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RESEARCH ARTICLE

Condyle modeling stability, craniofacial asymmetry and ACTN3 genotypes: Contribution to TMD prevalence in a cohort of dentofacial deformities

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Abstract

Craniofacial asymmetry, mandibular condylar modeling and temporomandibular joint disorders are common comorbidities of skeletally disproportionate malocclusions, but etiology of occurrence together is poorly understood. We compared asymmetry, condyle modeling stability and temporomandibular health in a cohort of 128 patients having orthodontics and orthognathic surgery to correct dentofacial deformity malocclusions. We also compared ACTN3 and ENPP1 genotypes for association to clinical conditions. Pre-surgical posterior-anterior cephalometric and panometric radiographic analyses; jaw pain and function questionnaire and clinical examination of TMD; and SNP-genotype analysis from saliva samples were compared to assess interrelationships. Almost half had asymmetries in need of surgical correction, which could be subdivided into four distinct morphological patterns. Asymmetric condyle modeling between sides was significantly greater in craniofacial asymmetry, but most commonly had an unanticipated pattern. Often, longer or larger condyles occurred on the shorter mandibular ramus side. Subjects with longer ramus but dimensionally smaller condyles were more likely to have self-reported TMD symptoms \( p = 0.023 \) and significantly greater clinical diagnosis of TMD \( p = 0.000001 \), with masticatory myalgia most prominent. Genotyping found two significant genotype associations for ACTN3 rs1671064 (Q523R missense) \( p = 0.02 \); rs678397 (intronic SNP) \( p = 0.04 \) and one significant allele association rs1815739 (R577X nonsense) \( p = 0.00 \). Skeletal asymmetry, unusual condyle modeling and TMD are common and interrelated components of many dentofacial deformities. Imbalanced musculoskeletal functional adaptations and genetic or epigenetic influences contribute to the etiology, and require further investigation.
1. Introduction

Growth and stability of the mandibular condyle is essential for attainment and maintenance of mandibular size and morphology. Agenesis, trauma, local infectious pathologies and juvenile idiopathic arthritis all produce similar and distinctive mandibular morphologic disruptions, due to decreased growth in length and normal attainment of transverse width. [1] These condylar growth deficiencies result in skeletal class II open bite malocclusions characterized by a downward and backward growth rotation at the joint articulation and a pronounced antegonial notch. Variability in diminished mandibular length and severity of the dysmorphology is directly related to the chronologic age at which condylar disturbance is first encountered, as demonstrated in case reports of patients with either infections or trauma. [2] In normal joint growth, the condyles are adaptive to variable forces produced by differences in jaw morphology and muscle function. [3] This can result in quite variable changes in length, area and orientation when jaw growth is imbalanced or disproportionate. [4] When transverse skeletal or dental imbalances develop, the condyles adapt by not obtaining normal growth in size, especially in the medio-lateral dimension. [5] These transverse adaptations are reportedly more at risk for development of condyle displacement within the joint and temporomandibular joint disorders (TMD). [6–8]

Dentofacial deformity patients develop the most disproportionate skeletal variations of normal growth and are most likely to have TMJ dysfunction and symptoms. [9] Orthodontic and orthognathic surgical treatments have recently been documented as effective therapies in restoring facial balance and relieving TMD signs and symptoms, especially for related arthralgia or myalgia. [10,11] TMD is also more likely to be associated with dentofacial deformities when a component of the malocclusion involves a significant imbalance in facial symmetry. [12] Well know arthritic conditions like idiopathic condylar resorption may produce skeletal malocclusions and TMD, but in most dentofacial deformity patients condylar modeling is more subtle, and therefore not always considered in treatment planning and outcomes. We recently developed a method for measuring normal condyle geometry variations in a group of patients with dentofacial deformities which revealed differences in condylar length or area between left and right sides. [13] Through genetic analysis we identified a genetic variant in the ENPP1 gene (rs937300) which associated with these variations as a potential causal factor, since it functions as an inhibitor of hydroxyapatite formation during mineralization. The finding indicates that some individuals may be more susceptible to condyle modeling due to both functional influences and inherent quality of bone adaptation.

When craniofacial asymmetry was present, these patients reported a significantly elevated level of pain and jaw dysfunction. [14] This coincided with significantly elevated clinical diagnosis of disc displacement with reduction, myalgia, arthralgia and TMD related headache. In discriminating between different patterns of asymmetry, we developed a new posterior anterior cephalometric analysis which distinguishes four anatomic subclassifications (group one—four), each with a different rate of TMD symptoms. The mandibular asymmetry categories are described in the Materials and Methods, Section 2.3. In group three, chin deviation is displaced to the side of the face which also has the longer ramus length. This unusual subclassification of asymmetry is very common and results in the highest rate of patient reported TMD symptoms. [14] Genetic analysis revealed that an additional variant in ENPP1 (rs858339) associated with this asymmetry pattern. Group two and three had the highest rates of reported TMD symptoms, and four had the lowest—even though skeletal imbalance was the most pronounced in this group. Therefore the posterior anterior cephalometric classification of asymmetry may indicate which groups are at higher risk for having or developing TMD, but does not discern which individuals within a group are predisposed. Although other predisposing factors such as
variations in the functional environment are arguably a primary factor influencing TMD, two possible explanations could be differences in condyle geometry variation, during or after growth, and genotype.

