méthodes : nous avons étudié la morphogenèse crâniofaciale chez le rat Dumbo par des techniques morphologiques et morphométriques, complétées par une approche cytogénétique. Le rat de la souche Wistar a été condsidéré comme l'organisme témoin. Pour les études morphologique et morphométriques, nous avons prélevé des embryons des deux souches depuis le 15ème jusqu'au 21ème jour du développement. Des embryons ont été colorés *in toto* au bleu alcian-sulfate d'alizarine. Les squelettes ainsi colorés ont été dessinés et observés à la *camera lucida*. Les mesures suivantes ont été prises dans le plan sagittal : longueur et

épaisseur de l'os zygomatique, longueur et épaisseurs antérieure et postérieure de la mandibule, longueur du maxillaire, position en hauteur de la région otique. L'analyse statistique a été réalisée à l'aide du test de Mann Whitney sous SPSS. Pour l'étude cytogénétique, les échantillons chromosomiques ont été préparés à partir de cultures lymphocytaires obtenues par voie sanguine chez les rats adultes de chaque lignée.

Résultats

Les embryons Dumbo présentent une hypoplasie des os zygomatiques, maxillaires et de la mandibule, ainsi qu'une micrognathie, pouvant évoquer certaines dysmorphoses observées dans l'espèce humaine. De surcroît, la position de l'ébauche otique est anormalement basse. Les différences quantitatives entre les deux groupes sont significatives. Cependant, l'étude cytogénétique ne démontre pas de différence entre les caryotypes des deux souches.

Conclusion

Les données démontrent que les différences morphométriques considérables affectant la région crâniofaciale dans les deux souches devraient résulter d'anomalies génétiques indétectables par la simple cartographie chromosomiques. Des études ultérieures histologiques et génétiques devraient permettre de préciser le déterminisme précoce du phénotype Dumbo.

Introduction

The Dumbo rat is characterized by craniofacial features that distinguish it from the normal Wistar rat. These characteristics evoke several human syndromes, as mandibulofacial dysostosis (Treacher-Collins syndrome) because of micrognathia, low position of the ears, hypoplasia of the zygomatic and mandibular bones. To the best of our knowledge, neither developmental biologists nor geneticists have measured or studied this mutation. Dumbo rats were purchased from the animal trade. This rat seems to be the product of the domestic breeding of rats (from Wistar origin) probably in the USA a few decades ago. Wistar rat have often been used for establishing the basic techniques of embryo manipulation (1,2) and were used as controls for this study. Moreover Wistar rat is an important model for human disease, and is extensively used for studying complex traits for example in the morphological, physiological and pharmacological analyses (3).

Dumbo rat was named "Dumbo" for its large ears. Upper and lower jaws appear shorter than in "normal" rats. The projection of the ears is below the line of the eyes, which is lower than in the normal rat. They are C-shaped and turned forwards, wider and less flexed in Dumbo rat than in other strains. These characteristics constitute the major reasons for its popularity among rodent lovers worldwide. Figure 1 shows the notable differences between Dumbo and Wistar rat at adult age.

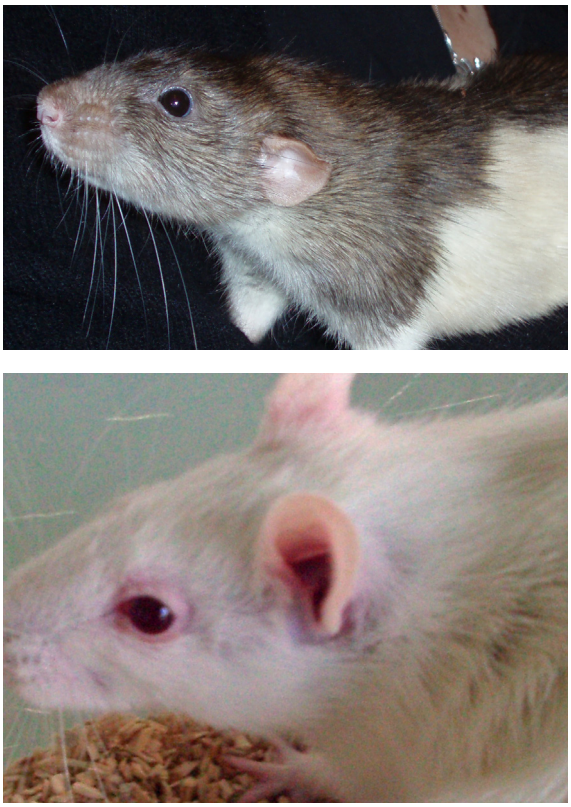

Fig 1: Differences between Dumbo (1) and Wistar (B) rats; micrognathia and low position of the ears in Dumbo rat.

