

# Early mandibular morphological differences in patients with FGFR2 and FGFR3-related syndromic craniosynostoses: a 3D comparative study

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#### 1 **Title page**

- 2 Early mandibular morphological differences in patients with FGFR2 and FGFR3-related
- 3 syndromic craniosynostoses: a 3D comparative study
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40 41 Abstract

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43 Syndromic craniosynostoses are defined by the premature fusion of one or more cranial and 44 facial sutures, leading to skull vault deformation, and midfacial retrusion. More recently, 45 mandibular shape modifications have been described in *FGFR*-related craniosynostoses, 46 which represent almost 75 % of the syndromic craniosynostoses. Here, further 47 characterisation of the mandibular phenotype in *FGFR*-related craniosynostoses is provided in 48 order to confirm mandibular shape modifications, as this could contribute to a better 49 understanding of the involvement of the FGFR pathway in craniofacial development.

50 The aim of our study was to analyse early mandibular morphology in a cohort of patients with 51 *FGFR2*- (Crouzon and Apert) and *FGFR3*- (Muenke and Crouzonodermoskeletal) related 52 syndromic craniosynostoses. We used a comparative geometric morphometric approach based 53 on 3D imaging. Thirty-one anatomical landmarks and eleven curves with sliding semi-54 landmarks were defined to model the shape of the mandible.

55 In total, 40 patients (12 with Crouzon, 12 with Apert, 12 with Muenke and 4 with 56 Crouzonodermoskeletal syndromes) and 40 age and sex-matched controls were included 57 (mean age: 13.7 months  $\pm$  11.9). Mandibular shape differed significantly between controls 58 and each patient group based on geometric morphometrics. Mandibular shape in FGFR2-59 craniosynostoses was characterized by open gonial angle, short ramus height, and high and 60 prominent symphysis. Short ramus height appeared more pronounced in Apert than in 61 Crouzon syndrome. Additionally, narrow inter-condylar and inter-gonial distances were 62 observed in Crouzon syndrome. Mandibular shape in FGFR3-craniosynostoses was 63 characterized by high and prominent symphysis and narrow inter-gonial distance. In addition, 64 narrow condylar processes affected patients with Crouzonodermoskeletal syndrome.

65	Statistical analysis of variance showed significant clustering of Apert and Crouzon, Crouzon
66	and Muenke, and Apert and Muenke patients (p<0.05).
67	Our results confirm distinct mandibular shapes at early ages in FGFR2- (Crouzon and Apert
68	syndromes) and FGFR3-related syndromic craniosynostoses (Muenke and
69	Crouzonodermoskeletal syndromes) and reinforce the hypothesis of genotype-phenotype
70	correspondence concerning mandibular morphology.
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72	Keywords
73	Craniosynostosis; FGFR2; FGFR3; mandibular shape; 3D geometric morphometrics
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#### 92 Introduction

93 Craniosynostoses – reported in 1:2,000 to 1:3,000 live births, comprising at least 20 % of
94 syndromic cases [1] – are defined by the premature fusion of one or more cranial and facial
95 sutures, leading to various craniofacial anomalies, among which: skull vault deformation,
96 potentially causing increased intracranial pressure, shallow orbits leading to exorbitism, and
97 midfacial retrusion that can be associated with obstructive sleep apnoea syndrome [2-4].

98 Various mutations have been reported in syndromic craniosynostoses and affect key 99 signalling pathways involved in craniofacial development, such as FGFRs/TWIST pathways, 100 MSX2, EFR, TCF12 and SMAD6 [5]. Mutations in genes from the FGFR (Fibroblast Growth 101 Factor Receptor) pathways are reported in almost 75% of syndromic craniosynostoses [6]. 102 Such *FGFR*-related conditions are due to specific activating heterozygous mutations localized 103 in FGFR1 (Pfeiffer syndrome type 1) [1], FGFR2 (Apert, Crouzon, Pfeiffer type 2 and 3 104 syndromes, Antley-Bixler, Beare-Stevenson cutis gyrata syndromes, Bent Bone Dysplasia and 105 Saethre-Chotzen like syndrome) [5-9] and FGFR3 genes (Muenke syndrome and 106 Crouzonodermoskeletal syndrome). Muenke syndrome is the most frequent FGFR-related 107 syndromic craniosynostosis [10]. Crouzonodermoskeletal syndrome is an exceedingly rare 108 syndrome sharing similarities with Crouzon syndrome and including skin lesions (acanthosis 109 nigricans) [11-14]. Most of these FGFR mutations are gain of function, inducing high activity 110 of the receptor in presence and in absence of its FGF ligands, thus disturbing bone formation.