Since mandibular morphology is a heritable trait, it is important to consider genetic and epigenetic (functional) influences upon condylar growth and adaptation. Fibroblast growth factor 2 (FGF-2) is a primary growth promoter of condylar cartilage growth during development. In animal models where lateral functional shift of the mandible are introduced, condylar FGF-2 expression is increased on the protruded ramus side and decreased on the contralateral retrusive side, introducing asymmetric changes in chondrocyte activity and cartilage morphology. FGF-2 promotes ENPP1 activity, resulting in enhanced subcondral bone mineralization. ENPP1 has at least 66 functional variants, some of which might respond differently to condylar environmental influences. Therefore, changes in left versus right condyle morphology demonstrated in condyle geometry variation could be the result of developing facial asymmetry during growth, rather than the primary cause. An additional influence on ENPP1 expression is ACTN3 genotype. In Actn3-/- mice ENPP1 gene expression is increased, resulting in lowered limb bone mineralization apposition rate, trabecular number and bone volume. We recently associated the common ACTN3 R577X mutation which results in lack of protein expression, with skeletal Class II malocclusion. The initial hypothesis is the lack of ACTN3 protein results in diminished subcondral bone growth or maintenance through increased ENPP1 activity.

To further understand variation in presentation of TMD signs and symptoms, we evaluated how different patterns of craniofacial asymmetry, asymmetric condyle geometry variation and ENPP1 or ACTN3 genotypes might interact, in a cohort of dentofacial deformities subjects already included in previous studies. These findings may be of diagnostic predictive value in counseling patients for their potential risk for developing or aggravation of TMD.

2. Materials and methods

2.1 Subjects

Subjects with dentofacial deformities who were undergoing elective orthognathic surgery for correction of dento-maxillo-facial dysmorphology (normal variations in jaw geometry which produce malocclusion and facial imbalance) were recruited for study from the Department of Oral and Maxillofacial Surgery, Roger Salengro Hospital, Lille France, after signing an informed consent to participate. The clinic serves an area of northern France of about 4 million inhabitants under the country’s National Health Service, and is the region’s primary center for maxillofacial surgery. The population for recruitment were non-growing adolescents or adults with a mean age of 26 years and 76% female. They were undergoing combined orthodontic and surgical treatments which included pre-surgical orthodontics, at least a mandibular bilateral sagittal split osteotomy, in conjunction with Lefort osteotomies of the midface as necessary, and a second round of post-surgical orthodontics to finalize occlusion. The study included subjects without other systemic conditions, and excluded those undergoing surgery for facial trauma, tumor, condylar hypertrophy or idiopathic resorption, rheumatoid or osteoarthitis, and congenital craniofacial syndromes or developmental conditions that might influence craniofacial growth. Clinical diagnoses of each patient were summarized at the time of surgery to include the sagittal and vertical malocclusion classification, based upon the extent of required sagittal, vertical and transverse repositioning of jaws estimated in the surgical treatment plan. De-identified information for study included radiographic and diagnostic images, calibrated for magnification, details of the surgery along with information for height, weight, race, ethnicity, age and sex. Subjects signed an informed consent form, and the research
The protocol was validated by the French independent ethical committee (Certificate CPP12/44), the Temple University Temple (Certificate 13438) and the University of Pittsburgh institutional review boards (Certificate PRO12080373).

2.2 Condyle geometry variation assessment

Although there is no widely accepted method to assess condyle modeling as part of normal growth or physiologic adaptation after maturation, the metric method (two dimensional radiographic measurements) have historically been utilized. [25,26] In our patient population, we recently developed a metric measurement method that compares morphometric differences between left and right condyle height or condyle area on panoramic radiographs. [13] Two lines were constructed to evaluate condylar height, one drawn tangential to the posterior edge of the mandible passing through the most posterior points of the condyle and mandibular ramus, and the perpendicular line passing through the lower end of the mandibular notch. Condylar height was measured perpendicular to the latter between the mandibular notch and the highest point of the condylar unit (Fig 1A). The surface of the condylar unit was measured, contouring the lowest point of the mandibular notch to the *lingula mandibulae*, then perpendicular to the rear edge of the mandibular ramus (Fig 1B). Bone modeling was determined by a differential measurement of condylar height or condylar surface defined by a percentage in relation to the larger side between right and left sides on a pre-surgical panoramic radiograph. From this patient data we were able to associate a genetic variant in *ENPP1* with mandibular condyle geometry variation. [13] For comparison, we utilized this existing data base, in combination with an assessment of craniofacial asymmetry to determine associations with TMD or genetic variations. Differences greater than 3% between sides were considered positive for condyle modeling and recorded as percentage difference between sides. Differences less than 3% were recorded as no difference between sides or 0%. Landmarks have been defined on

![Fig 1. Panoramic landmarks related to condyle modelling measurements.](https://doi.org/10.1371/journal.pone.0236425.g001)
calibrated radiographs, using a cephalostat. Data acquisition has been performed by two
observers, jointly defining the landmarks. All measures were done using ImageJ software
(National Institute of Health, Bethesda, MD, USA).

2.3 Asymmetry assessment and classification
Craniofacial asymmetry is a type of dentofacial deformity which has a unique set of morpho-
logic variations for which there have been many classification approaches. We recently de-
veloped a new diagnostic assessment based upon 17 anatomic landmarks on posteroanterior
cephalometric radiographs. [14] These landmarks were converted into 6 cephalometric metric
assessments which characterized four different asymmetry subtypes present in the population
(Fig 2). We characterized these as group 1: asymmetry of the mandibular body, but symmetry
in mandibular rami (sometimes termed “mandibular yaw”); group 2: differences in left and
right ramus heights, with mandibular chin deviated towards the shorter ramus height side
(what most clinicians would refer to as a typical facial asymmetry); group 3: differences in
ramus heights with mandibular chin point deviated towards the longer ramus height side (an
“atypical” facial asymmetry); and group 4; differences in left and right ramus heights, with
mandibular chin deviated towards the shorter ramus height side (as with group 2) but in addi-
tion with pronounced maxillary midfacial canting. From the cephalometric analysis patients
were classified as symmetric or asymmetric, and if asymmetric into subtypes. From these
patient groupings we previously found asymmetry group 2 and 3 had the highest incidence of
pre-surgical TMD, and groups 1, 3 and 4 had significant associations with genetic variants in
ENPP1 [14]. In the present study we compared these classifications for asymmetry to differ-
ences on condyle geometry, as determined in section 2.2.

