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ENPP1 and ESR1 genotypes influence temporomandibular disorders development and surgical-treatment response in dento-facial deformities

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Abstract

Dentofacial deformities are dys-morpho-functional disorders involving the temporomandibular joints (TMJ). Many authors have report a TMJ improvement in dysfunctional subjects with malocclusion after orthodontic or combined orthodontic and surgical treatment particularly for the relief of pain. In particular, few studies have highlighted the demographic and clinical predictors of response to surgical treatment. To date, no genetic factor has yet been identified as a predictor of response to surgical treatment. The aim of this cohort study is therefore to identify single-nucleotide polymorphisms associated with postoperative temporomandibular disorders (TMD) or with TMJ symptoms after orthognathic surgery. Here, we found the AA genotype of SNP rs1643821 (ESR1 gene) as a risk factor for dysfunctional worsening after orthognathic surgery. In addition, we have identified TT genotype of SNP rs858339 (ENPP1 gene) as a protective factor against TMD in a population of patients with dentofacial deformities. Conversely, the

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heterozygous genotype AT was identified as a risk factor of TMD with respect to the rest of our population. All these elements are particularly important to bring new screening strategies and tailor future treatment.

Keywords

Orthognathic Surgery; Temporomandibular Joint Disorders; Chronic Pain; estrogen receptor alpha; ectonucleotide pyrophosphatase phosphodiesterase 1

Introduction

Dentofacial deformities are anatomic distortions of the jaws, dentition and face which affect about 20% of the population. They can occur in conditions such as cleft lip and palate and other craniofacial anomalies, or can constitute normal anthropometric variation. Apart from their morphological and dental consequences, dentofacial deformities are dys-morpho-functional disorders involving the temporomandibular joints (TMJ). Subjects with dental malocclusion have a higher rate of temporomandibular disorders (TMD) compared to the population with normal occlusal relationships (Celic et al., 2002; Egermark et al., 2003; Miller et al., 2004; Thilander et al., 2002). This is especially true when increased vertical dimension is a component of malocclusion (Hwang et al., 2006; Nebbe et al., 1998).

Temporomandibular disorders are defined by the American Academy of Orofacial Pain (AAOP) as a group of musculoskeletal and neuromuscular disorders, which involve the masticatory musculature, the temporomandibular joints and associated structures (de Leeuw and Klasser, 2013). The contribution of occlusion in TMD has been debated for a long time (Pullinger et al., 1993). There appears to be a TMJ improvement in dysfunctional subjects with Class II malocclusion after orthodontic treatment (Henrikson et al., 2000; Mohlin et al., 2004). In subjects with more severe malocclusions requiring a combined orthodontic and surgical treatment plan, recent works have enriched the debate (Abrahamsson et al., 2013; Dujoncquoy et al., 2010). *Abrahamsson et al.* (Abrahamsson et al., 2013) have shown in a controlled study on patients with dentofacial deformities, a positive impact on TMJ health from combined orthodontic and surgical treatment, particularly for the relief of pain. Angle Class I occlusion thus seems beneficial to temporomandibular biomechanics, but few studies have highlighted the predictors of response to surgical treatment (Pusan Korea Pusan National University et al., 2015; Scolozzi et al., 2015; Valladares-Neto et al., 2014). Female sex, amount of mandibular advancement and degree counterclockwise rotation of the mandible appear to be the main factors influencing the TMJ health after bilateral sagittal split osteotomy (Valladares-Neto et al., 2014). Women with class II malocclusion and increased vertical dimension as indicated by a larger mandibular plane angle, in fact have less TMJ improvements and are more prone to postoperative condylar resorption.

The scientific advances in the field of genetics since 2005 have allowed identification of single-nucleotide polymorphisms (SNPs) associated with TMD (Diatchenko, 2004). This work has expanded to identify dozens of additional SNPs in the hope of identifying individuals at high-risk prior to onset of TMD and to devise new therapeutic approaches for these patients (Melis and Giosia, 2014). To date, no genetic factor has yet been identified as

a predictor of response to surgical treatment. The aim of this study is therefore to identify single-nucleotide polymorphisms associated with postoperative TMD or with TMJ symptoms after orthognathic surgery.

Materials & Methods

Subjects undergoing orthodontic and maxillofacial surgery treatment for correction of malocclusion were recruited between February 2013 and February 2014 from the Department of Oral and Maxillofacial Surgery at the University of Lille in France. They were recruited after signing an informed consent, and the research protocol was validated by the French independent ethical committee and the Temple University and the University of Pittsburgh institutional review boards. None of the subjects had systemic conditions, facial trauma, tumor, condylar hypertrophy, arthritis, or developmental conditions that might influence TMJ disorder.

Age, sex, facial biometrics and TMJ symptoms, were listed during the preoperative examination. Cephalometric data and classification were obtained by Delaire et al. (Delaire et al., 1981) analysis method. In that method, the line C3 is the superior line of the cranial base, drawn from M (junction of the nasofrontal, maxillofrontal and maxillonasal sutures) to Clp (apex of the posterior clinoid process). The line CF1 is perpendicular to line C3 registered at point FM, which is the intersect point of C3 and the line continuing to the anterior lacrimal crest. The adapted C1F1 angle represents the cranial angle corrected from abacus. Me Angle is defined as the angle between the line C3 and segment M-Me. Np Angle is defined as the angle between the line C3 and M-Npc (Nasopalatine canal). Anterior facial height is measured from Na (Nasion) to Me (Menton). After cephalometric analysis, malocclusion classification was also confirmed based upon the sagittal and vertical jaw repositioning required to execute the surgical treatment plan. The subjects were classified into 1 of 6 craniofacial morphologic groups that included 1 variation of sagittal skeletal jaw malocclusion, either Class II or Class III, and 1 variation of vertical skeletal jaw malocclusion — open bite, deep bite, or normal bite — relationship.

Facial anterior asymmetry was identified by measuring the deviation of mandibular dental midline relative to the vertical reference line on the posterior-anterior view. Vertical reference line is line passing through the crista galli and the superior part of the nasal septum, drawn perpendicular to the line between the intersections of the greater wings of the sphenoid bone and the lateral margins of the orbits. Facial posterior asymmetry was identified as described previously (Nicot et al., 2014). Regarding the comparative analysis, asymmetry was considered when ≥ 2 mm (no asymmetry vs asymmetry).

Saliva samples from all subjects were stored in Oragene kits (DNA Genotek, Ottawa, Ontario, Canada) and used for DNA extraction and posterior genotyping.

All subjects had at least a mandibular bilateral sagittal split osteotomy using Epker's technique (Epker, 1977). This osteotomy separates the ascending branch of the mandible from the dental arch, permitting repositioning in good occlusal position after adapted movement. Surgical mandibular anteroposterior movements influencing condylar position

during orthognathic surgery were noted. Mandibular advancements were distinguished from mandibular setbacks. The type of osteosynthesis was also noted due to its potential influence on TMJ. Its choice was mainly operator-dependent. The type of osteosynthesis was either “classic” – involving one or two titanium mini plates connecting the osteotomy site (Figure 1. A) – or one of increased rigidity, which had additional retromolar bi-cortical screws fixating the segments behind the mini plates – termed posterior rigid osteosynthesis (Figure 1. B).

Evaluation method of TMJ response to orthognathic surgery

TMJ response was evaluated using scores obtained from the “Jaw Pain Function” questionnaire (Clark et al., 1989; Gerstner et al., 1994; Undt et al., 2006) evaluated before and at 1 year after orthognathic surgery. The survey includes 8 questions pertaining to jaw function and 5 to jaw pain, with each question rated from 0 to 4 depending on the intensity of symptoms. A total score is thus obtained and an upper threshold or equal to 6 has been validated in the literature for the detection of TMD with a sensitivity of between 90.3% and 97.7% and a specificity between 95.7% and 100% (Gerstner et al., 1994). We used the scores in two independent ways to determine the presence or change in TMD symptoms as follows:

1. We first considered the JPF score one year after orthognathic surgery in order to select patients with severe TMJ symptoms postoperatively. Using the validated threshold to one postoperative year, we obtained a qualitative variable in two groups. Patients with JPF score ≥ 6 having TMD whereas patients with a JPF score < 6 were considered with no dysfunction.
2. We then generated a variable based on JPF score evolution. This allowed us to identify select patients who had worsening symptoms postoperatively. The variable was obtained by subtracting the postoperative score from the preoperative score. (JPF = JPF preoperative score - postoperative score)

Considering a threshold of -2 , we obtained a qualitative variable in two groups. Patients with JPF ≥ -2 represented the subpopulation worsening “significantly” while patients with JPF > 2 were classified in the subpopulation with no worsening.

SNPs in genes of interest were selected based upon our previous findings of their potential role in development of malocclusion and/or TMD, as described previously (Cheung et al., 2009; Deeley et al., 2015; Godel et al., 2014; Goding et al., 2003; Hottenstein et al., 2015; Kang et al., 2007; Kim et al., 2010; Liu et al., 2014; Nicot et al., 2014; Ribeiro-Dasilva et al., 2009; Sciote et al., 2015; Terkeltaub, 2001; Zebrick et al., 2014). These genes and their SNP characteristics are summarized in Table 1. Thirteen SNPs were selected for genotyping on all subjects and tested to determine whether specific allelic variants are correlated with changes in postoperative score using TaqMan chemistry (Life Technologies, Carlsbad, CA, USA) and end-point analysis in an automatic sequence-detection instrument (ABI Prism 7900HT; Applied Biosystems, Foster City, CA, USA), as described previously (Zuccherro et al., 2004).

The characteristics of the population were presented with the usual rules of descriptive statistics: frequencies and percentages for categorical variables; mean and standard deviation for quantitative variables. The association between Delta JPF or postoperative score and clinical or surgical characteristics of the population was assessed by Chi-2 test for categorical variables or Fisher exact test in the case of small numbers. Quantitative variables were analyzed using Student's t test. When the distribution of the variable did not follow a normal distribution, a nonparametric Wilcoxon test was performed. After ensuring compliance with the Hardy-Weinberg equilibrium, the association of different SNPs was also sought by chi-square or Fisher's exact tests. For each SNP, the analysis was performed by considering the three genotypes separately. In case of significance, analysis was completed to compare each genotype to the two others and odds ratios (OR) with 95% confidence intervals (95% C.I.) were calculated. The significance level was set at $p = 0.05$. The p-value was not given in the tables when the actual conditions were not met.