The Dumbo and related human abnormalities belong to a subset of craniofacial disorders caused by developmental abnormalities in the structures derived from the branchial arches. More specifically, these anomalies involve the mandibulary, maxillary and zygomatic bones, and the position of the ears. In vertebrates, these craniofacial structures are derived from the first pharyngeal arch, which receives crest cells from the midbrain and rhombomeres r1 and r2 migrate (4).

The craniofacial mesenchyme has a double origin, having been formed from cells resulting

from the rhombencephalic, mesencephalic and prosencephalic neural crests (mesectoderm), and from cephalic mesoderm. In mammals, tracing studies using whole embryo cultures have elucidated the migration pathways of cranial crest cells that form the craniofacial structures (5). The embryonic central nervous system (CNS) produces the cranial neural crest (CNC); one important characteristic of neural crest cells is their ability to give rise to a large variety of cells. From the cephalic neural crest are produced several tissues of the face and cranium, particularly the visceral skeleton, and the connective muscular tissue (6, 7, 9-14).

The cranial neural crest cells constitute a major contribution to the skeletal tissues of the skull, differentiating into both primary and secondary cartilage, as well as endochondral and membranous bone (10,15). Moreover, the formatting and emigrating neural crest temporarily possesses the ability to act on the central nervous system from where it emigrated, by expressing several trophic and mitotic factors (such as FGF) that are necessary for craniofacial development. The data summarized by Hanken and Gross (6) clearly show that neural crest cells possess some features that are highly conserved among many vertebrates.

Abnormalities in the reciprocal interaction between the central nervous system and the neural crest, or in the morphogenetic function of the neural crest in the face, lead to considerable facial malformation that may also involve the brain and its nerves. On the other hand, facial malformations could indicate central nervous system disorders (7) because that the embryonic central nervous system (CNS) itself produces the cranial neural crest (CNC), which generates the viscerocranium, and the connective tissues of craniofacial muscles (6, 7, 9-14). Neural crest cells express the same genes from their emigration from the CNS to their definitive maxillofacial location (18).

The first purpose of our study was to identify hypothetical cytogenetic abnormality. The first observations relative to the transmission of the Dumbo phenotype in our breeding suggest that this transmission is mendelian with a recessive mode. Cytogenetic study was performed in order to demonstrate or exclude any chromosomic change, in comparison to the Wistar strain.

The second aim was to compare prenatal skeletal development of Dumbo rats with Wistar rats using *in toto* staining.

Material and methods

Cytogenetic study

Twenty adult Dumbo rats and twenty Wistar rats were killed by carbon dioxide asphyxiation, as recommended by Belgian animal ethic's law. Chromosomes spreads were prepared from lymphocyte cultures obtained from peripheral blood by jugular vein puncture or it obtained by heart puncture as described in (19-20). Lymphocytes from 0.3-0.4 ml of blood were cultured for 72h at 37°C in 5 ml of DMEM (RPMI supplemented with 15% fetal calf serum, 1% glutamine, 0.5 % penistrepto, 2% PHA. Activated rat lymphocytes in their culture medium were added to 0.5 ml of colcemid (10% in RPMI) for 1h in order to prepare chromosomes during metaphase spread. The cell suspension was collected, treated with 10 ml KCl (5,592 g/l) and fixed with 10 ml of 3:1 methanol glacial: acetic acid. Plates were prepared by specking the solution per drip, stained in 10% Giemsa solution and examined with a light microscope (Axioscope, Zeiss, Germany), by the programme (Applied Imaging Cytovision). Twenty-two karyotypes were established for the two strains. A comparative karyotype of rat (Wistar) and (Dumbo) based on chromosome G-banding morphology was realized. The classification of the Wistar rat chromosomes was established following the recommendations of the committee for has standardized karyotype of *rattus norvegicus* (21).