2.4 Assessment of temporomandibular disorders
Temporomandibular joint functioning was assessed as a routine part of the pre-surgical eval-
uation using the Diagnostic Criteria for TMD (DC/TMD). [27] Overall this young population is
not presenting with fibromyalgia or pain related disability diagnosed in Axis II of the diagno-
sitic criteria. The three common Axis I disorders associated with asymmetry in the population
were disc displacement with reduction (DDR) (78%), myalgia (61%) and arthralgia (33%). [14]
We use the jaw pain and function (JPF) questionnaire to assess patient reported symptoms as
an indication of perceived severity before and one year after jaw surgery. [23] The JPF was
developed as a simple screening tool to determine presence of TMD. [28] It consists of eight
questions about jaw pain and five questions related to jaw function. The questionnaire has
been validated to reliably distinguish between normal (scores < 6) and TMD subjects
(scores ≥ 6) with up to 98% sensitivity and 100% specificity. [29] It has been validated in Euro-
pean translations [30] and we use a French version. [14,23] In this assessment, we included
TMD patients with positive diagnosis for DDR, myalgia and/or arthralgia. Patients with posi-
tive clinical diagnosis for other, less common forms of TMD were excluded from study since
they were insufficient number to investigate.

2.5 Comparing condyle variation with facial asymmetry
A total of 128 subjects had complete data sets for comparison of condyle variation with sym-
metry classification. We compared condyle height or condyle surface as percent differences
between sides, and which side, either left or right, was longer or larger to the symmetry classifi-
cation of patients. Symmetric subjects and those in asymmetry group 1 had equal left and right
mandibular ramus length. In the other three asymmetry groups one ramus was larger in length
and one smaller. In groups 2 and 4 the chin, as indicated by the mandibular menton landmark,
was deviated away from the facial midline towards the shorter ramus length side. In group 3 the
chin however was deviated away from the facial midline towards the longer ramus length
side.

In comparing condyle differences to these patterns of chin and ramus asymmetry, we antic-
ipated finding that the longer or larger condyle would be located on the same side as the longer
ramus. However, this was not true in the majority of patients. Rather, it was more likely that
increased condyle dimension was located on the side with the shorter ramus dimension.

Because of this unexpected finding, we further classified asymmetry groups into those who fol-
lowed the normal, expected pattern or those with an unexpected pattern as follows: (Table 1).

This criteria recognizes that in asymmetry group 3 the menton is deviated towards the
shorter ramus side, but since the ramus is longer on this side, it is anticipated that condyle
dimension would also be larger. Figs 3 and 4 compare of one subject in group 3 which had nor-
mal condyle modeling (1) and one with abnormal modeling (2).

Based upon this realization the anatomical investigation to study had two primary end-
points: 1) determine the frequency of condyle variation in patients with symmetry compared
to asymmetry and 2) determine if condyle variation could have contributed to differences in
TMD, in the differing patterns of asymmetry.

2.6 Genotyping
Saliva samples were collected during the pre-surgical evaluation and processed utilizing DNA
Genotek kits. Genomic DNA was used for profiling of polymorphisms using TaqMan chemis-
try [31] and for sequencing using an automatic sequence-detection instrument (ABI Prism
7900HT, Applied Biosystems). Seven single nucleotide polymorphisms were selected if geno-
typing: in ACTN3 rs1671064, rs1815739 and rs678397 [22] and in ENPP1 rs937300,
rs6569759, rs858339, and rs1409181. [23] The asymmetry population was compared for SNP
variants between normal versus abnormal modeling, as summarized in Table 1, section 2.5.

2.7 Statistical testing
Differences in condyle height or condyle area were compared between all symmetric and all
asymmetric subjects using an unpaired t test, and an ANOVA for comparison between the dif-
ferent asymmetry groups. For relationship to TMD, JPF scores were compared for each individ-
ual asymmetry group between normal growth and abnormal growth by individual
unpaired t tests. In cases where individual asymmetry group comparisons revealed no signifi-
cant differences for JPF, all asymmetry groups were averaged together (normal vs. abnormal

Table 1. Criteria for normal vs. abnormal condyle variation in asymmetry condyle height or area difference.

| same side as menton deviation | opposite side as menton deviation |
|-----------------------------|----------------------------------|
| Group 1—abnormal pattern    | Group 1—normal pattern           |
| Group 2—abnormal            | Group 2—normal                   |
| Group 3—normal              | Group 3—abnormal                 |
| Group 4—abnormal            | Group 4—normal                   |

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Normal differences in left vs right condyle length and surface area
For asymmetry group 3
condyle height and area), and compared by Student t tests to determine significance. For clinical diagnoses of TMD, Chi-square tests were used to compare individual and all TMD diagnoses between individual asymmetry groups, and for the number of TMD diagnosis between all normal vs. abnormal condyle height and area groups. Chi-square and Fisher’s exact tests were used to determine the over-representation of genotypes and alleles.

3. Results

3.1 Differences in condyle modeling between symmetry and asymmetry

Complete data was available from 128 subjects, 56 were classified within one of four craniofacial asymmetry groups from posterior anterior cephalometric analysis. When compared for differential bone modeling of mandibular condyles from panoramic radiographic analysis, there were very significant differences between symmetric vs asymmetric subjects. In the symmetric group there was a mean condyle height variation between sides of 7.37% ± 5.49, compared to 10.89% ± 7.39 for the asymmetric group, which was significantly different (p = 0.0025). The condyle area mean difference in symmetrics was 8.22% ± 5.53, while the asymmetric group difference was 10.35% ± 8.35, which was nearly significantly different at (p = 0.08).

Within the asymmetry population individual groups were compared to determine if there was a difference in the amount of left compared to right condyle height or area modeling. There was no significant differences for condyle height, but there was a significant difference for condyle area (p = 0.02). For condyle area, the amount of difference between sides increased as the severity of asymmetry became more pronounced. Group 1 had a mean area difference of 4.41%, group 2–11.62%, group 3–14.17% and group 4–16.56%.