Results

Between February 2013 and February 2014, 120 patients were included in the study. A total of 101 patients completed the postoperative questionnaire, constituting our analysis population. Patient selection is represented by the flow chart (Figure 2).

Our analysis population included a larger proportion of women (66.3%) and of young people (25.5 ± 11.3 years). Patients mainly had Class II dentofacial dysmorphism (68.3%), with Normal (39.6%) or Open bite (41.6%). The biometric characteristics of the population of analysis are listed in Table 2. One can also note that there were 26.7% of patients with TMD before the orthognathic surgery. Patients with TMD were classified according to the original Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) (Reiter et al., 2012; Schiffman et al., 2014). Regarding axis I, the most common pain-related temporomandibular disorders were distributed as follows : 19 patients (70.4%) presented local myalgia and 17 patients (63.0%) presented arthralgia. Concerning the most common intra-articular temporomandibular disorders, 14 patients (51.9%) presented a disc displacement with reduction and 6 (22.2%) patients presented a disc displacement with reduction with intermittent locking. There were no positive diagnosis to Axis II.

I. COMPARISON OF THE TWO GROUPS BY POSTOPERATIVE SCORE

There was no significant difference between the two groups regarding age, sex or biometric characteristics of patients, suggesting that the two populations were similar (Table 3). We found however more rigid fixation by bi-cortical retromolar screw in the <6 JPF score group (78.1%) compared to the ≥ 6 JPF score group (57.9%), however the findings were not statistically significant ($p=0.085$).

No SNP was found to be statistically associated with postoperative score (Table 4).

II. COMPARISON OF TWO GROUPS BY DELTA JPF SCORE

There were no significant differences by age, sex or biometric, clinical and surgical characteristics between the 2 groups for Delta Score, suggesting that the two populations were similar (Table 5).

Among the 13 SNPs studied, single-nucleotide polymorphism rs1643821 of *ESR1* gene had a statistically significant association with postoperative JPF score worsening ($p = 0.041$). There was especially a significantly greater proportion of subjects with the AA genotype in the JPF ≥ 2 group (postoperative symptomatic worsening (26.1%) as compared to JPF < 2 group (no postoperative symptomatic worsening) (7.8%) (Table 6). The AA genotype was a risk factor of developing a symptomatic worsening postoperatively as compared to the other two genotypes (OR = 4.176, 95% C.I. [1.198, 14.566], $p = 0.028$) (Table 7).

III. COMPARISON OF TWO GROUPS BY PREOPERATIVE SCORE

There were significantly ($p = 0.015$) more women in ≥ 6 preoperative JPF score group (85.2%) than in the < 6 group (59.5%). Patients were also significantly older in the preoperative ≥ 6 JPF score group (28.7 ± 10.3 years) as compared to < 6 group (24.4 ± 11.5 years) ($p = 0.018$).

There was no difference between the biometrical characteristics of the 2 groups of patients, suggesting that the two populations were comparable at the dentofacial level. Finally, 37% of patients were bruxers in the ≥ 6 preoperative JPF score group against 14.9% in the < 6 preoperative JPF score group ($p = 0.015$) (Table 8).

Of the 13 SNPs analyzed, only rs858339 (*ENPP1* gene) was associated with preoperative score ($p = 0.014$) (Table 9). There were proportionately more patients with the TT genotype in the < 6 preoperative JPF score group compared to the ≥ 6 preoperative JPF score group (preoperative TMD) and this significantly ($p = 0.008$).

In particular, the TT genotype was a protective factor against TMD (OR = 0.286, 95% C.I. [0.111, 0.735], $p = 0.008$). Conversely, the heterozygous genotype AT was a risk factor of TMD with respect to the rest of the population (OR = 3.910, 95% C.I. [1.526, 10.021], $p = 0.003$). Nevertheless, these p values have to be treated with some care considering the low number of subjects in AA group for rs858339 (Table 10).

Discussion

I. ROLE OF SNP rs1643821 (*ESR1* GENE) IN SYMPTOMATIC WORSENING OF TMJ AFTER ORTHOGNATHIC SURGERY

The role of estrogen in the occurrence of TMD has been investigated for many years. The prevalence of this condition in young women has presumed role for sex hormones (estrogen and progesterone) in TMJ remodeling (Arnett et al., 1996a, 1996b; Warren and Fried, 2001). The intensity of the painful symptoms appears to be greater in women for many anatomical locations, including the temporomandibular joints (Fillingim et al., 2009). Furthermore, there is a variation among women in the intensity of TMD related pain in the menstrual cycle; The intensity of pain is most extensive for the particular estrogen deficiency phase (menstrual phase) or during the phase of rapid change in estrogen levels (ovulatory phase and premenstrual phase) (LeResche et al., 2003). Supplementation in postmenopausal women by hormone replacement therapy as well as contraceptives in reproductive age women are also associated with an increased risk of developing a TMD (Fillingim and Edwards, 2001; LeResche, 1997; LeResche et al., 2003).

The biological activity of estrogen is mediated by specific receptors. The estrogen receptor is a protein of the steroid receptors family. There are two forms of this receptor, α and β . The α receptor is in particular found in the intra-articular cartilage and osteocytes and plays a role of intracellular mediators regulator. In rats, the α receptor is found in synovial cells, articular disc stromal cells and chondrocytes of the TMJ. There is an intensity gradient of age in favor of young subjects suggesting that estrogen is involved in the TMJ physiology differently depending on the age of the subjects (Yamada et al., 2003). In humans, Abubaker et al. (Abubaker et al., 1993) have highlighted the presence of estrogen receptors at the temporomandibular joint disk in both the subjects of male and female. These receptors are also found to a greater proportion in women with TMD than in subjects without TMD but these results can be highlighted significantly because of too low numbers. These results obtained by immunohistochemistry have subsequently been confirmed by transcriptomics (Ushiyama et al., 1999), in order to identify the estrogen receptor α as a fundamental biological mediator in the temporomandibular pathophysiology.

Recent studies have highlighted a significant association between single-nucleotide polymorphisms of ESR1 and symptoms of TMD (Kim et al., 2010) or TMJ osteoarthritis in women (Kang et al., 2007; Liu et al., 2014; Ribeiro-Dasilva et al., 2009). The gene encoding the α estrogen receptor is located on the long arm of chromosome 6 (6q25). It includes 7 introns and 8 exons on 140 kilobases (Han et al., 2011). The SNP rs1643821 of ESR1 gene is an alternative version with a transition from an A into a G at the intron 2 and having no known functional consequence. However, SNPs located at non-coding regions (introns) can affect splicing, transcription factors or may affect the production of non-coding RNA (Hull et al., 2007; Kwan et al., 2008). Similarly an SNP of the gene encoding the growth hormone (GH, Growth Hormone) affecting an intron is associated with physiological changes in hormone secretion (Millar et al., 2010), the rs1643821 variant could be another example of polymorphism affecting a functional intron by generating a specific modulation of transcription pathways related to estrogen. However, how this variant of the ESR1 gene modifies the articular symptomatic response to orthognathic surgery remains unknown. The rs1643821 single-nucleotide polymorphism of ESR1 gene has previously been significantly associated with the risk of osteoporotic fracture (Wang et al., 2012). Considering this work, our results do not show an association between rs1643821 and postoperative TMD. However, there is a significant association between this polymorphism and symptomatic worsening ($p = 0.041$). Especially, the AA homozygote mutated genotype is found as an aggravating risk factor for postoperative joint symptoms compared to wild-type genotype and the heterozygous genotype (OR = 4.176, 95% C.I. [1.198, 14.566], $p = 0.028$). These data allow us to strengthen the role of genetic polymorphism of ESR1 in the TMJ symptoms (Kang et al., 2007; Kim et al., 2010; Liu et al., 2014; Ribeiro-Dasilva et al., 2009).

On the biological level, the role of estrogen via the α receptor is well documented. It is involved in the pathophysiology of TMD through the inflammatory response, the bone mineralization and the nervous system (Craft, 2007). Via their α and β receptors in the peripheral nervous system as central nervous system (Laflamme et al., 1998; McEwen and Alves, 1999; Shupnik, 2002), estrogens are involved in the inflammatory response process and in the transmission of pain (Craft, 2007; Smith, 2006). Concerning the inflammatory response, estrogen negatively regulates the production of interleukin 1 (IL1) (Polan et al.,

1988), interleukin 6 (IL6) (Pottratz et al., 1994) and tumor necrosis factor α (TNF) (Ralston et al., 1990). IL1 and IL6- β cytokines are found in the synovial membrane of the temporomandibular joint during inflammation (Kubota et al., 1998), whereas TNF α and IL1 produced by monocytes / macrophages will promote cartilage resorption, inhibit proteoglycan synthesis and play a proinflammatory role in most of temporomandibular structures (Pettipher et al., 1986; Saklatvala, 1986). Treatment with estradiol seems then to have a protective effect on TMJ through reducing the number of monocytes in the articular synovial, and through reducing the production of proinflammatory cytokines (Guan et al., 2005; Pacifici, 1996, 1998). In rats, estrogen deficiency during puberty, induced by ovariectomy, predispose to alterations of TMJ by changes in serum levels of calcitonin and parathyroid hormone (Yasuoka et al., 2000). On the other hand, femoral bone mineral density is decreased in ovariectomized rats (Pajot et al., 2003). Estradiol is also involved in nociception mechanisms in TMJ. Using rats as model to study the pain has allowed identifying differences in masticatory muscle activity induced by glutamate (control of pain intensity) due to sexual hormones. Increasing concentrations of glutamate (the main excitatory neurotransmitters of central nervous system) seems to heighten awareness of TMD to noxious chemical stimuli (Cairns et al., 2002). In masticatory muscles, the increase of glutamate concentration plays an important role in the genesis of “myofascial pain syndrome” through activation of N-methyl D-aspartate receptors (NMDAr) (Castrillon et al., 2012).

