CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

Masticatory Muscle Defects in Hemifacial Microsomia: A New Embryological Concept

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Received 13 December 2010; Accepted 13 April 2011

First arch syndromes correspond to a wide spectrum of human latero-facial congenital anomalies affecting cranial neural crest cells (CNCCs) derivatives of the first pharyngeal arch (PA1). The abnormal traits display variable quantitative expression and are often unilateral. Mandibular skeletal defects are invariably accompanied by hypoplasia or agenesis of masticatory muscles, but no explanation has been proposed for this association. Indeed, during embryonic development, CNCCs give only rise to skeletal components of the head while muscles derive from cephalic myogenic mesodermal cells (CMMCs). Recent studies on animal models have shown that communication between CNCCs and CMMCs is essential for the development of masticatory muscles: genetic lesions affecting only CNCCs can prevent muscularization of the jaws. To evaluate the involvement of CNCC/CMMC interactions in human craniofacial development, we performed a quantitative analysis of masticatory muscle and mandibular bone volumes on craniofacial CT-scans from 8 children, ages 3 months to 16 years, affected by hemifacial microsomia. We found that: (1) in seven patients the masseter muscle is absent in the affected side; (2) the absence of masseter is correlated neither with the age of the patients nor with the volume and shape of the affected ramus; and (3) in all cases the pterygoid and the temporal muscles are either reduced or absent. Our findings suggest that an early developmental event is the origin of the muscular defects in these patients. We propose that the hypoplasia or agenesis of masticatory muscles derives from a defect in the CNCCs/CMMCs communication during early embryonic development.

Key words: hemifacial microsomia; CT-Scan; 3D reconstitution; mandible; masticatory muscles; craniofacial development; cranial neural crest cells; cephalic myogenic mesodermal cells

How to Cite this Article:
Heude E, Rivals I, Couly G, Levi G. 2011. Masticatory muscle defects in hemifacial microsomia: A new embryological concept. Am J Med Genet Part A 155:1991–1995.

INTRODUCTION

During jaw development of vertebrate embryos, cranial neural crest cells (CNCCs) from the posterior mesencephalic neural fold and the first rhombomeres migrate to the first pharyngeal arch (PA1) to give rise to the skeletal maxillo-mandibular elements [Couly et al., 2002]. In contrast, masticatory muscles are formed by PA1 cephalic myogenic mesodermal cells (CMMCs) [Couly et al., 1992; Trainor et al., 1994].

Although jaw muscles do not derive directly from CNCCs, these cells are a necessary source of molecular cues for the determination, differentiation, and patterning of CMMCs [Rinon et al., 2007; Grenier et al., 2009; Tokita and Schneider, 2009; Heude et al., 2010]. The CNCCs–CMMCs interaction is necessary to maintain the myogenic program in the CMMCs, leading to masticatory muscle formation.

First arch syndromes are human congenital malformations of the face resulting from defects of neural crest skeletal derivatives [Gorlin, 2001]. The specific type of first arch syndrome, hemifacial microsomia, is characterized by asymmetric defects of skeletal proximal PA1 derivatives. The most commonly affected skeletal structures include (1) the ascending ramus of the mandible, which is reduced or absent while distal mandibular components and teeth are not affected; (2) the temporo-mandibular joint; (3) the zygomatic arch; and (4) most components of the external and middle ear including the incus, the malleus, and the tympanic bone (see, e.g.,

Additional supporting information may be found in the online version of this article.

Grant sponsor: Association Française contre les Myopathies (AFM); Grant sponsor: Agence Nationale pour la Recherche (ANR) project DrOS and project Gendactyl.

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Published online 8 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.34095

Supplementary Fig. 1). The consequence is a lateral deviation of the mandible accompanied by malocclusion and hearing deficiency.