111 It has been demonstrated that *FGFR* genes are implicated in bone formation and both axial
112 and appendicular skeletal development as illustrated by the phenotype of chondrodysplasia
113 [15-18]. Skull vault, frontal bone and midfacial anomalies have been extensively described in
114 *FGFR*-related syndromic craniosynostoses [3,19-22]. Several studies also report mandibular

shape modifications in *FGFR2*- and *FGFR3*-related craniosynostoses [23-27] as well as in
achondroplasia (*FGFR3*) [28].

117 The mandible is a dermal bone derived from neural crest cells migrating into the first 118 pharyngeal arch [29-33]. Mandibular bone ossifies early during the 6<sup>th</sup> week of pregnancy 119 [34]. In mouse and chick models, *Fgfr2* and *Fgfr3* genes are expressed during mandibular 120 formation at the sites of primary (Meckel cartilage) and secondary cartilages (condyles, 121 symphysis) and have key roles in mandibular intramembranous and endochondral ossification 122 [35-39].

A better characterisation of the human mandibular phenotype is necessary in *FGFR*-related
craniosynostoses. This could help to better understand the implication of the FGFR pathway
in craniofacial development and highlight homologies between mouse models and human
phenotypes.

127 The aim of our study was to analyse mandibular morphology at early ages in a cohort of 128 patients with FGFR2-(Crouzon FGFR3and Apert) and (Muenke and 129 Crouzonodermoskeletal) related syndromic craniosynostoses, using a comparative geometric 130 morphometric approach based on 3D imaging, allowing shape comparisons between each 131 FGFR-craniosynostosis subtype and age and sex-matched controls, and between the different 132 FGFR-craniosynostosis subtypes.

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### 144 Patients and methods

145 Patients

146 We retrospectively included 40 patients presenting with genetically confirmed FGFR2- or 147 FGFR3-related syndromic craniosynostoses, and available craniofacial computed 148 tomographic (CT) scan. Only CT-scans performed at early ages before fronto-facial or fronto-149 orbital advancement, or in the immediate post-operative period after posterior vault expansion 150 (at post-operative days 1 or 2), were included, in order to avoid potential confounding factors 151 related to the effects of surgery on mandibular shape. The following clinical parameters were 152 collected: gender, type of mutation, age at CT-scan and at first surgical procedure and 153 tracheostomy. The database used to select patients covered the birth period 2003-2017. All 154 patients were initially managed in the National Referral Center for craniofacial malformations 155 (Centre de Référence Maladies Rares CRANIOST), located within our hospital. The study 156 was approved by the local Ethical Committee (Comité d'Éthique de Necker – Enfants 157 Malades, approval number: 2018 RK18).

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### 159 Controls

160 Controls were selected among age and gender-matched patients free of any reported
161 craniofacial anomalies who underwent CT-scans for the assessment of benign craniofacial
162 trauma or infections (mandible excluded). All CT-scans were extracted from the same
163 database (clinical database of Necker – Enfants Malades).

#### 165 Comparative study of mandibular morphology based on 3D geometric morphometrics

Segmentation and 3D reconstructions were performed based on DICOM raw data using 3D Slicer 4 [40] by isolating the mandible based on its cortical and trabecular bone density thresholds. Visual adjustments of the cortical bone density threshold were done for each CTscan, without smoothing factors to keep the correct contours and avoid any loss of information concerning anatomical variations. The 3D surface mesh obtained for each patient and control was saved in STL format.

Thirty-one anatomical landmarks and eleven curve with sliding semi-landmarks were defined in order to model the shape of the mandible, based on previous studies [41-43] and were placed using Viewbox 4 (dHal Software, Kifissia, Greece), by the same author (AM). Names and definitions of the landmarks and curves are provided on Figure 1. Using Morpho J v. 1.06 [44], a Procrustes ANOVA analysis for error measurement of 'factor repetition' showed no significant differences in landmarking positioning, allowing valid comparative analysis of shape variability among individuals (see Supplementary Table 1).