3.2 TMD differences in asymmetries between normal and abnormal condyle modeling

We investigated whether normal vs abnormal condyle growth modeling, based upon study criteria (Table 1), might contribute to differences in TMD prevalence. This revealed that abnormal condyle modeling was the most common finding throughout the classifications of asymmetry (Table 2). For condylar height, abnormal modeling ranged from 50 to 70 percent. For condylar area, modeling rates were higher in most groups, at rates between 50 and 75. Only asymmetry group 2 had and almost equal distribution of normal versus abnormal modeling.

We compared patient reported TMD symptoms using the JPF questionnaire between normal and abnormal condyle height or area, for each asymmetry group. The abnormal condyle height group had a mean JPF score or 6.3 while the normal group score was 4.7, demonstrating an almost significant difference in symptoms p = 0.055. Those with abnormal condyle area reported mean JPF score of 5.8 compared to the normal area group score of 5.5, resulting in no significant difference p = 0.75. When abnormal condyle height or area were grouped together for the entire population, there was an elevation in mean JPF score to 6.65 by comparison to 5.33 in normal modeling group (p = 0.023). Patient symptoms were greater in asymmetry groups 2 and 3, and either abnormal height or areas contributed to pain and functional differences at approximately the same rate.
Abnormal differences in left vs right condyle length and surface area
For asymmetry group 3
Clinical diagnosis of TMD was compared between normal and abnormal modeling for the population. Since multiple positive diagnoses were common, we first assessed if TMD was present or absent, regardless of single or multiple diagnoses in each patient, which we called “all TMD” patients. This overall positive versus negative comparison revealed a strong trend with a relative higher prevalence of “all TMD” in the abnormal group (p = 0.05) (Table 3). In individual TMD diagnoses of headache, myalgia, arthralgia, disc displacement with reduction and disc displacement without reduction there were no significant differences. However, there was a trend for diagnosis of myalgia (p = 0.06). When total, multiple individual diagnoses were grouped for overall comparison, there was affirming data that abnormal condyle modeling resulted in increased problems with TMD (p = 0.000001).

3.3 Genotype differences

For genotype comparisons we grouped all abnormal condyle height and abnormal condyle area modeling subjects together and compared them to those with normal modeling in each individual asymmetry group. For ENPP1 there were no significant differences in genotypes or alleles for SNPs rs937300 (p range values 0.29 to 0.88), rs6569759 (p range values 0.85 to 0.92) or rs858339 (p range values 0.37 to 0.51). For ACTN3 however there were significant differences in group 2 for genotypes rs1671064 (p = 0.02), rs678397 (p = 0.04) and alleles 1815739 (p = 0.00) (Table 4). There was a trend for rs1815739 genotypes (p = 0.08). Results were most likely positive in group 2 since it had the most subjects for comparison.

4. Discussion

4.1 Condylar role in mandibular modeling

Modeling is the process by which bone enlarges and takes shape during normal growth. Modeling is a complimentary process of resorption or deposition to generate new tissue during homeostasis or for modification of size and shape. The condyle contributes to both mandibular modeling and modeling during normal growth, and has inherent capacity to remodel after growth is completed through chondrocyte sensitivity to variations in mechanotransduction. [32] These effects are well documented in orthodontic treatment of Class II malocclusions with repositioning appliances, where condyles enlarge in anterior-posterior dimension, compared to untreated controls in adolescent or even young adult patients. Ramus modeling can also occur at the same time as changes in condyle dimensions. [33] This ability to adapt and transition from chondrogenesis to osteogenesis is a unique anabolic feature of condylar...
In environments where forces differ in the transverse occlusal plane due to crossbite, some reports have identified asymmetrical condylar modeling, while others have not. In most studies considering skeletal asymmetries of the mandible, condyles are reported to be asymmetric, with decreased cross-sectional area, surface size and ramal height on the deviated side. [34,35] As descriptive morphology of craniofacial asymmetries has advanced, several classification approaches which emphasize mandibular roll, pitch and yaw have established that ramus length, menton deviation and condylar morphological variations do not always match each other, and ramus height may be longer on the same facial side to menton deviation. [14] We recently developed a posterior–anterior cephalometric analysis utilizing six measurements, with four of these detecting mandibular differences between the body, width, ramus length and menton deviation. When viewed by principal component analysis, symmetric and asymmetric faces cluster as distinct groups. Variability in asymmetric groups 1–4 revealed that principal components clustered by differences between the left and right mandibular sides, indicating that a consistent geometric variability explained differences in morphology between them. [14] The main fluctuating variable between groups is the relationship of chin deviation in the transverse plane to left vs right ramus length differences between sides. With regard to these relationships, this study has demonstrated two distinct patterns of asymmetry which have not been commonly recognized previously. First, it is almost equally common to have longer ramus length on the same side as chin deviation as it is to have a longer ramus on the contralateral side. Second, the condyle on the longer ramus side may be geometrically smaller than on the shorter ramus side, regardless of the specific menton to ramus relationship. Insight into these different patterns of

