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Genetic response in masseter muscle after orthognathic surgery in comparison with healthy controls – A Microarray study

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ABSTRACT

One third of adult patients with orthognathic surgery of a prognathic or retrognathic mandible show relapse. The sagittal split osteotomy of the mandible leads to a displacement of both parts up to 10 mm without any changes of muscle attachment. Changed mandible length needs adaptation of muscle capacity because of changed force to moment ratio. The aim of this Microarray study was to analyze the general genetic response of masseter muscle in patients with retrognathism or prognathism of the mandible six months after surgery in comparison with healthy untreated controls. We found in tissue samples from masseter muscle a reduction of different entities between patients and controls but less in retrognathic than in prognathic patients (274/429). The different entities to controls in prognathia were reduced from 1862 to 1749 but increased in retrognathia from 1070 to 1563. We have to consider that the total amount of different entities to the controls is higher in patients with prognathic mandible (7364) because of their strong genetic controlled development compared with that in patients with retrognathic mandible (4126), which is more environmentally influenced.

It can be concluded that function follows form after surgical change with high inheritance. In retrognathic patients the adaptation could be delayed or the capacity of regeneration potential is not sufficient.

1. Introduction

Adult patients with severe craniofacial deformities must undergo orthognathic surgery. Displacement of the mandible up to 10 mm in a sagittal and/or vertical direction leads to stretching or compression effects in mastication muscles (Hunt et al., 2006). But the origin and insertion of the muscles, especially the masseter muscle, stayed in unchanged positions. Several factors are involved in the change of functional requirements, including alterations to the mechanical advantage of the muscle force to moment ratio (M/F ratio) associated with the surgically induced change in length of the mandible, i.e. lengthening in retrognathic and shortening in prognathic cases (Harzer et al., 2010). Similarly, orthognathic surgery improves the quality and efficiency of the occlusion and mastication, which may induce on the one hand, less muscle activity due to the improved number of occlusal contacts, but equally may facilitate the development of higher forces dissipated through the greater number of teeth. These changes require a functional adaptation in the masticatory muscles. The masseter pterygoideus muscle sling plays an important role in the vertical position of the teeth and the mandible (Profitt, 1978; Hunt et al., 2006). An optimal occlusion protects the mandible against sagittal movement.

Investigations showed an instability or relapse in 20–30% of cases after orthognathic surgery. Eggensperger et al., 2006 found in retrognathic patients with orthognathic surgery a relapse of 50% after 12 years. Reasons can be an incorrect occlusion or a missing adaptation of the mastication muscles (Ayoub et al., 1997; Proffit et al., 1991; Hunt and Cunningham, 1997). Carslon et al. (1987) and Ellis and Carlson (1983) showed in animal experiments that the suprahyoid musculature remains attached to the distal mandibular segment and may be stretched by the advancement procedure. Ayoub et al. (1997) postulated that this factor could have affected the amount of tension generated by the suprahyoid

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muscles, and therefore the amount of relapse is the magnitude of mandibular advancement and autorotation. But in the animal model the adaptation in lip posture and pressure as well as the equilibrium between resting pressure of tongue and lips following orthognathic surgery could not be considered. Fränkel (1969) emphasized the important role of active muscle training of orbicularis oris muscle for neutralization of tension generated by suprahyoid muscles in Class II and open bite cases with permanent mouth breathing. It is evident that after mandibular advancement the ability of lip closing and strengthening of the orbicularis oris muscle would be much improved. Profitt and Phillips (1988) found when soft tissues were relaxed as the mandible rotated forward following superior repositioning of the maxilla, resting pressures decreased and gave support for the equilibrium between tongue and lips.

The myosin heavy chain (MYH) is the important contractile protein encoded by a group of genes consisting of I (slow), IIa, IIb, IIx (fast), extraocular, embryonic and neonatal genes. In situations of stress, either the MYH expression in the fiber is able to change from one phenotype into another or satellite cells are stimulated (Gedrange et al., 2006). Studies have shown that the adult masseter muscle contains unusual myosin isoforms specific to early developmental stages in skeletal muscles, i.e. embryonic (MYH3) and fetal (MYH8) (Soussi-Yanicostas et al., 1990). It has been suggested that control of masseter muscle development involves mechanisms distinct from those of other skeletal muscles, perhaps as a consequence of their craniofacial innervations or different embryonic origin (Soussi-Yanicostas et al., 1990; Talmadge, 2000). Oukhai et al. (2011) found different up and down regulation of MYH3 and MYH8 in masseter muscle six months after surgery in prognathic and retrognathic patients in comparison with the level before operation. This means that the genetic response of the masseter muscle is different in patients with under or overdeveloped mandibles. The etiology of mandibular prognathism has been attributed more to genetic inheritance pattern and less to environmental factors than in retrognathism.