179 Comparative mandibular morphology was assessed based on 3D geometric morphometrics 180 [45] using R [46] for data processing. Landmarks coordinates were superimposed using a 181 Generalized Procrustes Analysis (GPA) [47]. Sliding semi-landmarks of curves have been 182 allowed to slide minimizing the bending energy [43] using the 'gpagen' function of the 183 'geomorph' package [48]. Coordinates after GPA were assessed using principal component 184 analysis (PCA) which gives information about raw variability. Both analyses, GPA and PCA 185 were performed using the 'gpagen' and 'plotTangentSpace' functions of the 'geomorph' 186 package [48]. Ten PC axis have been kept for statistical analysis representing 90 % of total 187 shape variation [49]. The 3D visualizations along the axes of the PCA have been made using 188 'Thin Plate Splines' (TPS) [50], from the average shape to the extremes shapes of the axes 189 thanks to the 'tps3d' function of the 'Morpho' package [50]. In a first step, the 3D model of a control specimen (representative of the median age of the studied population) has been
warped to the mean shape (corresponding to the mean coordinates of all specimens, defining
the area 'zero' of the PCA) to create a 3D model of the mean shape. In a second step, this 3D
mean shape has been deformed using TPS interpolation according to the extreme shape of the
PC axes.

195 Then, to assess comparisons between groups, Canonical Variate Analyses (CVA) have been 196 performed using the 'CVA' function of the 'Morpho' package' [51]. Eleven datasets were 197 created for mandibular shape comparisons within the total group of patients and controls, 198 between patients and controls for each syndrome independently, as well as between each 199 groups of patients.

200 Multiple ANalysis Of VAriance (MANOVAs) were performed considering 'type of 201 syndrome' and 'age at CT-scan' as classifiers, considering 90 % of total shape variability 202 (corresponding to ten axes of the PCA).

#### 203 Statistical analysis

204 Quantitative data were expressed as mean  $\pm$  Standard Derivation or median  $\pm$  Interquartile 205 range and were compared using the Kruskal-Wallis test. p < 0.05 was considered as 206 significant.

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- 219 Results
- 220 1. Patients

221 Forty patients were included into our study: 12 patients with Crouzon syndrome, 12 with 222 Apert syndrome, 12 with Muenke syndrome and 4 with Crouzon syndrome with acanthosis 223 nigricans, and 40 age and sex-matched controls. Male/female ratio was 16/24 and mean age at 224 inclusion (date of CT-scan) was 13.7 months  $\pm$  11.9, without statistical difference between the 225 four groups (p=0.15). Patients characteristics, types of mutations and of craniosynostosis, and 226 age at CT-scan for each group were summarized in Table 1. Seventeen patients (6/12 in 227 Crouzon, 4/12 in Apert, 4/12 in Muenke, and 3/4 in Crouzon with acanthosis nigricans 228 group) had benefited from posterior skull vault expansion prior to the date of CT-scan, at 229 post-operative days 1 or 2. Three patients had severe ventilation disorders requiring temporary 230 tracheostomy (two with Crouzon with *acanthosis nigricans* and one with Crouzon syndrome).

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#### 2. Comparative geometric morphometric analysis of mandibular shape

**233** GPA and PCA analyses were performed on the total group of patients and controls. PCA of **234** the mandible shapes showed a clear shape separation of patients and controls along PC2 while **235** PC1 comprised age related shape changes, as confirmed by linear regression tests ( $r^2=0.42$ ; **236**  $p<10^{-4}$ ). The plots of PC scores and 3D shape changes associated with PC1 and PC2 by **237** morphing the mean mandibular surface model are shown in Figure 2. Mean 3D mandibular **238** shape differed significantly between patients and controls ( $p<10^{-3}$ ). 3D mandibular shape **239** differences obtained after CVA are shown in Figure 3.