Several studies have shown that masticatory muscles are also affected in first arch syndromes. However, no explanation has been proposed for the correlation between skeletal and muscular defects [Marsh et al., 1989; Kane et al., 1997; Huisinga-Fischer et al., 2001; Takashima et al., 2003; Hirschfelder et al., 2004; Huisinga-Fischer et al., 2004]. The craniofacial and cardiovascular features of many patients with first arch syndrome suggest CNCCs developmental defects [Johnston and Bronsky, 1995; Kallen et al., 2004]. This hypothesis is supported by mouse models of first arch syndromes in which deregulation or mutation of genes expressed by CNCCs recapitulated the human phenotype [Dixon et al., 2006; Zhu et al., 2007]. Here, we analyzed craniofacial CT-scans of children affected by first arch syndrome, displaying hemifacial microsomia. In all cases, unilateral proximal mandibular bone defects are associated to hypoplasia or aplasia of masticatory muscles on the affected side. Extending the embryological finding of CNCCs/CMMCs interaction in the control of craniofacial myogenesis, we propose that CNCCs developmental anomalies might be the primary cause of the muscle defects observed in patients with first arch syndrome.

MATERIALS AND METHODS

Patients

The cohort includes eight patients (four males and four females, mean age 7 years and 11 months, range 4 months to 16 years and 7 months) with hemifacial microsomia (Table I). The right hemimandible is affected in two patients, while the left is affected in the remaining six. Patients were evaluated and managed at the Service de Chirurgie Maxillofaciale et Stomatologie of Hôpital Necker-Enfants Malades (Gérard Couly, Paris, France). Institutional review board approval was obtained. CT examinations were performed between 2006 and 2010 to evaluate individual malformations before surgical intervention and reconstruction.

CT-Scan Analysis

Quantitative analysis of the mandibular bone and masticatory muscle volumes (including the masseter, pterygoid, and temporal muscles) was performed on craniofacial CT-scans from eight children affected by hemifacial microsomia, and the volumes of the affected and non-affected components were measured. CT-scans were performed on either a Philips 16-slice or 40-slice detector (Philips Medical Systems, Cleveland, OH) with the acquisition of contiguous 0.6 mm axial sections. These axial images were then processed automatically into 2 or 3 mm axial, coronal, and sagittal reconstructions using volumetric analysis and a bone detail algorithm.

CT-scan data of each subject were analyzed using the OsiriX® v.3.5 imaging software (University Hospital of Geneva, Switzerland) to evaluate the volumes of hemi-mandibles and masticatory muscles and to carry out 3D reconstructions (Fig. 1 and Supplementary Fig. 1 online). For bone and muscle volume measurements, regions of interest (ROIs) were segmented in each axial slice (Fig. 1C). Then, the OsiriX software computed each ROI series to calculate the volume and to generate 3D interactive representation of the skull and masticatory muscle masses (Fig. 1D–E).

The lateral and the median pterygoid muscles were considered as a single pterygoid muscle mass due to the difficulty to resolve the two juxtaposed muscle masses.

All patients presented unilateral dysplasia. As the volume of masticatory muscles of the non-affected side does not show compensatory effect [Huisinga-Fischer et al., 2001; Huisinga-Fischer et al., 2004], the mandibular bone and masticatory muscles of the non-affected side were used as controls.

Statistical Analysis

To test the significance of the volume reduction when the muscle is present in the affected side, the ratio between the volumes of affected and unaffected components was estimated as the slope of a linear regression of the volumes of the affected side versus the

TABLE I. Characteristics of Patients With Hemifacial Microsomia and Masticatory Muscle and Hemi-Mandible Volumes

				Non-affected side				Affected side			
				Volumes (cm^3)				Volumes (cm^3)			
Patient number	Sex	Affected side	Age	Masseter muscle	Temporal muscle	Pterygoid muscle	Hemi-mandible	Masseter muscle	Temporal muscle	Pterygoid muscle	Hemi-mandible
1	Male	Right	3 months	1,25	2,82	1,61	5,89	0	1,75	0,76	4,84
2	Male	Left	6 months	2,15	4,23	1,99	5,97	0	0	0,21	4,21
3	Female	Left	5 years 5 months	4,69	10,33	3,69	10,03	0	7,33	0	3,09
4	Female	Left	6 years 4 months	9,57	19,67	9,54	19,37	0	4,49	6,62	11,68
5	Female	Left	6 years 10 months	14,11	24,03	11,14	19,85	6,77	17,39	7,73	16,47
6	Male	Left	11 years 5 months	13,37	24,34	16,27	20,99	0	0	0	5,94
7	Male	Left	15 years 11 months	24,09	30,18	13,47	32,04	0	16,68	4,65	23,32
8	Female	Right	16 years 7 months	22,3	21,14	15,3	25,12	0	15,51	7,58	12,78