Estradiol increases dose-dependently the trigeminal afferent discharge induced by NMDA injection into the masseter or temporomandibular joint in ovariectomized rats (Dong et al., 2007). These sex differences are due in part to an increase in the expression of peripheral NMDA receptors by neurons in the dorsal root ganglion mediated by estrogen (McRoberts et al., 2007). From a practical point of view, the discovery of a genetic marker of symptomatic worsening after orthognathic surgery allows us to glimpse new therapeutic drug targets (Lötsch et al., 2013). In this case, temporomandibular nociceptive pathways mediated by estrogen highlight the potential role of ketamine, an inhibitor of glutamate at NMDA receptors, in the treatment of selected patients. This treatment, used in the form of intraarticular injection, appears to have a different efficiency depending on the forms of TMJ dysfunction (Ayesh et al., 2008; Castrillon et al., 2012). Nevertheless its targeted use after orthognathic surgery in patients with a mutated genotype AA of SNP rs1643821 of the ESR1 gene could be a therapeutic application to study, given the role of estrogen in “myofascial pain syndrom” through activation of NMDA receptors.

Many studies have demonstrated a higher prevalence of pain among women. There are notably higher prevalence of musculoskeletal pain, orofacial pain and TMD (Fillingim et al., 2009). There are also differences in postoperative pain by sex published in many surgical fields and in particular in joint surgery (Fillingim et al., 2009). *Rosseland et al.* (Rosseland and Stubhaug, 2004), for example, have found an incidence of significant pain (at least moderate) greater in women immediately after surgery (2H) of a knee arthroscopy. This difference is no longer found at 1 year postoperatively while there is a prevalence of the most important pain in the elderly over 50 years old. In addition, the reduction in quality of life associated with this pain is most important in women at 1 year postoperatively (Rosseland et al., 2008). While the prevalence of pain appears to be greater in women in

many areas, pharmacological response (Gear et al., 1999) and non-pharmacological response (Krogstad et al., 1996) to the pain are more important, in particular in the orofacial field. For example there is a significant decrease of pain in women treated with a conservative treatment of TMD, whereas men show no symptomatological improvement (Krogstad et al., 1996). For our part, we have not found symptomatological changes by age or sex but these results may be related to a lack of power of the study.

II. CONTROVERSY OF VARIABLES TO BE EXPLAINED: POSTOPERATIVE JPF SCORE OR JPF SCORE?

Determining the influence of genetic polymorphisms on the articular dysfunctional symptomatology requires an evaluation criterion adapted to the dysfunctional articular symptoms and to integrate such diverse settings as symptoms worsening or symptomatic severity.

While the diagnostic criteria of TMD become more complex in order to separate various dysfunctional categories grouped under the same nosological framework (Schiffman et al., 2014), we wanted to use a validated score in the detection of TMD, in the broadest sense of the term. (Clark et al., 1989; Gerstner et al., 1994; Undt et al., 2006). Choosing this quantitative score with a pathological threshold defined in the literature then allowed us to consider both cases for which there was “severe” symptoms (TMD → 6 Postoperative JPF Score) and cases for which there was a dysfunctional symptoms worsening (−2 JPF Score).

One of the key criticisms of the variable named “postoperative JPF score” is that a 6 postoperative JPF score includes two kinds of patients: those with a score <6 and that have evolved after orthognathic surgery to a TMD and those who already had a 6 score and have remained highly symptomatic. Conversely, a <6 postoperative JPF score includes dysfunctional patients who were preoperatively and which are no longer under the criteria of the questionnaire postoperatively, but also patients who were not dysfunctional preoperatively and who have not presented a symptomatic evolution. In this way this criterion considers patients who are broader dysfunctional postoperatively without assuming their original status. Using this evaluation criterion is therefore to determine the SNP representing a risk factor for becoming or remaining dysfunctional postoperatively. Furthermore, it does not allow inclusion of patients whose score is below 6 postoperatively, while they sometimes can show an important variation of the score. A patient with a preoperative score of 0 who develops a postoperative score of 5, is for example not considered dysfunctional while he presents a larger change than a patient, with a preoperative score of 5, who develops a postoperative score of 6.

Therefore, the variable named “ JPF score” takes on its full meaning to determine patients with symptomatological worsening without these patients belonging to the TMD population. The threshold selected was determined arbitrarily and corresponds to a 30% worsening of the threshold defined in the literature for the detection of TMD. The main drawback of this endpoint is not to consider the intensity of the symptoms. Indeed, this criterion doesn’t allow us to distinguish a patient with a score moving from 0 to 2 of a patient with a score moving from 6 to 8, whereas these two patients have completely different symptoms.

A variable integrating both subjects with symptoms worsening and “severe” subjects would have to increase the power of the statistical test, but it would have suffered a less accurate interpretation because of encompassing a larger volume of patients too distinct from one other from a symptomatic point of view. The choice to use these two variables independently allows us to separate statistically these two categories of patients. Finally, JPF score does not distinguish between different subtypes of TMD as recommended by the International Association for Dental Research (IADR) and the International Association of Study of Pain (IASP) (Schiffman et al., 2014) or the American Academy of orofacial Pain (AAOP) (Reiter et al., 2012).

III. LIMITED CONFUSION FACTORS?

Although the link between TMD and orthognathic surgery is a subject of increased interest in the literature, a small number of quality studies have examined the predictors of symptomatic articular response to this surgical treatment (Dicker et al., 2015; Pusan Korea Pusan National University et al., 2015; Scolozzi et al., 2015; Valladares-Neto et al., 2014).

However, some clinical and surgical elements have been identified. From a demographic point of view, young age and female sex are factors influencing post-surgical joint symptoms (Valladares-Neto et al., 2014). Valladares-Neto et al. (Valladares-Neto et al., 2014) have also shown the importance of mandibular advancement, the degree of counterclockwise rotation of the mandible and the rigidity of fixation technique could be factors influencing the TMJ health. Thus, young adult females with mandibular retrognathism and increased mandibular plane angle would present a smaller joint improvement after orthognathic surgery and would be more prone to postoperative condylar resorption. Conversely, Sang-Yong et al. (Pusan Korea Pusan National University et al., 2015) noted an improvement of dysfunctional symptomatology and a good postoperative condylar stability after bimaxillary surgery in dysfunctional subjects with class III. These data are supported by Scolozzi et al. (Scolozzi et al., 2015) pointing out that the coexistence of maxillary retrusion and mandibular excess was an improving factor. Conversely, bimaxillary surgery in the broadest sense of the term is found in their work as a factor of symptoms worsening, as well as the existence of a “TMJ clicking”, a pain in the preoperative TMJ palpation or pain in the masseter palpation.

In our work, we did not find any significant difference between the two groups according to the JPF score or the postoperative JPF score for demographic, biometric or clinical characteristics and particularly for Class II or open bite malocclusion. Surgically, we did not find any significant association between the type of mandibular movement and the postoperative symptomatic aggravation. These results accord to those proposed by Dicker et al. (Dicker et al., 2015), which showed no significant changes in masseter or medial pterygoid muscles vectors and axial condylar rotation after a mandibular advancement. Moreover, unlike Valladares-Neto et al. (Valladares-Neto et al., 2014) our results noted a trend towards significance of the type of osteosynthesis by postoperative JPF score ($p = 0.07$). In other words, there is less rigid osteosynthesis in the dysfunctional group suggesting that a rigid fixation by bi-cortical retromolar screws could be a protective factor of postoperative TMD. These results will be developed on a larger population to be confirmed. It must be remembered that this type of osteosynthesis called “rigid” leaves little potential

for adaptation to the mandibular condyle and is experience-dependent; yet most orthognathic surgeons perform a condylar manual “repositioning” after sagittal split osteotomy of mandibular advancement. Although the evidence leads us to believe that the best position is the one involving the minimal stress of TMJ, analysis of the literature has not identified significant association between condylar positioning and condylar resorption (Ueki et al., 2012).

Bruxism supposed to be a confounding factor in the analysis of literature (Fernandes et al., 2015; Fillingim et al., 2009; Khawaja et al., 2015; LeResche, 1997; Manfredini and Lobbezoo, 2010; Ohrbach et al., 2011; Slade et al., 2011), is not found as a factor influencing the postoperative joint dysfunction or symptomatic worsening in our population. However, we found a significant association between bruxism and preoperative TMD confirming data already published. These results suggest that this factor, whose role seems to be important in the genesis of TMD, does not have a decisive role in the evolution of postoperative pain.

The absence of a significantly different in biometric and clinical features between the two groups according to the two variables to be explained (JPF score and postoperative JPF score) enables us to relativize the existence of confounding factor, limiting the need for multivariate analysis for these two variables. However, this study suffers from relatively low numbers weakening its statistical power. Therefore, some results showing a trend of significance (posterior rigid osteosynthesis) could become significant by increasing the analysis population.

IV. FACTORS ASSOCIATED WITH TMD IN OUR POPULATION

The results of our study have demonstrated a significant relationship between age ($p = 0.018$), sex ($p = 0.015$), bruxism ($p = 0.015$) and TMD. Our results are consistent with the distribution of TMD found in the literature according to age or sex (Fillingim et al., 2009; LeResche, 1997; Ohrbach et al., 2011; Slade et al., 2011). We found more female patients (85.2%; $p = 0.015$) in the preoperative TMD group with a higher median age between the second and the fourth decade (28.7 ± 10 , 3 years; $p = 0.018$). Bruxism was also associated with the presence of TMD ($p = 0.015$) confirming the literature data (Fernandes et al., 2015; Fillingim et al., 2009; Khawaja et al., 2015; LeResche, 1997; Manfredini and Lobbezoo, 2010; Ohrbach et al., 2011; Slade et al., 2011).