241	2.1. In FGFR2-related craniosynostoses
242	The plots of PC scores and wire frames of the first two PCs and 3D shape changes associated
243	to PC2 for patients and control groups are shown in Figure 4. ANOVA analyses showed
244	significant statistical shape differences along PC2 between patients and controls in Crouzon
245	and Apert groups ( $p < 10^{-2}$ ).
246	Mandibular shape in Crouzon patients was characterized by (1) open gonial angle, (2) short
247	ramus height, (3) narrow inter-gonial and inter-condylar distances and (4) slightly high and
248	prominent symphysis at the level of alveolar bone.
249	Mandibular shape in Apert patients was characterized by (1) open gonial angle, (2) short
250	ramus height and (3) slightly high and prominent symphysis at both basilar and alveolar
251	levels.
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253	2.2. In FGFR3-related craniosynostoses
253 254	<b>2.2. In</b> <i>FGFR3</i> -related craniosynostoses The plots of PC scores and wire frames of the first two PCs and 3D shape changes associated
253 254 255	<b>2.2. In FGFR3-related craniosynostoses</b> The plots of PC scores and wire frames of the first two PCs and 3D shape changes associated to PC2 for patients and controls group are shown in Figure 5. ANOVA analyses showed
253 254 255 256	<ul><li>2.2. In FGFR3-related craniosynostoses</li><li>The plots of PC scores and wire frames of the first two PCs and 3D shape changes associated</li><li>to PC2 for patients and controls group are shown in Figure 5. ANOVA analyses showed</li><li>significant statistical shape differences along PC2 between patients and controls in Muenke</li></ul>
253 254 255 256 257	<b>2.2. In</b> <i>FGFR3</i> -related craniosynostoses The plots of PC scores and wire frames of the first two PCs and 3D shape changes associated to PC2 for patients and controls group are shown in Figure 5. ANOVA analyses showed significant statistical shape differences along PC2 between patients and controls in Muenke group ( $p<10^{-3}$ ). The low number of patients in Crouzonodermoskeletal subgroup did not allow
253 254 255 256 257 258	<b>2.2. In</b> <i>FGFR3</i> -related craniosynostoses The plots of PC scores and wire frames of the first two PCs and 3D shape changes associated to PC2 for patients and controls group are shown in Figure 5. ANOVA analyses showed significant statistical shape differences along PC2 between patients and controls in Muenke group ( $p<10^{-3}$ ). The low number of patients in Crouzonodermoskeletal subgroup did not allow ANOVA analysis.
253 254 255 256 257 258 259	<ul> <li>2.2. In <i>FGFR3</i>-related craniosynostoses</li> <li>The plots of PC scores and wire frames of the first two PCs and 3D shape changes associated to PC2 for patients and controls group are shown in Figure 5. ANOVA analyses showed significant statistical shape differences along PC2 between patients and controls in Muenke group (p&lt;10<sup>-3</sup>). The low number of patients in Crouzonodermoskeletal subgroup did not allow ANOVA analysis.</li> <li>Mandibular shape in Muenke patients was characterized by (1) slightly high and prominent</li> </ul>
253 254 255 256 257 258 259 260	<ul> <li>2.2. In <i>FGFR3</i>-related craniosynostoses</li> <li>The plots of PC scores and wire frames of the first two PCs and 3D shape changes associated to PC2 for patients and controls group are shown in Figure 5. ANOVA analyses showed significant statistical shape differences along PC2 between patients and controls in Muenke group (p&lt;10<sup>-3</sup>). The low number of patients in Crouzonodermoskeletal subgroup did not allow ANOVA analysis.</li> <li>Mandibular shape in Muenke patients was characterized by (1) slightly high and prominent symphysis at the level of basilar border only and (2) open sigmoid notch with anteriorly</li> </ul>
253 254 255 256 257 258 259 260 261	<ul> <li>2.2. In FGFR3-related craniosynostoses</li> <li>The plots of PC scores and wire frames of the first two PCs and 3D shape changes associated to PC2 for patients and controls group are shown in Figure 5. ANOVA analyses showed significant statistical shape differences along PC2 between patients and controls in Muenke group (p&lt;10<sup>-3</sup>). The low number of patients in Crouzonodermoskeletal subgroup did not allow ANOVA analysis.</li> <li>Mandibular shape in Muenke patients was characterized by (1) slightly high and prominent symphysis at the level of basilar border only and (2) open sigmoid notch with anteriorly placed coronoid process and (3) narrow inter-gonial distance.</li> </ul>
253 254 255 256 257 258 259 260 261 262	<ul> <li>2.2. In FGFR3-related craniosynostoses</li> <li>The plots of PC scores and wire frames of the first two PCs and 3D shape changes associated to PC2 for patients and controls group are shown in Figure 5. ANOVA analyses showed significant statistical shape differences along PC2 between patients and controls in Muenke group (p&lt;10<sup>-3</sup>). The low number of patients in Crouzonodermoskeletal subgroup did not allow ANOVA analysis.</li> <li>Mandibular shape in Muenke patients was characterized by (1) slightly high and prominent symphysis at the level of basilar border only and (2) open sigmoid notch with anteriorly placed coronoid process and (3) narrow inter-gonial distance.</li> <li>Mandibular shape in Crouzonodermoskeletal patients was characterized by (1) prominent</li> </ul>
253 254 255 256 257 258 259 260 261 262 263	<ul> <li>2.2. In <i>FGFR3</i>-related craniosynostoses</li> <li>The plots of PC scores and wire frames of the first two PCs and 3D shape changes associated to PC2 for patients and controls group are shown in Figure 5. ANOVA analyses showed significant statistical shape differences along PC2 between patients and controls in Muenke group (p&lt;10<sup>-3</sup>). The low number of patients in Crouzonodermoskeletal subgroup did not allow ANOVA analysis.</li> <li>Mandibular shape in Muenke patients was characterized by (1) slightly high and prominent symphysis at the level of basilar border only and (2) open sigmoid notch with anteriorly placed coronoid process and (3) narrow inter-gonial distance.</li> <li>Mandibular shape in Crouzonodermoskeletal patients was characterized by (1) prominent symphysis at both alveolar and basilar levels and (2) narrow inter-condylar and inter-gonial</li> </ul>