### Table 3. Differences in TMD diagnosis between normal and abnormal condyle modeling.

| Condyle area + height | group 1 | group 2 | group 3 | group 4 | total |
|-----------------------|---------|---------|---------|---------|-------|
| abnormal (11)         | 3       | 1       | 13      | 8       | 28    |
| normal (5)            | 0       | 0       | 3       | 1       | 6     |
| normal (23)           | 3       | 1       | 8       | 5       | 27    |
| normal (23)           | 0       | 0       | 3       | 4       | 9     |
| normal (11)           | 2       | 0       | 15      | 6       | 27    |
| normal (9)            | 0       | 0       | 5       | 1       | 7     |
| normal (4)            | 0       | 0       | 0       | 0       | 1     |

| TMD diagnoses         | all TMD | headache | myalgia | arthralgia | DDR | DD w/o R |
|-----------------------|---------|----------|---------|------------|-----|----------|
| normal (28)           | 3       | 1        | 13      | 8          | 1   | 0        |
| normal (10)           | 1       | 0        | 3       | 1          | 1   | 2        |
| abnormal (23)         | 11      | 3        | 8       | 5          | 2   | 2        |
| abnormal (11)         | 2       | 0        | 15      | 6          | 1   | 1        |
| abnormal (9)          | 0       | 0        | 5       | 1          | 0   | 0        |
| abnormal (68)         | 28      | 10       | 10      | 8          | 9   | 8        |
| abnormal (43)         | 11      | 6        | 27      | 12         | 7   | 1        |

| Chi-squared test      | p       | p       | p       | p         | p    | p       |
|-----------------------|---------|---------|---------|-----------|------|---------|
| all TMD               | 0.05    | 0.41    | 0.06    | 0.42      | 0.19 | 0.11    |
| headache              |         | 0.41    |         |           |      |         |
| myalgia               |         |         | 0.06    |           |      |         |
| arthralgia            |         |         |         | 0.19      |      |         |
| DDR                   |         |         |         |           |      |         |
| DD w/o R              |         |         |         |           |      |         |

* (n) indicates total number of subjects per group; DDR = Disc displacement with reduction; DD w/o R = Disc displacement without reduction without limited opening. [36]

Table 4. Comparison of SNP genotypes by condyle modeling pattern.

| Gene | ACTN3 | rs1671064 (Q523R) | p value | rs1815739 (R577X) | p value | rs678397 (intrinsic) | p value |
|------|-------|-------------------|---------|-------------------|---------|---------------------|---------|
| SNP  | GG    | GA                | AA      | genotype/allele   |         | genotype/allele     |         |
| Group 1 | normal | 2 (15) | 7 (54) | 4 (31) | p = 0.15/0.08 | 2 (15) | 4 (31) | 7 (54) | p = 0.11/0.07 | 2 (15) | 4 (31) | 7 (54) | p = 0.15/0.07 |
|       | abnormal | 2 (66) | 1 (33) | 0       |         | 2 (66) | 0       | 1 (33) |         | 2 (66) | 0       | 1 (33) |         |
| Group 2 | normal | 5 (22) | 18 (78) | 0       | p = 0.02/0.08 | 9 (33) | 1 (4) | 17 (63) | p = 0.08/0.00 | 10 (40) | 1 (4) | 14 (56) | p = 0.04/0.08 |
|       | abnormal | 5 (26) | 9 (48) | 5 (26) |         | 5 (26) | 6 (32) | 8 (42) |         | 6 (31) | 6 (31) | 7 (38) |         |
| Group 3 | normal | 2 (8) | 14 (61) | 7 (31) | p = 0.69/0.49 | 2 (4) | 6 (33) | 16 (63) | p = 0.84/0.34 | 2 (10) | 7 (33) | 12 (57) | p = 0.72/0.64 |
|       | abnormal | 2 (12) | 11 (69) | 3 (19) |         | 2 (12) | 3 (19) | 11 (69) |         | 2 (15) | 3 (21) | 9 (64) |         |
| Group 4 | normal | 4 (40) | 6 (60) | 0       | p = 0.73/0.79 | 4 (40) | 0       | 6 (60) | p = 0.3/0.79 | 4 (40) | 2 (20) | 4 (40) | p = 0.62/0.45 |
|       | abnormal | 2 (50) | 2 (50) | 0       |         | 2 (50) | 0       | 2 (50) |         | 2 (50) | 0       | 2 (50) |         |

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symmetry most likely arise from the number of subjects we have been able to evaluate and compare, rather than previous studies with a more limited population from which these more subtle variations would be less possible to distinguish. These findings raise important clinical questions in patient diagnosis, treatment and management, since differences may relate to intrinsic genetic factors and risk of pre or post treatment TMD and stability.

4.2 Condyle modeling and signs or symptoms of TMD

In our population, asymmetric subjects have both higher clinical diagnoses of TMD and higher reported TMD symptoms, as indicated by the JPF survey. Yet within individual asymmetric groups, the standard deviation for mean values of patient reported symptoms are quite high. This is especially true for group three which had a mean JPF score of 9.11 ± 5.62. Likewise group two, also with elevated symptoms had JPF score of 6.94 ± 5.46. Therefore we evaluated if the pattern of condyle modeling might influence symptom variability by comparing expected versus unexpected geometry variations (Table 1). This resulted in a significantly elevated (p = 0.02) level of patient reported symptoms when modeling did not match with ramus height or area differences. For TMD diagnosis the same comparison revealed a significant difference for subjects with at least one positive finding (p = 0.05) (Table 3). While the individual types of TMD were not significant, when the total number of patient diagnoses were compared, there was very significantly elevated differences (p = 0.0001). This resulted since those with abnormal condyle modeling most often had multiple combinations of different types of TMD. The most common TMD diagnoses in both normal and abnormal groups were masticatory muscle myalgia and disc displacement with reduction, with abnormals almost three times more likely to have myalgia and twice as likely to have disc displacement. Arthralgia rates were almost equal between groups. In abnormal modeling arthralgia was not nearly as likely, with a ratio of 3:1 compared to myalgia. Since presence of arthritic conditions or condylar resorption were part of the study exclusion criteria, the study considered if differences in condylar modeling produced different rates of arthralgia, and found no difference.