We also found a significant association between rs858339 (ENPP1 gene) and TMD ($p = 0.014$). ENPP1 is a gene encoding for ectonucleotide pyrophosphatase / phosphodiesterase 1, a transmembrane ectoenzyme that hydrolyzes extracellular molecules having a phosphodiester or pyrophosphate bond. This rather ubiquitous protein, highly expressed in chondrocytes and osteoblasts, plays a fundamental role in the regulation of bone mineralization (Goding et al., 2003). It hydrolyzes the inorganic pyrophosphate (PPi), a leading inhibitor of calcification, which inhibits crystal growth of hydroxyapatite (Terkeltaub, 2001). The role of ectonucleotide pyrophosphatase / phosphodiesterase 1 in inhibiting calcification has been demonstrated in a mouse model of ttw phenotype / ttw (tiptoe walking), which carries a nonsense mutation in the ENPP1 gene (Okawa et al., 1998). The ENPP1 –/– mice develop a phenotype of hypermineralisation, calcification of tendons

and ligaments. These knock-out mice constitute a model for the human disease that is responsible for ossification of the posterior longitudinal ligament (OPLL) (Okawa et al., 1998). The phenotypic characteristics of ENPP1 $-/-$ mice include marked alterations in mineralization of the long bones and calvaria, and the peri-spinal soft tissue and arterial calcification (Mackenzie et al., 2012). Some SNPs in the ENPP1 gene have also been implicated in susceptibility, severity and progression of post-surgical OPLL (He et al., 2013). ENPP1 was also studied in the Framingham cohort, which identify polymorphisms associated with bone mineral density or bone morpho-biometrics. The SNP rs1974201 of ENPP1 gene is associated with bone morphology in the Framingham cohort (Cheung et al., 2009) while the SNP rs6569759 is associated with class III malocclusion (Deeley et al., 2015) suggesting that ENPP1 has a key role in bone morpho-biometrics. How these SNPs alter bone metabolism to affect bone formation remains unknown.

In our study, there was a greater proportion of subjects with TT wild genotype of rs6569759 (ENPP1 gene) in non-worsened group suggesting that this polymorphism was a protective factor dysfunction of TMD (OR = 0.286, 95% C.I. [0.111; 0.735], $p = 0.008$). Conversely, the heterozygous mutated polymorphism was a risk factor (OR = 3.910, 95% C.I. [1.526, 10.021], $p = 0.003$). The weak theoretical expected numbers for homozygous mutant genotype does not allow us to reach a conclusion due to the impossibility of using relevant statistical analyzes. As polymorphisms associated with surgical progression of OPLL, the presence of a mutant allele and a fortiori the homozygous mutant genotype of rs6569759 may thus have a role in regulating the TMJ bone mineralization by altering its function. Other ENPP1 polymorphisms have already been associated with TMD (Sciote et al., 2015). Our results are relatively consistent with those previously published making rs6569759 a key polymorphism of TMD. The results should, however, be confirmed with larger numbers to highlight the role of risk factor of homozygous mutant genotype.

V. RESEARCH PERSPECTIVE: Oppera COHORT AND POTENTIAL GENES OF INTEREST

Many other genes of interest could be potential factors influencing the dysfunctional response to orthognathic surgery, particularly genes of the Oppera cohort (Slade et al., 2013). Oppera is a prospective cohort of 3262 adult subjects (18–44 years old) volunteers, recruited in four US sites between May 2006 and November 2008. This is the largest cohort to date to study the etiopathogenic factors of TMD. 2737 subjects followed for 2.8 years, have completed questionnaires to identify the occurrence of 260 cases of TMD.

Among the various research areas within this cohort, a component concerned predictive genes of TMD. Thus, 3295 SNP representing 358 genes known to be involved in pain perception systems were studied. None SNPs has been identified as significantly associated with the occurrence of TMD. However, they were many SNPs correlated with intermediate phenotypes TMD (Slade et al., 2013; Smith et al., 2011, 2013). On the other hand, polymorphisms of COMT genes, ESR1 genes and GCH1 genes interact to influence the enzymatic activity of catechol-O-methyltransferase, musculoskeletal pain, and mood constituting a phenomenon of epistasis (Smith et al., 2014). The study of CACNA2D1, a gene of interest in the cohort Oppera encoding subunit alpha-2 / delta-1 of voltage dependent calcium channels implicated in neuropathic pain, has a differential expression in the

masseter muscles according and sex between women with or without TMD myalgia-type (Godel et al., 2014; Sciote et al., 2015). All of these genes constitute a gene pool of interest in the study of polymorphisms that may influence the dysfunctional worsening after orthognathic surgery.

Conclusion

Although long debated, the combined orthodontic and surgical treatment of dentofacial dysmorphism seems beneficial to temporomandibular symptoms. Several demographic, clinical or surgical features influencing the joint response were found in recent clinical studies. We have identified the AA genotype of SNP rs1643821 (ESR1 gene) as a risk factor for dysfunctional worsening after orthognathic surgery. In addition, we have identified TT genotype of SNP rs858339 (ENPP1 gene) as a protective factor against TMD in a population of patients with dentofacial deformities. Conversely, the heterozygous genotype AT was identified as a risk factor of TMD with respect to the rest of our population. All these elements are particularly important to bring new screening strategies and tailor future treatment. Results should, however, be completed on a larger cohort.

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References

- Abrahamsson C, Henrikson T, Nilner M, Sunzel B, Bondemark L, Ekberg E. TMD before and after correction of dentofacial deformities by orthodontic and orthognathic treatment. *Int J Oral Maxillofac Surg.* 2013; 42:752–758. [PubMed: 23159168]
- Abubaker AO, Raslan WF, Sotereanos GC. Estrogen and progesterone receptors in temporomandibular joint discs of symptomatic and asymptomatic persons: a preliminary study. *J Oral Maxillofac Surg.* 1993; 51:1096–1100. [PubMed: 7692023]
- Arnett GW, Milam SB, Gottesman L. Progressive mandibular retrusion—idiopathic condylar resorption. Part I. *Am J Orthod Dentofacial Orthop.* 1996a; 110:8–15. [PubMed: 8686682]
- Arnett GW, Milam SB, Gottesman L. Progressive mandibular retrusion—idiopathic condylar resorption. Part II. *Am J Orthod Dentofacial Orthop.* 1996b; 110:117–127. [PubMed: 8760837]
- Ayesh EE, Jensen TS, Svensson P. Effects of intra-articular ketamine on pain and somatosensory function in temporomandibular joint arthralgia patients. *Pain.* 2008; 137:286–294. [PubMed: 17923328]
- Cairns BE, Sim Y, Bereiter DA, Sessle BJ, Hu JW. Influence of sex on reflex jaw muscle activity evoked from the rat temporomandibular joint. *Brain Res.* 2002; 957:338–344. [PubMed: 12445976]
- Castrillon EE, Cairns BE, Wang K, Arendt-Nielsen L, Svensson P. Comparison of glutamate-evoked pain between the temporalis and masseter muscles in men and women. *Pain.* 2012; 153:823–829. [PubMed: 22336721]
- Celic R, Jerolimov V, Panduric J. A study of the influence of occlusal factors and parafunctional habits on the prevalence of signs and symptoms of TMD. *Int J Prosthodont.* 2002; 15:43–48. [PubMed: 11887598]

- Cheung CL, Livshits G, Zhou Y, Meigs JB, McAteer JB, Florez JC, Cupples LA, Demissie S, Kiel DP, Karasik D. Hip Geometry Variation is Associated With Bone Mineralization Pathway Gene Variants: The Framingham Study. *J Bone Miner Res.* 2010; 25:1564–1571. [PubMed: 19888898]
- Clark GT, Seligman DA, Solberg WK, Pullinger AG. Guidelines for the examination and diagnosis of temporomandibular disorders. *J Craniomandib Disord.* 1989; 3:7–14. [PubMed: 2606995]
- Craft RM. Modulation of pain by estrogens. *Pain.* 2007; 132:S3–S12. [PubMed: 17951003]
- Deeley K, Vieira AR, Burnheimer J, Kim S, Sciote JJ. ACTN3 and ENPP1 contribute to sagittal craniofacial differences in Humans. *J Dent Res.* 2015; 94(A):2114589.
- Delaire J, Schendel SA, Tulasne JF. An architectural and structural craniofacial analysis: a new lateral cephalometric analysis. *Oral Surg Oral Med Oral Pathol.* 1981; 52:226–238. [PubMed: 6945527]
- Diatchenko L. Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Hum Mol Genet.* 2004; 14:135–143. [PubMed: 15537663]
- Dicker GJ, Castelijns JA, Tuinzing DB, Stoelinga PJW. Do the changes in muscle mass, muscle direction, and rotations of the condyles that occur after sagittal split advancement osteotomies play a role in the aetiology of progressive condylar resorption? *Int J Oral Maxillofac Surg.* 2015; 44:627–631. [PubMed: 25636702]
- Dong XD, Mann MK, Kumar U, Svensson P, Arendt-Nielsen L, Hu JW, Sessle BJ, Cairns BE. Sex-related differences in NMDA-evoked rat masseter muscle afferent discharge result from estrogen-mediated modulation of peripheral NMDA receptor activity. *Neuroscience.* 2007; 146:822–832. [PubMed: 17382479]
- Dujoncqoy JP, Ferri J, Raoul G, Kleinheinz J. Temporomandibular joint dysfunction and orthognathic surgery: a retrospective study. *Head Face Med.* 2010; 6:27. [PubMed: 21083902]
- Egermark I, Magnusson T, Carlsson GE. A 20-year follow-up of signs and symptoms of temporomandibular disorders and malocclusions in subjects with and without orthodontic treatment in childhood. *Angle Orthod.* 2003; 73:109–115. [PubMed: 12725365]
- Epker BN. Modifications in the sagittal osteotomy of the mandible. *J Oral Surg.* 1977; 35:157–159. [PubMed: 264514]
- Fernandes G, van Selms MKA, Gonçalves DAG, Lobbezoo F, Camparis CM. Factors associated with temporomandibular disorders pain in adolescents. *J Oral Rehabil.* 2015; 42:113–119. [PubMed: 25244610]
- Fillingim RB, Edwards RR. The association of hormone replacement therapy with experimental pain responses in postmenopausal women. *Pain.* 2001; 92:229–234. [PubMed: 11323144]
- Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL. Sex, Gender, and Pain: A Review of Recent Clinical and Experimental Findings. *J Pain.* 2009; 10:447–485. [PubMed: 19411059]
- Gear RW, Miaskowski C, Gordon NC, Paul SM, Heller PH, Levine JD. The kappa opioid nalbuphine produces gender- and dose-dependent analgesia and antianalgesia in patients with postoperative pain. *Pain.* 1999; 83:339–345. [PubMed: 10534607]
- Gerstner GE, Clark GT, Goulet JP. Validity of a brief questionnaire in screening asymptomatic subjects from subjects with tension-type headaches or temporomandibular disorders. *Community Dent Oral Epidemiol.* 1994; 22:235–242. [PubMed: 7924238]
- Godel JH, Foley BF, Nicot R, Horton MJ, Barton ER, Ferri J, Raoul G, Vieira AR, Sciote JJ. Associations between ACTN3 and OPPERA pain-related genes in malocclusion. *Mol Pain.* 2014; 10:P4.
- Goding JW, Grobбен B, Slegers H. Physiological and pathophysiological functions of the ecto-nucleotide pyrophosphatase/phosphodiesterase family. *Biochim Biophys Acta.* 2003; 1638:1–19. [PubMed: 12757929]
- Guan G, Kerins CC, Bellinger LL, Kramer PR. Estrogenic effect on swelling and monocytic receptor expression in an arthritic temporomandibular joint model. *J Steroid Biochem Mol Biol.* 2005; 97:241–250. [PubMed: 16153820]
- Han J, Jiang T, Bai H, Gu H, Dong J, Ma H, Hu Z, Shen H. Genetic variants of 6q25 and breast cancer susceptibility: a two-stage fine mapping study in a Chinese population. *Breast Cancer Res Treat.* 2011; 129:901–907. [PubMed: 21528353]