266 2.3. Comparison of mandibular shape between the groups of *FGFR2* and *FGFR3*267 craniosynostoses

To analyse shape differences between each patient group, a MANOVA test was performed and showed statistically significant differences between Crouzon and Apert (p=0.04), Crouzon and Muenke ( $p<10^{-3}$ ) and Apert and Muenke patients ( $p<10^{-3}$ ). 3D mandibular shape differences between patient groups obtained after CVA are shown in Figure 6.

Short ramus height appeared more pronounced in Apert than in Crouzon syndrome and did not obviously affect patients with FGFR3-syndromes. In Crouzon syndrome, condylar processes appeared narrower when compared to Apert and Muenke syndromes. The angular processes were also narrower in Crouzon and Muenke syndromes, when compared with Apert syndrome. Coronoid processes were anteriorly displaced in Muenke syndrome when compared with the other groups. The position of the symphysis did not differ obviously among groups. No statistically significant shape separation was observed between Crouzonodermoskeletal patients (n=4) and the other groups (data not shown).

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#### 295 Discussion

Mandibular shape results from genetic and epigenetic factors, thus explaining the high phenotypic heterogeneity among individuals [52]. Mandibular shape is also constantly modified along life, related to numerous anatomical and functional factors such as dental eruption, tooth loss, mastication, and lingual function [41,42,53]. Determining intrinsic mandibular anomalies in craniosynostosis syndromes is thus a technical challenge, where mandibular shape is also affected by skull base changes.

302 High genetic variability characterizes Crouzon syndrome, with at least 47 distinct mutations 303 described to date [54], while Apert syndrome is characterized by a very small set of mutations 304 (FGFR2<sup>S252W</sup>, FGFR2<sup>P253R</sup>, and FGFR2<sup>S252F</sup> in a lesser extent) [55,56] and Muenke and 305 Crouzonodermoskeletal syndromes are caused by a unique mutation  $(FGFR3^{P250R})$  and 306  $FGFR3^{A391E}$ , respectively) [10,11]. Although based on a relatively small sample of genotyped 307 patients, our cohort was representative of the main mutations usually reported in these 308 syndromic craniosynostoses. Only four patients diagnosed with the extremely rare 309 Crouzonodermoskeletal syndrome with confirmed A391E mutation could be included in this 310 study.