Morphologic and morphometric studies

Collection of embryos

The vaginal liquid was examined each morning and the day on which spermatozoa were observed was regarded as day 0. At different gestational stages mothers were asphyxiated with CO2 and the embryos were collected at ages E15 to 21. The embryos were immediately placed in physiological serum. They were examined and drawn using a stereomicroscope with *camera lucida*. Some measures were realized before the preparation of skeleton.

We prepared and compared 88 skeletons of both strains. From 4 to 6 Dumbo embryos were used at the ages of E15 to 21. For Wistar rats, 6 to 8 embryos were used at each age. The embryonic skeletons were stained *in toto* by Blue Alcian-Alizarin. This staining allows the observation of the cartilaginous and osseous structures: cartilaginous structures are stained blue and osseous structures pink.

Procedure for preparing the skeletons stained in toto by Blue Alcian-Alizarin as described by Watson (21)

The skin and internal organs of the embryos were removed. The embryos were fixed in 95% ethanol for 2-3 days and stained with Alcian blue for 24 hours, or for longer if necessary. The skeletons were again placed in 95% ethanol for six days, changing the ethanol daily. They were then placed in 1% KOH for 30 minutes, and then in alizarin staining solution (alizarin in 1% KOH). When the skeletons become clearly visible, the embryos were transferred to 50% glycerin, and tow days later they were transferred to 80% glycerin. They were kept in 80% glycerin, with a change every two days. Finally, the skeletons are transferred to pure glycerin for preservation.

The skeletons of the embryos were examined and drawn in *norma sagittalis* under a *camera lucida* (Wild Typ 308700 M32, Heerbrugg Switzerland), and the following measurements were taken from the drawings. These measurements were the zygomatic length and thickness, the length of the mandible and its anterior and posterior thicknesses, the length of the maxillary, and the petrous bone height. Figure 2 shows the measurements realized on the images taken by the *camera lucida*.

Despite their interest, measurements in *norma frontalis* were not performed because of problems due to the length of the snout. More accurate techniques as scanning electron microscopy and digitalized direct measurements would be used to complete the present analyzis.

Fig 2: *In toto* measurement landmarks 1: zygomatic bone length, 2: zygomatic bone thickness, 3: mandible length, 4: posterior mandible thickness, 5: anterior mandible thickness, 6: inner ear height, 7: maxillary bone length.

The ratio of each of these measurements to the overall length of the embryo was calculated. Then the means and standard errors of these ratios were calculated. Also p-levels were calculated using MANN WHITNEY test in SPSS. Inter- and intra-observer measurements were made for reliability purposes. The mean difference (d) and the mean measurement (m) were calculated by statistical methods of Bland and Altman. The percentage d/m x 100 was calculated for inter-and intra-observer measurements. These percentages represent inter-and intra-observer variability.

Results

Karyotype and cytogenetic study

This work provides the first description of the chromosome formula of Dumbo rat. G-banded karyotypes of Wistar and Dumbo fibroblast cells at the late metaphase are presented. The G-banding pattern of Wistar shows close resemblance to that of *Rattus norvegicus* obtained by 'Committee for a standardized karyotype of *Rattus norvegicus* (22).

The comparative analysis of Dumbo chromosomes with those of Wistar puts forward the analogies between these two strains. The diploid number is 42 in both strains. Comparison of G-banding patterns revealed no differences between *Rattus norvegicus* and Dumbo rat. Figures 3 and 4 highlight the analogies, which exist between the chromosomes of Dumbo and those of Wistar.

Comparison of morphologic and morphometric analyses

Overall length of the embryos

There is not significant difference at the level of the overall length between both strains of rats in the same embryonic age.