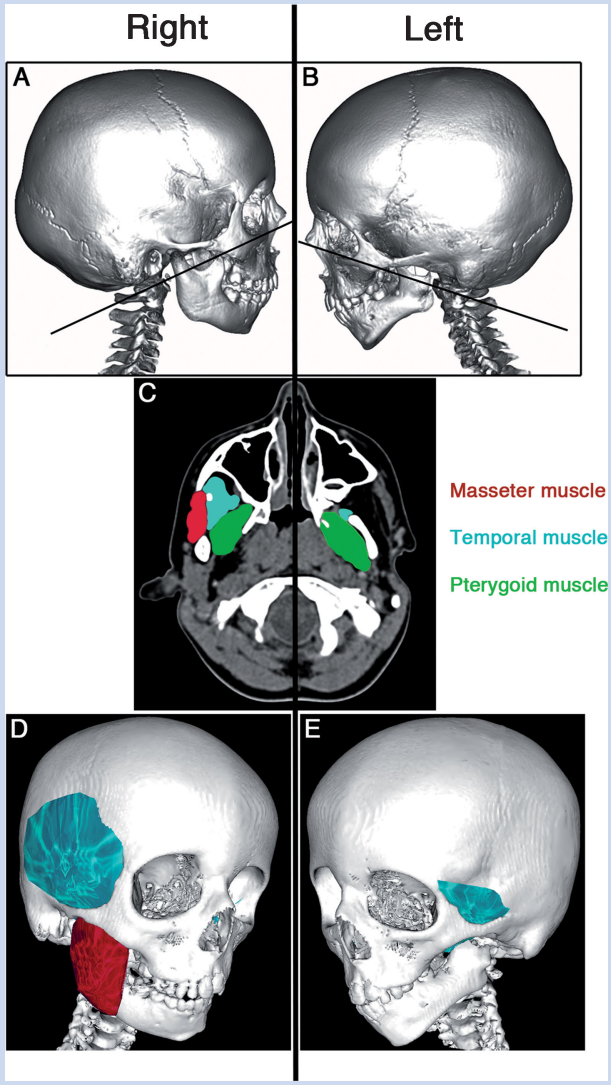

FIG. 1. Craniofacial CT-scan and 3D reconstructions of a patient with hemifacial microsomia. The patient is a 6-year-old girl (Patient 4) affected by hemifacial microsomia. A,B: right (unaffected side) and left (affected side) view of a skull 3D reconstruction. C: CT-scan section (at the indicated level in A and B) with the defined masticatory muscles. D,E: right and left view of masticatory muscle 3D reconstruction. Note the absence of masseter muscle (in red) on the affected side and the strong reduction of the temporal muscle (in blue). On this specific section the pterygoid muscle (in green) does not appear reduced, but its global volume is reduced by 31% (see Supplementary Table I online). The patient is the same as depicted in Supplementary Figure 1 online.

unaffected side (Fig. 2). We performed a weighted least squares analysis. The volumes on both sides are subject to measurement uncertainty that is proportional to the volumes themselves. As the standard deviation of this uncertainty is much smaller than the volume range, the uncertainty on the input (the volume on the unaffected side) can be neglected. However, the increasing variances of the output (the volume on the affected side) with the volume value are taken into account by weighting the measurements by the inverse of the volume values on the affected side. Except for the masseter muscle for which a single non-zero volume is available in the affected side, a 95% confidence interval (c.i.) can be computed in addition to the point estimate of the slope.

RESULTS

The shape of craniofacial bones and of the masseter, pterygoid, and temporal muscles was reconstructed from serial CT-scan sections (Fig. 1). All patients presented a unilateral reduction of the ascending ramus and temporo-mandibular joint of the mandible, defects of the zygomatic arch, and abnormal middle and external ear (see, e.g., Supplementary Fig. 1). The severity of the mandibular defect was very variable ranging from virtual absence to mild reduction of proximal components.