311 Our results showed significant distinct mandibular shapes at early ages in Crouzon, Apert, 312 Muenke and Crouzonodermoskeletal syndromes when compared with age and sex-matched 313 controls and highlight genotype-phenotype correspondence in *FGFR2*- and *FGFR3*-related 314 syndromic craniosynostoses. Although Crouzon and Apert patients displayed comparable mandibular shapes (open gonial angle, short ramus height, slightly high and prominent
symphysis), anatomical variations were stronger in the Apert than in the Crouzon subgroup,
which is consistent with previous findings for the upper third of the face, the midface
[3,21,57,58] and the mandible [59,60].

319 differences in mandibular shapes Significant were observed in Muenke and 320 Crouzonodermoskeletal subgroups relative to controls. However, changes did not obviously 321 affect ramus height and gonial angulation, unlike FGFR2-craniosynostoses. These 322 morphological differences between FGFR2- and FGFR3-craniosynostoses reinforce the 323 hypothesis that activating mutations in FGFR2 and FGFR3 genes may affect membranous 324 and endochondral ossification processes within different manners, and hence generate 325 variable craniofacial and mandibular phenotypes. Concerning mandibular formation, FGFR2 326 and FGFR3-activating mutations may have differential intrinsic effects on the primary 327 (Meckel cartilage) and secondary condylar and symphyseal cartilages, then affecting 328 mandibular development and morphology. In addition, it has been shown that patients 329 affected by achondroplasia (linked to activating mutations in FGFR3 gene), presented 330 defective size and orientation of the ramus [28]. Overall, this underlines the different effects 331 of the distinct mutations in FGFR3 gene - responsible for craniosynostosis and 332 chondrodysplasia – on mandibular formation and morphology.

Of note, we observed anteriorly displaced coronoid processes in Muenke subgroup relative to
controls and other patient groups. This morphological feature may be induced by the cranial
shape modifications, particularly affecting the temporal region, then influencing temporal
muscles dynamics at both temporal and coronoid insertions levels.

In all subgroups, tendency for prominent and high symphysis was observed. Symphysis shape
is determined by the combined effects of dental eruption and occlusion, and the position of
the mandible relative to the maxilla, as well as tongue function and suprahyoid muscles

340 insertion sites [41, 61]. We could not control all potential influencing factors of mandibular 341 shape in this retrospective study. In addition, we could not confirm that prominent symphysis 342 reflected true prognathism, as all patients had moderate to extreme midfacial retrusion. The 343 severity of midfacial retrusion varies among individuals with reported genotype-phenotype 344 correlations [3]. In fact, midfacial retrusion appeared more pronounced in Apert than in 345 Crouzon syndrome, more obvious in Apert cases carrying FGFR2<sup>S252W</sup> mutation relative to 346 those carrying  $FGFR2^{P253R}$  mutation, the latter leading to more severe midfacial retrusion than 347 does *FGFR3*<sup>P250R</sup>, responsible for Muenke syndrome [3,62].

Crouzon syndrome is characterized by its wide spectrum of craniofacial features, from normal phenotype to severe cloverleaf skull malformations. In our series, we also observed high variability of craniosynostosis, from no synostosis at the time of the study (in one patient) to severe cloverleaf skull shape, and moderate to severe fronto-maxillary retrusion. Noticeably, phenotypic features may be absent at birth and evolve gradually during the first years of life [63-65]. However, we observed homogeneous mandibular shapes in the group of Crouzon patients even though six distinct mutations were observed in this group.

355 Several authors have reported various and sometimes divergent mandibular phenotypic 356 features in FGFR2-related syndromic craniosynostoses, in terms of proportions and shapes 357 [23-27,60]. First, these divergent results may be explained by the heterogenous ages at 358 inclusion in the different series. Secondly, mandibular morphology was analysed by various 359 methods, often after craniofacial surgery procedures, thus challenging the comparisons 360 between the studies. Of note, the mandibular shape and particularly intercondylar width, 361 depend on the distance between the condylar fossa on the skull base, as shown in Crouzon and 362 Apert syndromes [27]. Similar results have been found in artificially deformed skulls, where 363 external mechanical constraints had been intentionally exerted on the skull vault with 364 different deforming devices as observed worldwide [66-69]: secondary skull base

365 modifications induced a shift in the position of the glenoid fossa, thus influencing mandibular 366 shape [67,69]. In syndromic craniosynostoses, intrinsic skull base deformations could thus act 367 as potential secondary influencing factors on mandibular shape. We showed a slight 368 narrowing of the condylar processes in Crouzon and Crouzonodermoskeletal syndromes when 369 compared with controls, which was consistent with previous findings [23,27]. This finding 370 thus most probably resulted from the combined effects of (1) repercussions of the vault 371 deformation on the skull base, (2) intrinsic skull base deformations and (3) intrinsic 372 mandibular shape characteristics.