Macroscopic descriptions of "dumbo" malformation

Macroscopic descriptions of "dumbo" malformation were realized on the embryos and adults. We noted that the Dumbo embryos presented snout shortness, as well as, hypoplasia of the zygomatic bone, micrognathia and microstoma, which was confirmed by measurement of the mandibular and maxillar

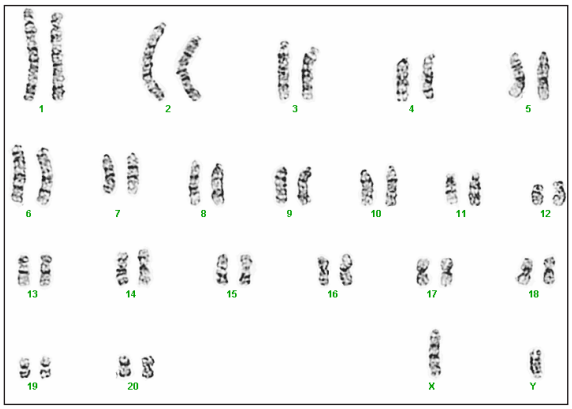

Fig 3: karyotype of the Dumbo rat

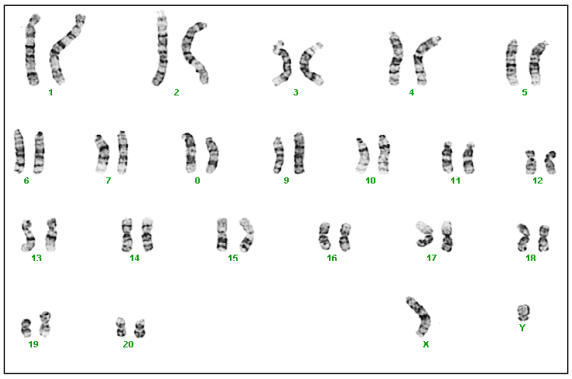

Fig 4: karyotype of the Wistar rat

bones. Moreover, the position of the preliminary ear was abnormally low. Middle ear ossicles blastemata were normal and the shape of otic capsule appeared also similar to that in control embryos. Twenty Dumbo embryos that have different ages showed exophthalmia, which was probably due to hypodevelopment of the zygomatic bone. Figure 5 shows these characteristics at gestational day 17. All the malformations in the Dumbo embryos were clearly evident in the adult Dumbo rat.

Examination of embryonic skulls stained in toto with Alcian blue and alizarin sulphate

Further, examination of embryonic skulls stained in toto with Alcian blue and alizarin sulphate at different ages from E15 to 21, revealed net hypoplasia of the mandible, maxillar and zygoma associated with abnormalities of the ear position in Dumbo embryos. The shortness of the maxillar and mandibular bones give the mouth of the Dumbo rat a specific form (microstomia).

E15

At this age, the ossification was not observed in either Wistar or Dumbo rats.

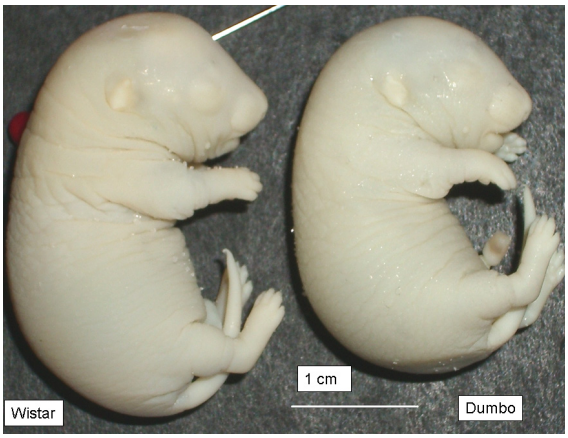

Fig 5: comparison between E17 Dumbo (left) and Wistar (right) embryos drawn under a Lucida camera. Bar: 1cm

E16

The first ossification centers appeared at the vicinity of the cartilage of Meckel in both strains. The inner ear primordium was low situated in the Dumbo rat, but its shape of appeared similar to that in control embryos. At this age the other structures of skull are still not ossified in both strains of rats.

E17

The osseous growth of the mandible is evident, with increases in its length and thickness. In both races, we saw ossification that invaded several craniofacial regions, representing the beginning of the formation of the frontal, parietal, maxillar and zygomatic bone that usually grow more slowly in Dumbo rat. Figures 8 highlights these differences.