Patient 6 presents the most severe mandibular volume reduction (72% reduction of the affected mandible, see supplementary Table I online). In this patient all masticatory muscles are absent on the affected side.

In 7 of 8 patients the masseter muscle was absent on the affected side. The masseter absence did not correlate with the mandibular reduction, this muscle was absent both with mild (e.g., Patients 1 and 2) and severe (e.g., Patients 3 and 6) mandibular phenotypes.

The temporal and pterygoid muscles were invariably reduced or absent (Table I, Supplementary Table I online).

Linear regressions for the mean ratio between the affected and unaffected side volumes resulted in estimates and 95% c.i. of 0.49 [0.24; 0.74] for the temporal muscle, 0.43 [0.21; 0.66] for the pterygoid muscle, and 0.51 [0.33; 0.69] for the hemi-mandible (Fig. 2). The size of hypoplastic structures was, therefore, approximately 50% reduced. As no c.i. includes one, all volumes are significantly reduced on the affected side.

DISCUSSION

We have shown here that the mandibular skeletal defects in hemifacial microsomia are associated with hypoplasia or aplasia of masticatory muscles. In particular, the masseter muscle was absent in the affected side in six of seven patients.

Recently, we demonstrated that PA1 CNCCs, which give rise to skeletal element of the jaws, are required for the differentiation and patterning of PA1 CMMCs leading to masticatory muscle formation [Heude et al., 2010]. Based on experimental results from animal models [Rinon et al., 2007; Grenier et al., 2009; Tokita and Schneider, 2009; Heude et al., 2010], we hypothesize that the CNCCs developmental anomaly involved in first arch syndrome is at the origin of the muscle defects observed in the patients. The CNCCs may have lost the ability to induce the CMMCs to form masticatory muscles (Fig. 3).

The absence of the masseter was not directly correlated to the severity of the mandibular bone involvement. If indeed the muscular defect results from a CNCC/mesoderm communication problem, our evidence would suggest that muscle malformation occurs during early development, prior to the morphogenesis of the neural crest-derived mandibular bone.

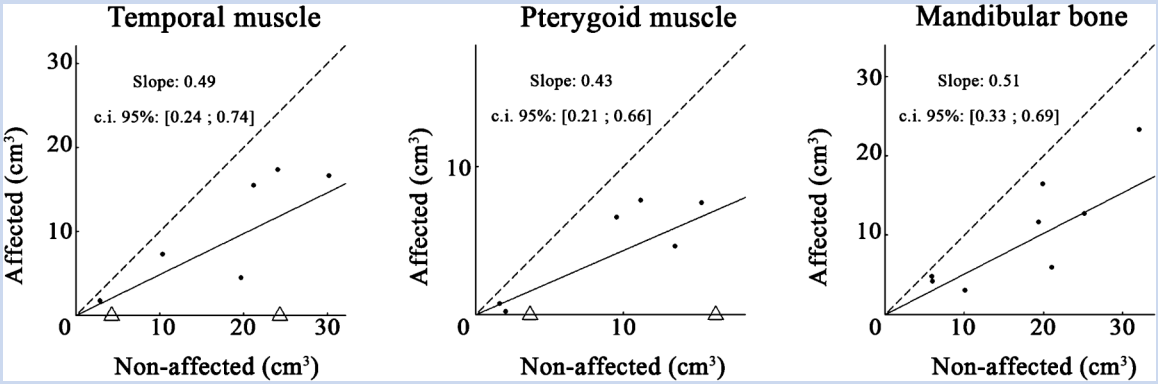

FIG. 2. Linear regression analysis of the volumes of affected versus unaffected masticatory muscles and hemi-mandibles. The analysis includes only hypoplastic, and not aplastic muscles, which are indicated as triangles on the ordinates. The slope and the 95% confidence interval (c.i.) are presented for each linear regression. The masseter muscle was not included as it was absent in 7 of 8 patients. None of the c.i. includes one, which means that all volume reductions are statistically significant (the dashed line indicates the bisector with unit slope).