An opposite finding occurred for myalgia and disc displacement with reduction, which are much more likely with abnormal condyle modeling. Myalgia was the only individual TMD diagnosis which was almost significantly different for condyle modeling (p = 0.06), and emerged as the solitary clinical diagnosis most related to abnormal modeling (Table 3). The asymmetric patients under study had significant skeletal imbalances, when evaluated by posterior-anterior cephalometric analysis, with the greatest skeletal variation being ramal height differences at p < 0.0001. Muscle functioning is also imbalanced in craniofacial asymmetry, with a significant increase in fast twitch skeletal muscle fibers on the side to which the menton was deviated, when analyzed from masseter muscle biopsy during surgery. Imbalanced force during whole muscle contraction in repetitive athletic activity is a well-known cause of myalgia, especially in the lower back and shoulder. Women athletes with hip strength asymmetry, a type of hip muscle imbalance, are more likely to develop occurrences of low back pain. For male wheelchair athletes, weakness in humeral head depressors, a shoulder muscle imbalance, can result in development of rotator cuff impingement syndrome. Muscle rather than joint pain may also arise from repetitive, unvaried, continuous locomotion and often affect women more than men in the upper extremities.

The TMJ is a unique craniofacial joint since there are three articulations, each joint and the occlusion. Postural and functional position of the jaws is coordinated by afferent input from muscle spindles throughout the head and neck and imbalances in the trigeminal motor system can produce imbalanced stress distribution throughout the cervical spine. Facial skeletal asymmetry produces functional imbalances in masticatory muscles with greater activation on
the longer ramus side. This produces an uneven stress distribution in the mandible, which may occur due to either differences in masticatory muscle forces or skeletal geometry. [38] The TMJ can buffer imbalanced mechanical stress by alteration in rates of chondrogenesis, which may be the etiology of abnormal condyle modeling. In animal models, imbalance in masticatory muscle activity results in asymmetric growth of subcondral bone to normalize stress distributions. [39] This presents the interesting possibility that individual patients adapt better to craniofacial asymmetry if condyle geometry differences provide positive stress support within the joint. Imbalances may also explain why patients experience high rates of myalgia, since increased peripheral activation of masseter muscle spindles can contribute to and help maintain chronic muscle pain. [40]

4.3 ACTN3 anpd ENPP1 genotypes

ENPP1 is a trans membrane glycoprotein which synthesizes inorganic phosphate from extracellular ATP, inhibiting hydroxyapatite formation. SNPs in ENPP1 are associated with a large number of bone diseases and abnormal bone and joint morphology. Different mechanical strain environments change ENPP1 expression which can lead to either protection or calcification of endplate cartilage chondrocytes. [41] In the mandible we recently found the rs937300 SNP associated with variation in condyle geometry between left and right sides. [13] The GG genotype was protective against condyle height reduction. Therefore it is likely that both genotype and functional variations contribute to the pattern of condyle modeling. Two SNPs also associated with either group 1 or group 3 craniofacial asymmetry. [14] Therefore, we evaluated the possibility that ENPP1 variants also contributed to abnormal condyle modeling, but have so far found no associations.

ENPP1 and ACTN3 are connected in bone adaptation by an unknown biologic mechanism. Nevertheless, in Actn3-/- mice osteoblasts have up-regulated expression of ENPP1 which may lead to differences in mineralization rates. [21] This in vitro connection is consistent with disruption of normal mineralization resulting in an overall decrease in bone mass in α-actinin-3 deficiency. In humans the ACTN3 R577X (rs1815739) null polymorphism associates with higher serum levels of modeling markers, which may make bone more susceptible to geometry variations. [42] We found a very significant association (p < 0.0001) for R577X allele differences, with the X allele (null polymorphism) elevated in the normal condyle modeling group. There were additional significant associations for ACTN3 rs1671064 and rs678397 genotypes. rs1671064 is the Q523R polymorphism which produces an A to G transition not known to have functional consequences. Q523R however has been found to have linkage disequilibrium with R577X, [43] and this may indicate that R577X being tested is in close proximity (in linkage disequilibrium) with Q523R, which we could speculate is the actual genetic variant (mutation) that is leading to condyle modeling and TMD symptoms. rs678397 is an intronic SNP which has previously been identified as having a very significant association (p = 0.003) with skeletal class II malocclusions, most likely through variations in condylar growth. [22] All of these findings indicate that both ENPP1 and ACTN3 genotypes associate with varying patterns of condyle modeling in ways which are not yet understood. ACTN3 genotypes can influence ENPP1 expression, as can changes in cartilage mechanical strain environments. [40] Differing biomechanical forces as epigenetic factors and intrinsic genetic differences, both contribute to the pattern of condyle modeling stability or instability, and require further investigation.

4.4 Study shortcomings

The study used conventional posterior anterior cephalograms and panoramic radiographs to determine morphologic differences in the pattern of craniofacial asymmetry and condyle
modeling. These imaging modalities are routinely utilized in radiographic evaluation of dental patients. Although commuted tomography (CBCT) is more precise, current clinical guidelines from the American Dental Association and American Association and Pediatric Dentistry recommend prescription of panoramic radiographs for routine periodic imaging.

Although the study investigated a relatively large number of patients, the study protocol separated participants almost in half for symmetry, and the asymmetric subjects were eventually subdivided into 8 groups for asymmetry type and condyle remolding differences. This resulted in statistical comparisons between limited numbers of patients between groups, and the most important study shortcoming. Future directions will include ongoing studies with larger subject numbers to further understand how condyle modeling and craniofacial asymmetry arise and interact.

4.5 Conclusions

In dentofacial deformity subjects, craniofacial asymmetry, abnormal patterns of condyle modeling and TMD are common comorbidities. Condyle geometry variations between mandibular sides and TMD are more common if asymmetry is present as part of the deformity. Often, asymmetric condyle geometry variation does not match differences in ramus length, found in different classifications of facial asymmetry. TMD signs and symptoms are more likely when condyle variations and ramus asymmetry do not match. The most common TMD diagnosis is masticatory muscle myalgia, which likely results from unequal force distributions. ACTN3 genotypes under study associate with asymmetric condyle modeling and Q523R (mis-sense) may be in linkage disequilibrium with R577X, the common null polymorphism.