E18

The figure 6 shows the embryonic skulls of Wistar and Dumbo rat. The osseous development of the mandibular, maxillar and zygomatic bone becomes clearer, as shown by measurements carried out using the camera Lucida (Figs 8). These measurements show

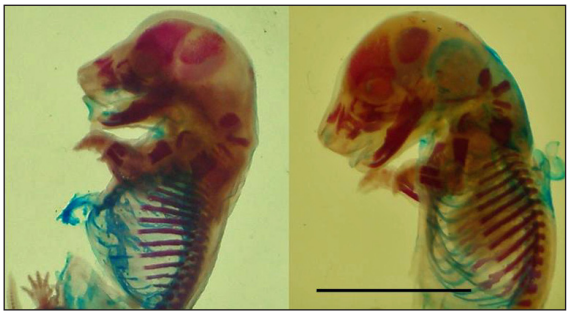

Fig 6: comparison between *in toto* staining of E 18 Wistar (left) and Dumbo (right) embryos. Bar: 1 cm.

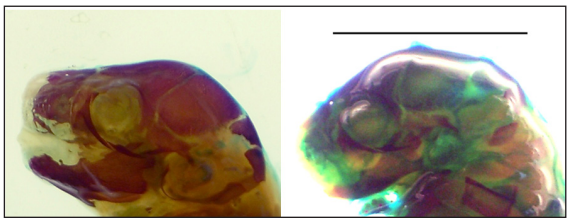

Fig 7: comparison between in toto stainings of E21 Wistar (left) and Dumbo (right) embryos. Bar: 1 cm.

differences like those we previously found between the two strains. These differences involve several components, such as the mandibular, maxillar and zygomatic bones, which usually grow more slowly in Dumbo rat. The position of the petrous bone is all always lower in the Dumbo rat.

E19, E20, E21

The differences between Dumbo and Wistar rats in craniofacial growth and development are maintained at all embryonic ages (Figs 8). Figure 7 shows the growth osseous of mandible, maxillar, and zygoma, which are more slowly, and the position of the petrous bone is all always lower in the Dumbo rat. The graphics elucidate the differences between Dumbo and Wistar rats in craniofacial development at gestational days 16 to 21.

Statistical analyses

Concerning the mean of length and thickness of the zygomatic bone, there are larger in the Wistar rat than in the Dumbo rat. Therefore, the difference in the zygomatic length between the two groups of rats is significant, always ($P< 0.05$). On the other hand, the difference in the zygomatic thickness is not significant.

Concerning the length of the mandible and its anterior and posterior thicknesses, there are larger in Wistar rat than in Dumbo rat. Consequently, the difference between the two groups of rats in its mean of length is significant, in all the ages and always ($P< 0.05$).

The difference in the anterior thickness of the mandible is not significant at E16, but becomes more significant since E18 ($P< 0.05$).

The same phenomenon is observed with the posterior thickness of the mandible ($P< 0.05$).

The length of the maxillary bone is larger in Wistar rat than in Dumbo rat, this difference becomes significant ($P< 0.05$) at E20 and E21.

Concerning the height of the petrous bone, it is higher in Dumbo rat than Wistar, this difference between the two groups is always significant, ($P< 0.001$). Table 1 shows p-level for several structures in different ages.

For total measurements, the percentage of the intra-observer variability was 0.5% whereas; the percentage of the inter-observer variability was 1.2%.

Discussion

The Dumbo rat exhibits some characteristics; shortness of the maxillar, zygomatic and mandibular bones, low position of the ears, evoking human dysmorphogenesis such as the mandibulofacial dysostosis (Treacher-Collins syndrome, (TCS)). This syndrome is characterized by micrognathia, deformity and low position of the ears, and hypoplasia of the mandibular and zygomatic arches. Conductive hearing loss and cleft palate are often pre-