Another fact supporting an early defect is continued growth of the hypoplastic muscles during postnatal development (as seen in Table I), suggesting that their basic physiology is preserved and that they do not regress due to the abnormal skeleton.

This new embryological concept could contribute to improved understanding of the aetiology for hemifacial microsomia, and it might be useful for prenatal diagnosis. In human embryos, CNCC colonize the first pharyngeal arch at around 4 weeks [O'Rahilly and Muller, 2007] and first arch syndromes are diagnosed at around 20 weeks on the basis of skeletal and external evidences [Castori et al., 2006]. With significant advances in prenatal ultrasound examination including high-resolution ultrasound, 3D and 4D

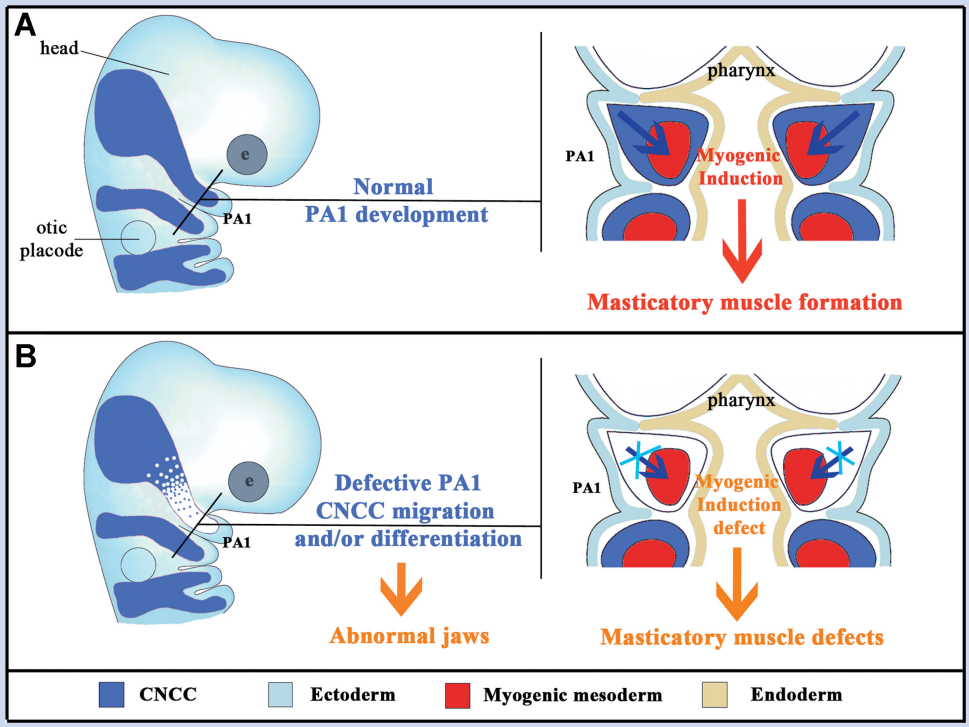

FIG. 3. Schematic view of CNCC–CMMC interactions during jaw development. On the left, the migratory routes of the CNCCs (blue) during early head formation are shown. The right part of the diagram represents a frontal section of either a normal embryo (A), or an embryo in which CNCCs migration did not occur normally (B), as it might happen in hemifacial microsomia. During normal development CNCCs (blue) provide signals to the myogenic mesoderm (red) in order to induce its differentiation into masticatory muscles.

technology, recognition of fetal facial movements and muscular growth, and shape may permit detection of early abnormal development as early as 12–13 weeks. At early stages these defects could be more easily detectable than skeletal abnormalities, because ossification of the mandible is still incomplete. Moreover, detection of muscle aplasia is important to direct postnatal surgical strategies and the choice of costochondral or iliac grafts used for mandibular reconstruction [Miloro et al., 2004].

ACKNOWLEDGMENTS

We thank Dr. Y. Gitton for helpful comments. This work has been supported by grants in aid by the Association Française contre les Myopathies (AFM), the Agence Nationale pour la Recherche (ANR) project DrOS, and Gendactyl. E.H. is recipient of a doctoral fellowship from the Fondation pour la Recherche Médicale (FRM).

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