These findings further diagnostic precision for interpreting which individual patients might have or develop TMD with or without symptoms. Those in asymmetry groups two and three with imbalanced condyle geometry variation seem to be most at risk, and this is useful diagnostic information. As patients develop asymmetries during maturation, evaluating these features could be important considerations in patient counseling regarding risks for not undergoing surgical treatment to correct skeletal discrepancies.

Supporting information

S1 Data.
(XLS)

Author Contributions

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References

1. Prowler JR, Glassman S. Agenesis of the mandibular condyles: Diagnostic findings and treatment of deformity by polyethylene implant. *Oral Surg Oral Med Oral Pathol* 1954; 7: 133–139. https://doi.org/10.1016/0030-4220(54)90043-2 PMID: 13133340

2. Rabey G. Craniofacial morphogenesis. *Proc R Soc Med* 1971; 64:103–111. PMID: 5548917

3. Mizoguchi I, Toriya N, Nakao Y. Growth of the mandible and biological characteristics to the mandibular condylar cartilage. *Jap Dent Sci Rev* 2013; 49:139–150.

4. Bjork A. Facial growth in man, studied with the aid of metallic implants. *Acta Odontol Scand* 1955; 13:9–34. https://doi.org/10.3109/0001635509028170 PMID: 14398173

5. Tadej G, Engstrom C, Borrman H, Christiansen EL. Mandibular condyle morphology in relation to malocclusions in children. *Angle Orthod* 1988; 59:187–194.

6. Mohlin B, Ingervall B, Thilander B. Relationship between malocclusion and mandibular dysfunction in Swedish men. *Eur J Orthod* 1980; 2:229–238. https://doi.org/10.1093/ejo/2.4.229 PMID: 6961042

7. Williamson EH. Oriented lateral TMJ laminograph symptomatic and nonsymptomatic joints compared. *Angle Orthod* 1983; 53:228–233. https://doi.org/10.1043/0003-3219(1983)053<0228:OLTJLJ>2.0.CO;2 PMID: 6579872

8. Al-Rawi NH, Uthman AT, Sodeify SM. Spatial analysis of mandibular condyles in patients with temporomandibular disorders and normal controls using cone beam computed tomography. *Eur J Dent* 2018; 155:99–104.

9. Dhalberg G, Petersson A, Westesson PL, Eriksson L. Disk displacement and temporomandibular joint symptoms in orthognathic surgery patients. *Oral Surg Oral Med Oral Pathol* 1995; 79:273–277.

10. Abrahamsson C, Henrikson T, Nilner M, Sunzel B, Bondemark L, Ekberg EC. TMD before and after correction of dentofacial deformities by orthodontic and orthognathic treatment. *Int J Oral Maxillofac Surg* 2013; 42:252–258.

11. Al-Moraissi EA, Wolford LM, Perez D, Laskin DM, Ellis E 3rd. Does Orthognathic Surgery Cause or Cure Temporomandibular Disorders? A Systematic Review and Meta-Analysis. *J Oral Maxillofac Surg* 2017; 75:1835–1847. https://doi.org/10.1016/j.joms.2017.03.029 PMID: 28419845

12. Miyatake E, Miyawaki S, Morishige Y, Nishiyama A, Sasaki A, Takano-Yamamoto T, et al. Class III malocclusion with severe facial asymmetry, unilateral crossbite, and temporomandibular disorders. *Am J Orthod Dentofacial Orthop* 2003; 124:435–445. https://doi.org/10.1016/s0889-5406(03)00562-6 PMID: 14560275

13. Constant M, Nicot R, Vieira AR, Raoul G, Sciote JJ, Ferri J, et al. Condylar geometry variation is associated with ENPP1 variant in a population of patients with dento-facial deformities. *J Craniomaxillofac Surg* 2017; 45:826–830. https://doi.org/10.1016/j.jcms.2017.02.020 PMID: 28381371

14. Chung K, Richards T, Nicot R, Vieira AR, Cruz CV, Raoul G, et al. ENPP1 and ESR1 genotypes associated with subclassifications of craniofacial asymmetry and severity of temporomandibular disorders. *Am J Orthod Dentofac Orthop* 2017; 152:631–645.

15. Lobb WK. Craniofacial morphology and occlusal variation in monozoigotic and dizygotic twins. *Angle Orthod* 1987; 57:219–233. https://doi.org/10.1043/0003-3219(1987)057<0219:CMAOVI>2.0.CO;2 PMID: 3477969

16. Šidlauskas M, Šalomskenė L, Andriuškevičiūtė I, Šidlauskienė M, Labanauskas Ž, Vasiliauskas A, et al. Heritability of mandibular cephalometric variables in twins with completed craniofacial growth. *Eur J Orthod* 2016; 38:493–502. https://doi.org/10.1093/ejo/cjv062 PMID: 26503948

17. Aoyama Y, Ochiai T, Shen FC, Hasegawa H. Subcutaneous basic FGF-injection accelerates the development of mandibular condyle of newborn mice during lactation period. *J Hard Tissue Biol* 2013; 22:293–300.

18. Fuentes MA, Opperman LA, Buschang P, Bellinger LL, Carlson DS, Hinton RJ, et al. Lateral functional shift of the mandible: Part II. Effects on gene expression in condylar cartilage. *Am J Orthod Dentofac Orthop* 2003; 123:160–166.

19. Nam HK, Liu J, Li Y, Kragor A, Hatch NE. Ectonucleotide pyrophosphatase/phosphodiesterase-1 (Enpp1) regulates osteoblast differentiation. *J Biol Chem* 2011; 286:39059–71. https://doi.org/10.1074/jbc.M111.221689 PMID: 21930712
20. Stella J, Buers I, van de Wetering K, Höhne W, Rutsch F, Nitschke Y, et al. Effects of different variants in the ENPP1 gene on the functional properties of ectonucleotide pyrophosphatase/phosphodiesterase family member 1. Hum Mut 2016; 37:1190–1201. https://doi.org/10.1002/humu.23057 PMID: 27467858

21. Yang N, Schindeler A, McDonald MM, Seto JT, Houweling PJ, Lek M, et al. α-Actinin-3 deficiency is associated with reduced bone mass in human and mouse. Bone 2011; 49: 790–798. https://doi.org/10.1016/j.bone.2011.07.009 PMID: 21784188

22. Zebrick B, Teeramongkolgul T, Nicot R, Horton MJ, Raoul G, Ferri J, et al. ACTN3 R577X genotypes associate with Class II and deep bite malocclusions. Am J Orthod Dentofac Orthop 2014; 146:603–611.