	E16	E17	E18	E19	E20	E21
zygomatic length		0.003	0.03	0.01	0.003	0.04
zygomatic thickness		0.37	0.8	0.9	0.3	0.3
mandible length	0.01	0.003	0.02	0.01	0.04	0.01
anterior mandible thickness	0.39	0.01	0.02	0.04	0.04	0.04
posterior mandible thickness	0.17	0.02	0.01	0.01	0.003	0.04
maxillary length		0.1	0.3	0.2	0.04	0.04
petrous height	0.006	0.003	0.001	0.006	0.003	0.003

Table 1: p-levels

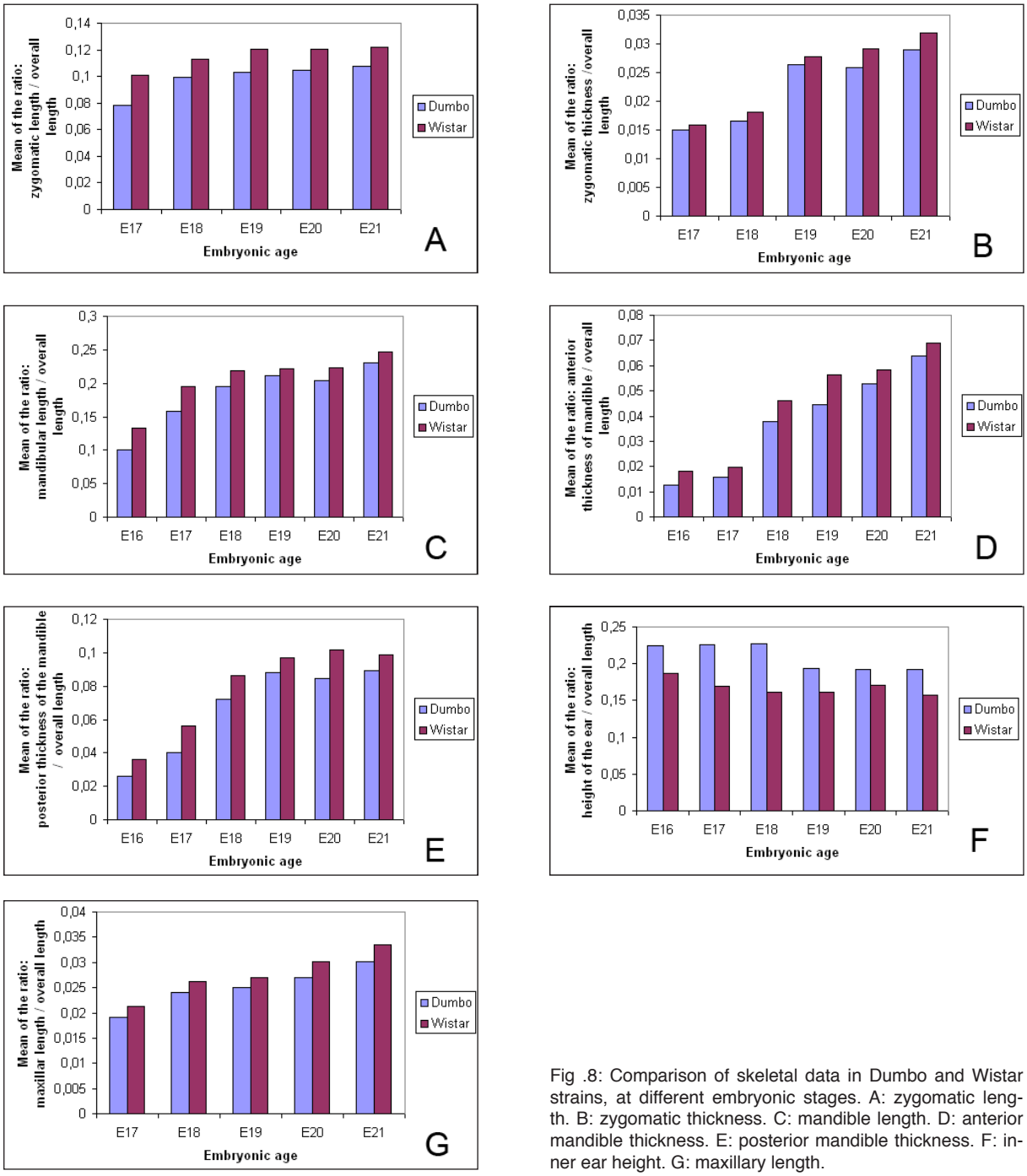

Fig .8: Comparison of skeletal data in Dumbo and Wistar strains, at different embryonic stages. A: zygomatic length. B: zygomatic thickness. C: mandible length. D: anterior mandible thickness. E: posterior mandible thickness. F: inner ear height. G: maxillary length.