- He Z, Zhu H, Ding L, Xiao H, Chen D, Xue F. Association of NPP1 polymorphism with postoperative progression of ossification of the posterior longitudinal ligament in Chinese patients. *Genet Mol Res.* 2013; 12:4648–4655. [PubMed: 24222241]
- Henrikson T, Nilner M, Kurol J. Signs of temporomandibular disorders in girls receiving orthodontic treatment. A prospective and longitudinal comparison with untreated Class II malocclusions and normal occlusion subjects. *Eur J Orthod.* 2000; 22:271–281. [PubMed: 10920559]
- Hottenstein MK, Horton MJ, Sciote JJ, Nicot R, Raoul G, Ferri J. Differential Expression of PITX2 in Adults with Facial Asymmetry. *J Dent Res.* 2015; 94(A):2114583.
- Hull J, Campino S, Rowlands K, Chan MS, Copley RR, Taylor MS, Rockett K, Elvidge G, Keating B, Knight J, et al. Identification of Common Genetic Variation That Modulates Alternative Splicing. *PLoS Genet.* 2007; 3:e99. [PubMed: 17571926]
- Hwang CJ, Sung SJ, Kim SJ. Lateral cephalometric characteristics of malocclusion patients with temporomandibular joint disorder symptoms. *Am J Orthod Dentofacial Orthop.* 2006; 129:497–503. [PubMed: 16627175]
- Kang SC, Lee DG, Choi JH, Kim ST, Kim YK, Ahn HJ. Association between estrogen receptor polymorphism and pain susceptibility in female temporomandibular joint osteoarthritis patients. *Int J Oral Maxillofac Surg.* 2007; 36:391–394. [PubMed: 17391927]
- Khawaja SN, McCall W, Dunford R, Nickel JC, Iwasaki LR, Crow HC, Gonzalez Y. Infield masticatory muscle activity in subjects with pain-related temporomandibular disorders diagnoses. *Orthod Craniofac Res.* 2015; 18:137–145. [PubMed: 25865542]
- Kim BS, Kim YK, Yun PY, Lee E, Bae J. The Effects of Estrogen Receptor α Polymorphism on the Prevalence of Symptomatic Temporomandibular Disorders. *J Oral Maxillofac Surg.* 2010; 68:2975–2979. [PubMed: 20656393]
- Krogstad BS, Jokstad A, Dahl BL, Vassend O. The reporting of pain, somatic complaints, and anxiety in a group of patients with TMD before and 2 years after treatment: sex differences. *J Orofac Pain.* 1996; 10:263–269. [PubMed: 9161231]
- Kubota E, Kubota T, Matsumoto J, Shibata T, Murakami KI. Synovial fluid cytokines and proteinases as markers of temporomandibular joint disease. *J Oral Maxillofac Surg.* 1998; 56:192–198. [PubMed: 9461144]
- Kwan T, Benovoy D, Dias C, Gurd S, Provencher C, Beaulieu P, Hudson TJ, Sladek R, Majewski J. Genome-wide analysis of transcript isoform variation in humans. *Nat Genet.* 2008; 40:225–231. [PubMed: 18193047]
- Laflamme N, Nappi RE, Drolet G, Labrie C, Rivest S. Expression and neuropeptidergic characterization of estrogen receptors (ER α and ER β) throughout the rat brain: anatomical evidence of distinct roles of each subtype. *J Neurobiol.* 1998; 36:357–378. [PubMed: 9733072]
- de Leeuw, R.; Klasser, G. Orofacial pain: guidelines for assessment, diagnosis, and management. 5. Quintessence; Chicago: 2013. p. 127-186.
- LeResche L. Epidemiology of temporomandibular disorders: implications for the investigation of etiologic factors. *Crit Rev Oral Biol Med.* 1997; 8:291–305. [PubMed: 9260045]
- LeResche L, Mancil L, Sherman JJ, Gandara B, Dworkin SF. Changes in temporomandibular pain and other symptoms across the menstrual cycle. *Pain.* 2003; 106:253–261. [PubMed: 14659508]
- Liu W, Shao FM, Yan L, Cao HX, Qiu D. Polymorphisms in the gene encoding estrogen receptor α are associated with osteoarthritis in Han Chinese women. *Int J Clin Exp Med.* 2014; 7:5772–5777. [PubMed: 25664105]
- Lötsch J, Doehring A, Mogil JS, Arndt T, Geisslinger G, Utsch A. Functional genomics of pain in analgesic drug development and therapy. *Pharmacol Ther.* 2013; 139:60–70. [PubMed: 23567662]
- Mackenzie NCW, Huesa C, Rutsch F, MacRae VE. New insights into NPP1 function: Lessons from clinical and animal studies. *Bone.* 2012; 51:961–968. [PubMed: 22842219]
- Manfredini D, Lobbezoo F. Relationship between bruxism and temporomandibular disorders: a systematic review of literature from 1998 to 2008. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2010; 109:e26–e50. [PubMed: 20451831]
- McEwen BS, Alves SE. Estrogen actions in the central nervous system. *Endocr Rev.* 1999; 20:279–307. [PubMed: 10368772]

- McRoberts JA, Li J, Ennes HS, Mayer EA. Sex-dependent differences in the activity and modulation of N-methyl-d-aspartic acid receptors in rat dorsal root ganglia neurons. *Neuroscience*. 2007; 148:1015–1020. [PubMed: 17693030]
- Melis, M.; Giosia, MD. The role of genetic factors in the etiology of temporomandibular disorders: a review. 2014. CRANIO® 2151090314Y.000
- Millar DS, Horan M, Chuzhanova NA, Cooper DN. Characterisation of a functional intronic polymorphism in the human growth hormone (GH1) gene. *Hum Genomics*. 2010; 4:289–301. [PubMed: 20650818]
- Miller JR, Burgess JA, Critchlow CW. Association between mandibular retrognathia and TMJ disorders in adult females. *J Public Health Dent*. 2004; 64:157–163. [PubMed: 15341139]
- Mohlin BO, Derweduwen K, Pilley R, Kingdon A, Shaw WC, Kenealy P. Malocclusion and temporomandibular disorder: a comparison of adolescents with moderate to severe dysfunction with those without signs and symptoms of temporomandibular disorder and their further development to 30 years of age. *Angle Orthod*. 2004; 74:319–327. [PubMed: 15264641]
- Nebbe B, Major PW, Prasad NG. Adolescent female craniofacial morphology associated with advanced bilateral TMJ disc displacement. *Eur J Orthod*. 1998; 20:701–712. [PubMed: 9926637]
- Nicot R, Hottenstein M, Raoul G, Ferri J, Horton M, Tobias JW, Barton E, Gele P, Sciote JJ. Nodal pathway genes are down-regulated in facial asymmetry. *J Craniofac Surg*. 2014; 25:e548–e555. [PubMed: 25364968]
- Ohrbach R, Fillingim RB, Mulkey F, Gonzalez Y, Gordon S, Gremillion H, Lim P-F, Ribeiro-Dasilva M, Greenspan JD, Knott C, et al. Clinical Findings and Pain Symptoms as Potential Risk Factors for Chronic TMD: Descriptive Data and Empirically Identified Domains from the OPPERA Case-Control Study. *J Pain*. 2011; 12:T27–T45. [PubMed: 22074750]
- Okawa A, Nakamura I, Goto S, Moriya H, Nakamura Y, Ikegawa S. Mutation in Npps in a mouse model of ossification of the posterior longitudinal ligament of the spine. *Nat Genet*. 1998; 19:271–273. [PubMed: 9662402]
- Pacifici R. Estrogen, cytokines, and pathogenesis of postmenopausal osteoporosis. *J Bone Miner Res*. 1996; 11:1043–1051. [PubMed: 8854239]
- Pacifici R. Editorial: cytokines, estrogen, and postmenopausal osteoporosis—the second decade. *Endocrinology*. 1998; 139:2659–2661. [PubMed: 9607769]
- Pajot J, Ressot C, Ngom I, Woda A. Gonadectomy induces site-specific differences in nociception in rats. *Pain*. 2003; 104:367–373. [PubMed: 12855347]
- Pettipher ER, Higgs GA, Henderson B. Interleukin 1 induces leukocyte infiltration and cartilage proteoglycan degradation in the synovial joint. *Proc Natl Acad Sci U S A*. 1986; 83:8749–8753. [PubMed: 3490671]
- Polan ML, Daniele A, Kuo A. Gonadal steroids modulate human monocyte interleukin-1 (IL-1) activity. *Fertil Steril*. 1988; 49:964–968. [PubMed: 2967196]
- Pottratz ST, Bellido T, Mocharla H, Crabb D, Manolagas SC. 17 beta-Estradiol inhibits expression of human interleukin-6 promoter-reporter constructs by a receptor-dependent mechanism. *J Clin Invest*. 1994; 93:944. [PubMed: 8132780]
- Pullinger AG, Seligman DA, Gornbein JA. A multiple logistic regression analysis of the risk and relative odds of temporomandibular disorders as a function of common occlusal features. *J Dent Res*. 1993; 72:968–979. [PubMed: 8496480]
- Yoon S-Y, Song J-M, Kim Y-D, Chung I-K, Shin S-H. Pusan Korea Pusan National University. Clinical changes of TMD and condyle stability after two jaw surgery with and without preceding TMD treatments in class III patients. *Maxillofac Plast Reconstr Surg*. 2015; 37:9. [PubMed: 25729744]
- Ralston SH, Russell RG, Gowen M. Estrogen inhibits release of tumor necrosis factor from peripheral blood mononuclear cells in postmenopausal women. *J Bone Miner Res*. 1990; 5:983–988. [PubMed: 2281828]
- Reiter S, Goldsmith C, Emodi-Perlman A, Friedman-Rubin P, Winocur E. Masticatory muscle disorders diagnostic criteria: the American Academy of Orofacial Pain versus the research diagnostic criteria/temporomandibular disorders (RDC/TMD). *J Oral Rehabil*. 2012; 39:941–947. [PubMed: 22882635]