23. Nicot R, Vieira AR, Raoul G, Delmotte C, Duhamel A, Ferri J, et al. ENPP1 and ESR1 genotypes influence temporomandibular disorders development and surgical-treatment response in dento-deformities. J Craniofac Surg 2016; 44:1226–1237. https://doi.org/10.1016/j.jcsm.2016.07.010 PMID: 27519661

24. McCarthy JG, Cutting CB. The timing of surgical intervention in craniofacial anomalies. Clin Plast Surg 1990; 17:161–182. PMID: 2406094

25. Bjork A, Skieller V. Normal and abnormal growth of the mandible. A synthesis of longitudinal cephalometric implant studies over a period of 25 years. Eur J Orthod 1983; 5:1–46. https://doi.org/10.1093/ejo/5.1.1 PMID: 6572593

26. Dibbets K, Muller B, Krop F, van der Welle L. Deformed condyles and craniofacial growth: findings of the Groningen longitudinal study. Sem Orthod 2013; 19:71–80.

27. Schiffman E, Ohrbach R, Truelove E, Look J, Anderson G, Goulet JP, et al. Diagnostic criteria for temporomandibular disorders (DC/TMD) for clinical and Research Applications. J Oral Facial Pain Headache 2014; 28:6–27. https://doi.org/10.11607/jop.1151 PMID: 24482784

28. Clark GT, Seligman DA, Solberg WK, Pullinger AG, et al. Validity of a brief questionnaire in screening asymptomatic subjects from subjects with tension-type headaches or temporomandibular disorders. Comm Dent Oral Epidemiol 1994; 22:105–112. https://doi.org/10.1111/j.1409-9940.1994.tb00924.x PMID: 8245021

29. Papachristou DJ, Papachroni KK, Papavassiliou GA, Pirttiniemi P, Gorgoulis VG, Piperi C, et al. Functional alterations in mechanical loading of condylar cartilage induces changes in the bony subcondylar region. Arch Oral Biol 2009; 54:1035–1045. https://doi.org/10.1016/j.archoralbio.2009.04.041 PMID: 19775676

30. Rüf S, Panchez H. Temporomandibular joint remodeling in adolescents and young adults during Herbst treatment: A prospective longitudinal magnetic resonance imaging and cephalometric radiographic investigation. Am J Orthod Dentofacial Orthop 1999; 115: 607–618. https://doi.org/10.1016/s0889-5046(99)70285-4 PMID: 10358242

31. Kilic N, Kili A, Oktay H. Condylar asymmetry in unilateral posterior crossbite patients. Am J Dentofacial Orthop 2008; 133: 382–387. https://doi.org/10.1016/j.ajodo.2006.04.041 PMID: 18331937

32. Iodice G, Danzi G, Cimino R, Paduano S, Michelotti A. Association between posterior crossbite, skeletal, and muscle asymmetry: a systematic review. Eur J Orthod 2016; 38:638–651. https://doi.org/10.1093/ejo/jcw003 PMID: 26823371

33. Nadler SF, Malanga GA, Feinberg JH, Prybcien M, Stitik TP, DePrince M, et al. Relationship between hip muscle imbalance and occurrence of low back pain in collegiate athletes. A prospective study. Am J Phys Med Rehabil 2001; 80:572–577. https://doi.org/10.1097/00002060-200108000-00005 PMID: 11475476

34. Veiersted KB, Westgaard RH, Andersen P. Electromyographic evaluation of muscular work pattern as a predictor of trapezius myalgia. Scand J Work Environ Health 1993; 19:284–290. https://doi.org/10.5271/sjweh.1472 PMID: 8235518

35. Shimazaki T, Motoyoshi M, Hosoi K, Namura S. The effect of occlusal alteration and masticatory imbalance on the cervical spine. Eur J Orthod 2003; 25:457–463. https://doi.org/10.1093/ejo/25.5.457 PMID: 14609013
39. Miyazaki M, Yonemitsu I, Takei M, Kure-Hattori I, Ono T. The imbalance of masticatory muscle activity affects the asymmetric growth of condylar cartilage and subchondral bone in rats. *Arch Oral Biol* 2016; 63:22–31. https://doi.org/10.1016/j.archoralbio.2015.11.020 PMID: 26669214

40. Lund JP, Sadeghi S, Athanassiadis T, Caram Salas N, Auclair F, Thivierge B, et al. Assessment of the potential role of muscle spindle mechanoreceptor afferents in chronic muscle pain in the rat masseter muscle. *Plos One* 2010; 5:e11131. https://doi.org/10.1371/journal.pone.0011131 PMID: 20559566

41. Xu HG, Hu CJ, Wang H, Liu P, Yang XM, Zhang Y, et al. Effects of mechanical strain on ANK, ENPP1 and TGF-β1 expression in rat endplate chondrocytes in vivo. *Mol Med Report* 2011; 4:831–835.

42. Levinger I, Yan X, Bishop D, Houweling PJ, Papadimitriou I, Munson F, et al. The influence of α-actinin-3 deficiency on bone remodeling markers in young men. *Bone* 2017; 98:26–30. https://doi.org/10.1016/j.bone.2017.02.010 PMID: 28254467

43. Paparini A, Ripani M, Giordano GD, Santoni D, Pigozzi F, Romano-Spica V, et al. ACTN3 genotyping by real-time PCR in the Italian population and athletes. *Med Sci Sport Exerc* 2007; 39:810–815.