sent (23-25). Teber et al (25) identified TCOF1 mutations in 28 of 36 (78%) patients with a clinically unequivocal diagnosis of TCS. Dixon (26) showed that this syndrome is an autosomal dominant disorder caused by inherited or spontaneous mutations in the TCOF1 gene located on human chromosome 5tq31-q34 (23-28). The mutations in the TCOF1 gene lead to diminution of Treacle, the protein encoded by TCOF1 gene. This protein is required for normal proliferation of crest cells because; it controls the production of mature ribosomes. Therefore, TCOF1/ Treacle is a spatiotemporal regulator of ribosome biogenesis, a deficiency that causes apoptosis and disrupts neural crest cell formation and proliferation, leading to craniofacial abnormalities (29).

Furthermore, both the Di George syndrome, which is caused by deletion 22q11 (30), and the Nager syndrome, which probably involves

locus 9q32 (31) are characterized by shortness of the maxillary, zygomatic and mandibular bones, and low position of the ears. The similarities between macroscopic findings of these syndromes and the specific characteristics of the Dumbo rat indicate that they all can involve similar disturbances in morphogenesis.

Our study shows that the Dumbo embryos presented microstoma comparable with that seen in the human malformation known as hemifacial microsomia (Goldenhar syndrome or HFM) and in *Hfm mice* (transgenic mutation of a locus termed *Hfm* on chromosome 10). *Hfm* mice, which were generated by crossbreeding wild type and heterozygous adults from the 643 transgenic lineage (32, 33), may provide insight into the causes of the Goldenhar syndrome. It has been hypothesized that at least some *HFM* anomalies have a genetic basis and are mediated by mesenchymal disruptions and possibly embryonic hemorrhages (34). Otani et al (35) conclude that in *Hfm* embryos the branchial arch adjacent to the hemorrhage site displayed normal cell density but reduced dimensions following phagocytosis of the hematoma. This implies a reduction in arch mesenchymal cell numbers. The low position of the ears and developmental anomaly of the external ear in the Dumbo rat resembles that in the *Lse* mouse mutant. The *Lse* gene in mice is located on chromosome 7 but chromosomal preparations of the *Lse* mutant appear to be normal (35). Low-set ears (Lse) are a dominant mutation and pharyngeal arch disorder in mice that is characterized by malformed and malpositioned external ears and eye defects. Lse mice also have reduced growth after birth and reduced viability. Lse was first observed in 1983 in a male mouse issuing from a cross between a female ovarian transplant recipient utilized to maintain dreher-J, and a male from the inbred strain C3HeB/FeJLe-a/a. Theiler and Sweet (35) studied *Lse* and suggested that the Lse phenotype is only the superficial manifestation of a gene, which when mutated has deleterious effects on other organ systems also derived from the branchial arches. Other study (36) showed that Lse mouse features have their parallel in many human syndromes, including mandibulofacial dysostosis (Treacher-Collins syndrome). This suggests that *Lse* gene may be function on the neural crest cell proliferation and apoptosis pathways as TCOF1 gene.

Furthermore, the Dumbo characteristics are similar to those provoked in C57BL mice by the administration of 400 mg/kg of retinoic acide (RA) on gestation day 9 (17). They note that administration of RA induces cephalic abnormalities in the embryos, simulating the human TCS; microstomia, hypoplasia of the zygomatic and mandibular bones, micrognathia, auricular abnormalities, low position of ear. Poswillo (38) showed that administration of RA to rats in gestation induces mandibulofacial dysostosis TCS. Morriss-Kay and her co-workers showed that administration of RA to mice induces abnormalities in the branchial arches (39-41). They attributed these abnormalities induced in mouse by RA to abnormal migration of the neural crest cells and to an abnormal pattern of the expression of the Hox genes in the branchial mesenchyme diverted of neural crests. Moreover, RA activates the Hox genes differently and the degree of activation depends on the location of gene in the cluster Hox, time of exposition to the retinoic acid and concentration of this acid (42). The retinoic acid is an important physiological regulator of cellular differentiation, proliferation, apoptosis, reproduction, and embryonic development in many species, and that its excess or a deficiency during gestation results abnormal tissues whose origins depend on the establishment of the central body axis, the organization of the animal along the anterior/posterior axis (43-44). Later, Holland and Holland (45) showed that (RA) changes the pattern of Hoxb-1 expression in mice.