- Ribeiro-Dasilva MC, Peres Line SR, Leme Godoy dos Santos MC, Arthuri MT, Hou W, Fillingim RB, Rizzatti Barbosa CM. Estrogen Receptor- α Polymorphisms and Predisposition to TMJ Disorder. *J Pain*. 2009; 10:527–533. [PubMed: 19411060]
- Rosseland LA, Stubhaug A. Gender is a confounding factor in pain trials: women report more pain than men after arthroscopic surgery. *Pain*. 2004; 112:248–253. [PubMed: 15561379]
- Rosseland LA, Solheim N, Stubhaug A. Pain and disability 1 year after knee arthroscopic procedures. *Acta Anaesthesiol Scand*. 2008; 52:332–337. [PubMed: 18269385]
- Saklatvala J. Tumour necrosis factor alpha stimulates resorption and inhibits synthesis of proteoglycan in cartilage. *Nature*. 1986; 322:547–549. [PubMed: 3736671]
- Schiffman E, Ohrbach R, Truelove E, Look J, Anderson G, Goulet JP, List T, Svensson P, Gonzalez Y, Lobbezoo F, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: recommendations of the International RDC/TMD Consortium Network* and Orofacial Pain Special Interest Groupdagger. *J Oral Facial Pain Headache*. 2014; 28:6–27. [PubMed: 24482784]
- Sciote JJ, Vieira AR, Nicot R, Ferri J, Raoul G, Horton MJ. ENPP1 and CACNA2D1 associate with TMD-related Masticatory Muscle Pain. *J Dent Res*. 2015; 94(A):2115516.
- Scolozzi P, Wandeler PA, Courvoisier DS. Can clinical factors predict postoperative temporomandibular disorders in orthognathic patients? A retrospective study of 219 patients. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2015; 119:531–538. [PubMed: 25767066]
- Shupnik MA. Oestrogen receptors, receptor variants and oestrogen actions in the hypothalamic-pituitary axis. *J Neuroendocrinol*. 2002; 14:85–94. [PubMed: 11849367]
- Slade GD, Bair E, By K, Mulkey F, Baraian C, Rothwell R, Reynolds M, Miller V, Gonzalez Y, Gordon S, et al. Study Methods, Recruitment, Sociodemographic Findings, and Demographic Representativeness in the OPPERA Study. *J Pain*. 2011; 12:T12–T26. [PubMed: 22074749]
- Slade GD, Fillingim RB, Sanders AE, Bair E, Greenspan JD, Ohrbach R, Dubner R, Diatchenko L, Smith SB, Knott C, et al. Summary of Findings From the OPPERA Prospective Cohort Study of Incidence of First-Onset Temporomandibular Disorder: Implications and Future Directions. *J Pain*. 2013; 14:T116–T124. [PubMed: 24275219]
- Smith YR. Pronociceptive and Antinociceptive Effects of Estradiol through Endogenous Opioid Neurotransmission in Women. *J Neurosci*. 2006; 26:5777–5785. [PubMed: 16723535]
- Smith SB, Maixner DW, Greenspan JD, Dubner R, Fillingim RB, Ohrbach R, Knott C, Slade GD, Bair E, Gibson DG, et al. Potential Genetic Risk Factors for Chronic TMD: Genetic Associations from the OPPERA Case Control Study. *J Pain*. 2011; 12:T92–T101. [PubMed: 22074755]
- Smith SB, Mir E, Bair E, Slade GD, Dubner R, Fillingim RB, Greenspan JD, Ohrbach R, Knott C, Weir B, et al. Genetic Variants Associated With Development of TMD and Its Intermediate Phenotypes: The Genetic Architecture of TMD in the OPPERA Prospective Cohort Study. *J Pain*. 2013; 14:T91–T101. e1–3. [PubMed: 24275226]
- Smith SB, Reenilä I, Männistö PT, Slade GD, Maixner W, Diatchenko L, Nackley AG. Epistasis between polymorphisms in COMT, ESR1, and GCH1 influences COMT enzyme activity and pain. *Pain*. 2014; 155:2390–2399. [PubMed: 25218601]
- Terkeltaub RA. Inorganic pyrophosphate generation and disposition in pathophysiology. *Am J Physiol Cell Physiol*. 2001; 281:C1–C11. [PubMed: 11401820]
- Thilander B, Rubio G, Pena L, de Mayorga C. Prevalence of temporomandibular dysfunction and its association with malocclusion in children and adolescents: an epidemiologic study related to specified stages of dental development. *Angle Orthod*. 2002; 72:146–154. [PubMed: 11999938]
- Ueki K, Moroi A, Sotobori M, Ishihara Y, Marukawa K, Takatsuka S, Yoshizawa K, Kato K, Kawashiri S. A hypothesis on the desired postoperative position of the condyle in orthognathic surgery: a review. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2012; 114:567–576. [PubMed: 22819333]
- Undt G, Murakami KI, Clark GT, Ploder O, Dem A, Lang T, Wiesinger GF. Cross-cultural adaptation of the JPF-Questionnaire for German-speaking patients with functional temporomandibular joint disorders. *J Craniomaxillofac Surg*. 2006; 34:226–233. [PubMed: 16621587]
- Ushiyama T, Ueyama H, Inoue K, Ohkubo I, Hukuda S. Expression of genes for estrogen receptors alpha and beta in human articular chondrocytes. *Osteoarthritis Cartilage*. 1999; 7:560–566.

- Valladares-Neto J, Cevidanes LH, Rocha WC, de Almeida GA, de PAIVA JB, Rino-Neto J. TMJ response to mandibular advancement surgery: an overview of risk factors. *J Appl Oral Sci.* 2014; 22:2–14. [PubMed: 24626243]
- Wang C, Zhang Z, Zhang H, He JW, Gu JM, Hu WW, Hu YQ, Li M, Liu YJ, Fu WZ, et al. Susceptibility genes for osteoporotic fracture in postmenopausal chinese women. *J Bone Miner Res.* 2012; 27:2582–2591. [PubMed: 22807154]
- Warren MP, Fried JL. Temporomandibular disorders and hormones in women. *Cells Tissues Organs.* 2001; 169:187–192. [PubMed: 11455113]
- Yamada K, Nozawa-Inoue K, Kawano Y, Kohno S, Amizuka N, Iwanaga T, Maeda T. Expression of estrogen receptor alpha (ER alpha) in the rat temporomandibular joint. *Anat Rec A Discov Mol Cell Evol Biol.* 2003; 274:934–941. [PubMed: 12973717]
- Yasuoka T, Nakashima M, Okuda T, Tatematsu N. Effect of estrogen replacement on temporomandibular joint remodeling in ovariectomized rats. *J Oral Maxillofac Surg.* 2000; 58:189–196. discussion 196–197. [PubMed: 10670598]
- Zebreck B, Teeramongkolgul T, Nicot R, Horton MJ, Raoul G, Ferri J, Vieira AR, Sciote JJ. ACTN3 R577X genotypes associate with Class II and deepbite malocclusions. *Am J Orthod Dentofacial Orthop.* 2014; 146:603–611. [PubMed: 25439211]
- Zuccherro TM, Cooper ME, Maher BS, Daack-Hirsch S, Nepomuceno B, Ribeiro L, Caprau D, Christensen K, Suzuki Y, Machida J, et al. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. *N Engl J Med.* 2004; 351:769–780. [PubMed: 15317890]

Perspective

This study allows us to identify sub-populations at high risk of developing postoperative temporomandibular disorders after orthognathic surgery procedures. Many other genes of interest could be potential factors influencing the dysfunctional response to orthognathic surgery, particularly genes of the Opera cohort.

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Figure 1.