The aim of this Microarray study was to check in general whether function follows form in both diverse mandible growth patterns.

2. Material and methods

This study was approved by the ethics committee of the Technical University of Dresden, which authorized all surgical and study procedures (No. EK 24608-2009). Tissue samples of masseter muscle were taken from six adult patients with orthognathic surgery (3 prognath/3 retrognath) before and six months after surgery and from three healthy controls during extraction of third molar without orthodontic treatment and orthognathic surgery. An orthopantograph (OPT), lateral cephalogram, and plaster casts were taken before and 6 months after operation from all Class II and Class III patients. Digital lateral photographs were taken from every control in the standard manner (Frankfurt plane horizontal and teeth in centric occlusion, defined by Schwarz, 1958). Lips and chin should be within the jaw profile field between nasion and orbital verticale, e.g. maxilla and mandible are in a neutral position. Overbite and overjet were measured at the models.

The inclusion criterion for Class II patients was an ANB angle of >3° (SNB <77°, SNA >80°, ML/NL 18–26°), and criteria for Class III patients were an ANB angle of <0° (SNB >80°, SNA >79°, ML/NL 18–26°) and sagittal displacement of >3 mm in both groups. Inclusion criteria for the controls were neutral jaw position and occlusion, e.g. lips and chin were within the profile field. Overjet and overbite should be >2 mm and <3 mm. The sexes should be balanced. Patients with cleft lip and syndromes were excluded. The bimaxillary osteotomy, a combination of LeFort I and Obwegeser/Dal Pont procedures, or mono-maxillary osteotomy of the mandible, were done by the same surgeon for all patients. The insertion and origin of the masseter muscle remained unchanged (Breuel et al., 2013).

Sample sizes were 2 × 3 × 3 mm with a wet weight of 21.7 mg. From each patient a biological replicate was stored. They were shock frozen in liquid nitrogen immediately after removal and then stored at −80 °C.

Total RNA from the muscle biopsies was isolated with peqGOLD TriFast™ (peqLab, Erlangen Germany). Reverse-transcribed PCR was performed with RevertAid™ First Strand cDNA Synthesis Kit (Fermentas GmbH, St.Leon-Rot, Germany) according to the manufacturer's instructions. Analysis was carried out with Microarray technique (SurePrint G3 Human Gene Expression 8 × 60K Microarray Design ID 02800). ANOVA and unpaired t-test ($p < 0.05$) were used for statistics in the Microarrays.

To determine correlations and significant differences among the investigated genes, SPSS Vers.19 for Windows was used. The linear correlation between absolute values at T1 to T2 of all examined genes was shown with the Pearson correlation coefficient.

Statistical analysis for group comparisons was performed with Mann–Whitney U-test. Means and standard errors were calculated from individual values by standard procedures. Significance was set at $p < 0.01$ and 0.05.

3. Results

An overall reduction of gene expression differences between controls and patients after surgery was shown in the Microarray study. The reduction of 429 entities (units of gene expression changes) in prognathic patients was higher than in retrognathic patients (274) (Fig. 1). This means that function follows form but the adaptation is determined by the morphological origin.

The expression differences from pre to six months post surgery were demonstrated between both patient groups versus controls with an unpaired t-test under the condition gene expression >2 fold change ($p < 0.05$) (Fig. 2a and b). The different entities in prognathia were reduced from 1862 to 1749 (Fig. 2a) but increased in retrognathia from 1070 to 1563 before and after surgery (Fig. 2b). However, it has to be considered that the total number of different entities is higher in prognathic than in retrognathic patients.

In a 3D-model it was demonstrated that the three controls are located very close together, which is a sign of genetic similarity (Fig. 3, right corner). The spots of the orthognathic patients move closer six months after surgery, but the difference to the entities of the controls is higher in retrognathic than in prognathic patients.

It is remarkable that after selection of special genes (fold change >2) responsible for inflammatory or immunologically mediation, that in Class III the down regulation is higher than in Class II (Table 1).

4. Discussion

The etiology of nonsyndromic mandibular prognathism has been attributed to various genetic inheritance patterns and some environmental factors. The more dominant inheritance in prognathic patients gives support for the greater number of different entities in masseter muscle compared to the controls. The data for the functional adaptation procedures after surgery were unexpected from the genetic point of view and could be summarized in a hypothetic scheme:

Prognathic mandible → shortening → lower M/F ratio → decrease of different entities to the controls

Surgery normal length → functional adaptation?