The Hox genes regulate anteroposterior (A-P) identity of the cranial neural crest (CNC). This suggests that some aspects of the CNC developmental program are encoded in the premigratory CNC cells (12, 46). While the Hox genes apparently can specify A-P properties of the CNC for the second and more posterior arches, other genes that are expressed in the central nervous system anterior of rhombomere 3, such as Otx-2, must be essential for regulating development of the first arch. Otx-2 is expressed in the mesencephalic neural plate (47), which contributes CNC, to the first arch (11, 13). Mice with a heterozygotes Otx-2 mutation have abnormal bones derived from the mandibular and maxillary components of the first arch (48). Further, Msx genes are an immediate-early response gene to BMP4 and act in dorsal-ventral and head-trunk patterning of the embryo (49-50). During NC induction, the Msx1 gene is upregulated at the neural border (51).

Other genes are likely to be responsible for programming development along the proximodistal (P-D) and mediolateral (M-L) dimensions of the arches. At least six Dlx genes (Dlx-1, -2, -3, -5, -6 and –7) are expressed in spatially restricted patterns in craniofacial mesenchyme and ectoderm of vertebrates (52-64). Using mutant (transgenic) mice showed that the Dlx genes participate in P-D patterning (65).

Conclusion

Our comprehension of craniofacial malformations has been hindered by the diversity of the phenotypes and by the obscurity of their aetiology. The Dumbo rat which, is reminiscent of some characteristics of human dysmorphogenesis, may help clarify the process of malformed development. The disorders observed in Dumbo rat may be due to trouble in the information carried by the neural crests cells, leading to abnormal proliferation, migration, or apoptosis of these cells, and subsequently to anomalies in the rostro-caudal gradient of embryogenesis.

The chromosomal data revealed a similarity between the Dumbo and Wistar rats and did not reveal any chromosomal malformation in the Dumbo rat. However, we cannot exclude the possibility that genetic mutations play a role in the morphological differences between the two rats. Point mutations, and short deletions, insertions, and substitutions in the DNA sequence go undetected by traditional cytogenetic analysis even though they may lead to morphological differences. This may be particularly true if a regulatory gene participating in developmental processes is affected. Even more extensive deletions, insertions or rearrangements of DNA also may not be observed microscopically.

Our data indicate that the significant morphometric differences in the craniofacial structures between Dumbo and Wistar rats might be due to genetic mutations that are undetectable by chromosome mapping. To identify the genetic basis of these differences, other types of investigations would be needed, such as studying the expression of genes implicated in craniofacial morphogenesis, e.g. Msx1, Msx2, Dlx1, Dlx2, Dlx4, and Pax1. This can be done by PCR or by immunodetection. Genes identified as potential contributors to the Dumbo phenotype may be sequenced to identify possible mutations.

A microscopic study of the histological sections could be interesting for examine the early phases of the craniofacial development, and analyze the craniofacial chondrogenesis and osteogenesis. Morphometric sagittal and frontal analysis of the adult skull of both strains must also be performed, as well as a frontal cephalometry in fetuses using more accurate techniques than *camera lucida drawings.* Elucidation of the mechanism underlying the Dumbo phenotype is expected to contribute to better understanding of some human facial malformations.

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Correspondence to:
S. Louryan, MD PhD
Laboratory of Anatomy and Embryology
Université Libre de Bruxelles
C.P.619, Route de Lennik, 808
B-1070 Brussels
Belgium
Tel: 32 2 555 6376
Fax: 32 2 555 6378
E-mail: Slouryan@ulb.ac.be