Panoramic radiographs representing a “classic” osteosynthesis (A) involving one or two titanium mini plates connecting the osteotomy site and one of increased rigidity (B) which had additional retromolar bi-cortical screws (white arrow) fixating the segments behind the mini plates. *Note that osteosynthesis technique is also different for genioplasty. It does not represent a confounding factor given the absence of biomechanical role on occlusion or TMJ.*

Figure 2.
Flow chart of patients

Table 1

Description of analyzed SNP (source: *NCBI variation viewer*)

Gene	Chromosome	SNP	Location	Allelic variation	Protein change	SNP type	1000 Genomes	Minor Allele Frequency	Mechanism	References
<i>ACTN3</i>	11	rs1671064	66,560,202	A	Arg523Gln	Missense		G = 0.4135	- Muscle determinism	(25–27)
		rs1815739	66,560,624	T	Arg577Ter	Nonsense		T = 0.4008	- Dentofacial deformities	
		rs678397	66,557,112	C		Intron variant		T = 0.4203	- TMD	
<i>ENPP1</i>	6	rs9373000	131,900,566	G		Intron variant		G = 0.3594	- Bone mineralization	(27–31)
		rs6569759	131,811,976	G		Intron variant		A = 0.3880	- Dentofacial deformities	
		rs858339	131,832,757	A		Intron variant		A = 0.2810	- TMD	
		rs1409181	131,828,160	G		Intron variant		G = 0.4806		
<i>ESR1</i>	6	rs1643821	151,862,416	A	Ser10=	Intron variant		A = 0.4852	- TMD	(32–35)
		rs3020318	151,968,635	C		Intron variant		C = 0.4105		
		rs3020377	151,951,263	A		Intron variant		A = 0.3804		
		rs2077647	151,807,942	C		Synonymous		C = 0.4665		
<i>PITX1</i>	5	rs1131611	135,029,306	T	Arg140=	Synonymous		T = 0.1793	- mandibular development	(20,36)
<i>PITX2</i>	4	rs2595110	110,624,167	G		Intron variant		G = 0.1516	- Facial Asymmetry	

Table 2

Description of analysis population

Characteristics of analysis population	N=101
Age <i>my (DS)</i>	25.5 (11.3)
Females <i>n (%)</i>	67 (66.3)
Biometrical characteristics:	
Sagittal <i>n (%)</i>	
- Class I	1 (1)
- Class II	69 (68.3)
- Class III	31 (30.7)
Vertical <i>n (%)</i>	
- Normal Bite	40 (39.6)
- Open Bite	42 (41.6)
- Deep Bite	19 (18.8)
Asymmetry <i>n (%)</i>	
- No asymmetry	34 (33.6)
- Minor asymmetry	23 (22.8)
- Asymmetry > 2 mm	39 (38.6)
- Facial posterior asymmetry	5 (5)
Me Angle (°) <i>my (DS)</i>	85.4 (6.3)
Np Angle (°) <i>my (DS)</i>	87.0 (4.5)
Adapted C1F1 (°) <i>my (DS)</i>	86.9 (3.0)
Facial height (Na-Me in mm) <i>my (DS)</i>	121.3 (10.0)
Preoperative JPF score 6 <i>n (%)</i>	27 (26.7)

N : number of observation; my : mean ; DS : standard deviation; n (%) : number of observation (percentage)

Table 3

Comparison of patients by postoperative JPF score: <6 postoperative JPF score group vs ≥6 postoperative JPF score group (postoperative TMD)

	JPF score <6 N=82	JPF score ≥6 N=19	p
Age <i>my (DS)</i>	26.1 (11.9)	23.1 (7.8)	0.734
Females <i>n (%)</i>	52 (63.4)	15 (79.0)	0.197
Biometrical characteristics:			
Sagittal <i>n (%)</i>			
- Class II	54 (65.9)	16 (84.2)	0.118
- Class III	28 (34.1)	3 (15.8)	
Vertical <i>n (%)</i>			
- Normal Bite	31 (37.8)	9 (47.4)	0.546
- Open Bite	34 (41.5)	8 (42.1)	
- Deep Bite	17 (20.7)	2 (10.5)	
Asymmetry <i>n (%)</i>			
- No asymmetry	27 (32.9)	7 (36.8)	0.745
- Asymmetry	55 (67.1)	12 (63.2)	
Me Angle <i>my (DS)</i>	85.6 (6.0)	84.6 (7.3)	0.350
Np Angle <i>my (DS)</i>	87.0 (4.3)	86.9 (5.4)	0.966
Adapted C1F1 <i>my (DS)</i>	87.0 (3.0)	86.8 (3.2)	0.958
Facial Height (mm) <i>my (DS)</i>	121.9 (10.4)	118.9 (8.2)	0.258
Bruxism <i>n (%)</i>	16 (19.5)	5 (26.3)	0.536
Surgical characteristics:			
Type of osteosynthesis:			
- Posterior rigid osteosynthesis	64 (78.1)	11 (57.9)	0.085
Type of mandibular movement:			
- Mandibular advancement	58 (70.7)	16 (84.2)	0.232

N : number of observation; my : mean ; DS : standard deviation; n (%) : number of observation (percentage); p : p-value

Table 4

Comparison of SNP genotypes by postoperative JPF score: <6 postoperative JPF score group vs 9 postoperative JPF score group (postoperative TMD)

Gene	SNP	N	Genotype	<6 postoperative JPF score	6 postoperative JPF score	Groups	p
<i>ACTN3</i>	rs1671064	100	AA n (%)	15 (18.5)	4 (21.1)	AA vs GA vs GG	0.723
			GA	50 (61.7)	10 (52.6)		
			GG	16 (19.8)	5 (26.3)		
	rs1815739	101	CC	17 (20.7)	4 (21.1)	CC vs TC vs TT	1
			TC	48 (58.6)	11 (57.8)		
			TT	17 (20.7)	4 (21.1)		
	rs678397	95	CC	17 (22.1)	4 (22.2)	CC vs CT vs TT	0.939
			CT	42 (54.5)	9 (50)		
			TT	18 (23.4)	5 (27.8)		
<i>ENPP1</i>	rs9373000	100	AA	49 (60.5)	9 (47.4)	AA vs AG vs GG	0.545
			AG	27 (33.3)	8 (42.1)		
			GG	5 (6.2)	2 (10.5)		
	rs6569759	98	GG	19 (23.8)	4 (22.2)	GG vs AG vs AA	0.177
			AG	46 (57.5)	7 (38.9)		
			AA	15 (18.7)	7 (38.9)		
	rs858339	93	TT	45 (59.2)	6 (35.3)	TT vs AT vs AA	
			AT	27 (35.5)	10 (58.2)		
			AA	4 (5.3)	1 (5.9)		
	rs1409181	101	CC	13 (15.9)	4 (21)	CC vs CG vs GG	0.530
			CG	46 (58.1)	12 (63.2)		
			GG	23 (28)	3 (15.8)		
<i>ESR1</i>	rs1643821	100	GG	33 (40.7)	7 (36.9)	GG vs AG vs AA	0.902

Gene	SNP	N	Genotype	<6 postoperative JPF score	6 postoperative JPF score	Groups	p
	rs3020318	99	AG	38 (46.9)	10 (52.6)	TT vs CT vs CC	0.175
			AA	10 (12.4)	2 (510.5)		
			TT	9 (11.2)	5 (26.3)		
			CT	37 (46.3)	9 (47.4)		
			CC	34 (42.5)	5 (26.3)		
	rs3020377	99	GG	7 (8.6)	3 (16.7)	GG vs AG vs AA	0.139
			AG	31 (38.3)	10 (55.5)		
			AA	43 (53.1)	5 (27.8)		
	rs2077647	101	TT	28 (34.2)	4 (21.0)	TT vs CT vs CC	0.511
			CT	41 (50)	12 (63.2)		
			CC	13 (15.8)	3 (15.8)		
<i>PITX1</i>	rs1131611	100	GG	60 (74.1)	16 (80.2)	GG vs GT vs TT	0.381
			GT	20 (24.7)	2 (10.5)		
			TT	1 (1.2)	1 (5.3)		
<i>PITX2</i>	rs2595110	100	AA	43 (53.1)	12 (63.2)	AA vs AG vs GG	0.381
			AG	30 (37.0)	4 (21.0)		
			GG	8 (9.9)	3 (15.8)		

N : number of observation; my : mean ; DS : standard deviation; n (%) : number of observation (percentage); p : p-value

Table 5Comparison of patients by JPF score : >-2 JPF group vs -2 JPF group (*symptomatic worsening*)

	>-2 JPF N=78	-2 JPF N=23	p
Age <i>my (DS)</i>	26.0 (10.9)	23.9 (12.6)	0.208
Females <i>n (%)</i>	53 (68.0)	14 (60.9)	0.528
Biometrical characteristics:			
Sagittal <i>n (%)</i>			
- Class II	54 (69.2)	16 (69.6)	0.976
- Class III	24 (30.8)	7 (30.4)	
Vertical <i>n (%)</i>			
- Normal Bite	33 (42.3)	7 (30.4)	0.480
- Open Bite	30 (38.5)	12 (57.2)	
- Deep Bite	15 (19.7)	4 (17.4)	
Asymmetry <i>n (%)</i>			
- No asymmetry	29 (37.2)	5 (21.7)	0.168
- Asymmetry	49 (62.8)	18 (78.3)	
Me Angle <i>my (DS)</i>	85.4 (6.3)	85.7 (6.2)	0.833
Np Angle <i>my (DS)</i>	86.9 (4.1)	87.1 (5.6)	0.868
Adapted C1F1 <i>my (DS)</i>	86.7 (2.8)	87.7 (3.6)	0.281
Facial height (mm) <i>my (DS)</i>	121.4 (9.6)	120.8 (11.6)	0.995
Bruxism <i>n (%)</i>	18 (23.1)	3 (13.0)	0.389
Surgical characteristics:			
Type of osteosynthesis :			
Posterior rigid osteosynthesis	56 (71.8)	19 (82.6)	0.297
Type of mandibular movement:			
Mandibular advancement	58 (74.4)	16 (69.6)	0.648

N : number of observation; my : mean ; DS : standard deviation; n (%) : number of observation (percentage); p : p-value

Table 6
Comparison of SNP genotypes by JPf score : >-2 JPf group vs -2 JPf (*symptomatic worsening*)

Gene	SNP	N	Genotype	>-2 JPf	-2 JPf	Groups	p
<i>ACTN3</i>	rs1671064	100	AA n (%)	16 (20.8)	3 (13.0)	AA vs GA vs GG	0.188
			GA	48 (62.3)	12 (52.2)		
			GG	13 (16.9)	8 (34.8)		
	rs1815739	101	CC	18 (23.1)	3 (13.1)	CC vs TC vs TT	0.348
			TC	46 (59.0)	13 (56.5)		
			TT	14 (18.0)	7 (30.4)		
<i>ENPP1</i>	rs678397	95	CC	18 (24.7)	3 (13.6)	CC vs CT vs TT	0.101
			CT	41 (56.1)	10 (45.5)		
			TT	14 (19.2)	9 (40.9)		
	rs9373000	100	AA	44 (57.1)	14 (60.9)	AA vs AG vs GG	0.842
			AG	27 (35.1)	8 (34.8)		
			GG	6 (7.8)	1 (4.3)		
<i>ESR1</i>	rs569759	98	GG	18 (23.7)	5 (22.7)	GG vs AG vs AA	0.186
			AG	44 (57.9)	9 (40.9)		
			AA	14 (18.4)	8 (36.4)		
	rs858339	93	TT	43 (56.6)	8 (47.1)	TT vs AT vs AA	
			AT	31 (40.8)	6 (35.3)		
			AA	2 (2.6)	3 (17.6)		
<i>ESR1</i>	rs1409181	101	CC	12 (15.4)	5 (21.7)	CC vs CG vs GG	0.752
			CG	46 (59.0)	12 (52.2)		
			GG	20 (25.6)	6 (26.1)		
	rs1643821	100	GG	34 (44.2)	6 (26.1)	GG vs AG vs AA	0.041

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Gene	SNP	N	Genotype	>-2	JPF	-2	JPF	Groups	p		
	rs3020318	99	AG	37 (48.0)	11 (47.8)						
			AA	6 (7.8)	6 (26.1)						
			TT	10 (13.1)	4 (17.4)					TT vs CT vs CC	0.869
			CT	36 (47.4)	10 (43.5)						
			CC	30 (39.5)	9 (39.1)						
	rs3020377	99	GG	8 (10.4)	2 (9.2)	GG vs AG vs AA	0.908				
			AG	31 (40.3)	10 (45.4)						
			AA	38 (49.3)	10 (45.4)						
	rs2077647	101	TT	24 (30.1)	8 (34.8)	TT vs CT vs CC	0.215				
CT			44 (56.4)	9 (39.1)							
CC			10 (12.8)	6 (26.1)							
PITX1	rs1131611	100	GG	58 (75.3)	18 (78.3)	GG vs GT vs TT					
			GT	18 (23.4)	4 (17.4)						
			TT	1 (1.3)	1 (4.3)						
PITX2	rs2595110	100	AA	42 (54.5)	13 (56.5)	AA vs AG vs GG	0.922				
			AG	26 (33.8)	8 (34.8)						
			GG	9 (11.7)	2 (8.7)						

N : number of observation; my : mean ; DS : standard deviation; n (%) : number of observation (percentage); p : p-value

Table 7Comparison of rs1643821 genotypes (*ESR1* gene) by JPF score: >-2 JPF score group vs -2 JPF score group (symptomatic worsening)

SNP	N	Genotype	-2 JPF	>-2 JPF	Groups	p	OR	95% IC
rs1643821	100	GG n (%)	6 (26.1)	34 (44.2)	GG vs AG vs AA	0.041		
		AG	11 (47.8)	37 (48.0)				
		AA	6 (26.1)	6 (7.8)				
		GG	6 (26.1)	34 (44.2)	GG vs (AG + AA)	0.121	0.446	0.159 1.255
		AG + AA	17 (73.9)	43 (55.8)				
		AG	11 (47.8)	37 (48.1)	AG vs (GG + AA)	0.985	0.991	0.390 2.517
		GG + AA	12 (52.2)	42 (51.9)				
		AA	6 (26.1)	6 (7.8)	AA vs (GG + AG)	0.028	4.176	1.198 14.566
		GG + AG	17 (73.9)	71 (92.2)				

N : number of observation; n (%) : number of observation (percentage) ; p : p-value ; OR : odds ratio ; 95% IC : confidence interval of 95%

Table 8

Comparison of patients by preoperative JPF score : <6 JPF score group vs 6 JPF score group (preoperative TMD)

	<6 JPF score N=74	6 JPF score N=27	p
Age <i>my (DS)</i>	24.4 (11.5)	28.7 (10.3)	0.018
Females <i>n (%)</i>	44 (59.5)	23 (85.2)	0.015
Biometrical characteristics:			
Sagittal <i>n (%)</i>			
- Class II	49 (66.2)	21 (77.8)	0.265
- Class III	25 (33.8)	6 (22.2)	
Vertical <i>n (%)</i>			
- Normal Bite	29 (39.2)	11 (40.7)	0.990
- Open Bite	31 (41.9)	11 (40.7)	
- Deep Bite	14 (18.9)	5 (18.6)	
Asymmetry <i>n (%)</i>			
- No asymmetry	24 (32.4)	10 (34.0)	0.665
- Asymmetry	50 (67.6)	17 (63.0)	
Me Angle <i>my (DS)</i>	85.7 (6.2)	84.7 (6.5)	0.225
Np Angle <i>my (DS)</i>	87.1 (4.6)	86.6 (4.2)	0.611
Adapted C1F1 <i>my (DS)</i>	87.2 (3.2)	86.1 (2.4)	0.142
Facial height (mm) <i>my (DS)</i>	122.0 (10.5)	119.5 (8.4)	0.271
Bruxism <i>n (%)</i>	11 (14.9)	10 (37.0)	0.015

N : number of observation; my : mean ; DS : standard deviation; n (%) : number of observation (percentage); p : p-value

Table 9

Comparison of SNP genotypes by preoperative JPF score: <6 preoperative JPF score group vs 6 JPF score group (preoperative TMD)

Gene	SNP	N	Genotype	<6 preoperative JPF score	6 preoperative JPF score	Groups	p
<i>ACTN3</i>	rs1671064	100	AA n (%)	13 (17.8)	6 (22.2)	AA vs GA vs GG	0.333
			GA	42 (57.5)	18 (66.7)		
			GG	18 (54.7)	3 (11.1)		
	rs1815739	101	CC	15 (20.3)	6 (22.2)	CC vs TC vs TT	0.345
			TC	41 (55.4)	18 (66.7)		
			TT	18 (24.3)	3 (11.1)		
	rs678397	95	CC	15 (21.4)	6 (24)	CC vs CT vs TT	0.244
			CT	35 (50)	16 (64)		
			TT	20 (28.6)	3 (12)		
	<i>ENPP1</i> rs9373000	100	AA	44 (60.3)	14 (51.9)	AA vs AG vs GG	0.555
			AG	25 (34.2)	10 (37.0)		
			GG	4 (5.5)	3 (11.1)		
	rs6569759	98	GG	16 (22.5)	7 (25.9)	GG vs AG vs AA	0.937
			AG	39 (55.0)	14 (51.9)		
			AA	16 (22.5)	6 (22.2)		
	rs858339	93	TT	42 (63.6)	9 (33.3)	TT vs AT vs AA	0.014
			AT	20 (30.3)	17 (63.0)		
			AA	4 (6.1)	1 (3.7)		
	rs1409181	101	CC	11 (14.9)	6 (22.2)	CC vs CG vs GG	0.658
			CG	43 (58.1)	15 (55.6)		
			GG	20 (27.0)	6 (22.2)		
	<i>ESR1</i> rs1643821	100	GG	28 (38.4)	12 (44.4)	GG vs AG vs AA	0.859

Gene	SNP	N	Genotype	<6 preoperative JPF score	6 preoperative JPF score	Groups	p
	rs3020318	99	AG	36 (49.3)	12 (44.4)	TT vs CT vs CC	0.745
			AA	9 (12.3)	3 (11.2)		
			TT	10 (13.9)	4 (14.8)		
			CT	32 (44.4)	14 (51.9)		
			CC	30 (41.7)	9 (33.3)		
	rs3020377	99	GG	6 (8.2)	4 (15.4)	GG vs AG vs AA	0.500
			AG	32 (43.8)	9 (34.6)		
			AA	35 (48.0)	13 (50)		
	rs2077647	101	TT	25 (33.8)	7 (25.9)	TT vs CT vs CC	0.734
			CT	38 (51.3)	15 (55.6)		
			CC	11 (14.9)	5 (18.5)		
<i>PITX1</i>	rs1131611	100	GG	58 (78.4)	18 (69.2)	GG vs GT vs TT	
			GT	15 (20.3)	7 (26.9)		
			TT	1 (1.3)	1 (3.9)		
<i>PITX2</i>	rs2595110	100	AA	38 (52.1)	17 (63.0)	AA vs AG vs GG	0.586
			AG	26 (35.6)	8 (29.6)		
			GG	9 (12.3)	2 (7.4)		

N : number of observation; my : mean ; DS : standard deviation; n (%) : number of observation (percentage); p : p-value

Table 10

Comparison of rs858339 genotypes (*ENPP1* gene) by preoperative JPF score: <6 preoperative JPF score group vs 6 preoperative JPF score group (TMD)

SNP	N	Genotype	<6 preoperative JPF score	6 preoperative JPF score	Groups	p	OR	95% IC
rs858339	93	TT n (%)	42 (63.6)	9 (33.3)	TT vs AT vs AA	0.014		
		AT	20 (30.3)	17 (63.0)				
		AA	4 (6.1)	1 (3.7)				
		TT	42 (63.6)	9 (33.3)	TT vs (AT + AA)	0.008	0.286	0.111 0.735
		AT + AA	24 (36.4)	18 (66.7)				
		AT	20 (30.3)	17 (63.0)	AT vs (TT + AA)	0.003	3.910	1.526 10.021
		TT + AA	46 (69.7)	10 (37.0)				
		AA	4 (6.1)	1 (3.7)	AA vs (TT + AT)			
		TT + AT	62 (93.9)	26 (96.3)				

N : number of observation; n (%) : number of observation (percentage) ; p : p-value ; OR : odds ratio ; 95% IC : confidence interval of 95%