Of note, all included CT-scans were performed at early ages before fronto-facial and frontoorbital surgery, thus ruling out potential effects of surgery on mandibular shape. In fact,
fronto-facial surgery involved maxillary advancement [22,70,71] and could thus influence
mandibular morphology.

377 Concerning mean age at selected CT-scan, no significant differences were observed between 378 groups. We confirmed by metric measurements, wider angles in Crouzon and Apert patient 379 group when compared with controls (p=0.03 and p=0.003, respectively). Although mean ages 380 were not statistically different between groups, quantifying mandibular size differences with 381 metric values based on linear measurements would require a different study design, with 382 strictly the same ages in each group, which was not fully the case in our population of 383 growing children. We aimed to detect shape differences among groups, independently of 384 mandibular size, which varies among individuals, particularly with age. We thus performed a 385 General Procrustes Analysis, as it allows valid shape comparisons for objects of different 386 sizes and detection of proportional changes of one anatomical region relatively to another.

387 Concerning results of PCA, while PC1 comprised age-related changes, PC2 clearly separated
388 patients from controls, thus excluding the sole effect of age on mandibular morphological
389 variations. Finally, comparisons between each patients group showed statistically significant

morphological differences between Crouzon and Apert, Crouzon and Muenke, and Apert and
Muenke patients. Overall, our results strengthen the hypothesis that mandibular shape in *FGFR*-related syndromic craniosynostoses results from the *FGFR* activating mutation
disturbing ossification processes and reinforce the hypothesis of genotype-phenotype
correspondence. No statistical difference was found for morphological comparisons between
Crouzonodermoskeletal patients and the other groups, most probably due to the small sample
of this extremely rare syndromic craniosynostosis.

Early tracheostomy was required for two patients with Crouzonodermoskeletal syndrome and
one patient with Crouzon syndrome. These patients presented severe midfacial retrusion, and
the mandible appeared as 'prognathic' relative to the maxilla. Although this small sample size
did not allow statistical comparisons, linear measurements of the mandibles could not reveal
significant differences between patients with or without tracheostomy.

402 FGFR-related syndromic craniosynostoses are due to gain-of-function activating mutations of 403 FGFR genes. The impact of a constitutive activation in FGFR2 and FGFR3 on endochondral 404 ossification has been first shown for long bones (FGFR3) and skull base synchondrosis 405 (FGFR2 and FGFR3) with altered chondrocyte proliferation and differentiation [72-80]. The 406 impact of activating mutations in FGFR2 and FGFR3 on mandibular formation has been more 407 recently studied [28,82-84]. Mandibular bone is formed by intramembranous ossification 408 induced before birth at the site of Meckel cartilage, and by endochondral ossification at the 409 site of condylar, angular and symphyseal cartilages, serving as growth centres after birth 410 [38,81]. Mandibular shape differences have been observed in mouse models for Apert  $(Fgfr2^{+/S252W})$ , and  $Fgfr2^{+/P253R}$ ) and Crouzon syndromes  $(Fgfr2c^{C342Y/+})$ , with affected ramus 411 412 morphology in all models. The greatest magnitude of morphological changes was for 413  $Fgfr2^{+/S252W}$  mice, while the Crouzon model showed the most limited changes; interestingly, 414 anterior body shape changes were only seen in this Crouzon model [82]. This study also

415 demonstrated increased Meckel cartilage volume and increased proliferation of osteoblasts 416 and chondrocytes forming this cartilage in  $Fgfr2^{+/S252W}$  mice [82]. Concerning *FGFR3* 417 activating mutations, a mouse model for Muenke syndrome,  $Fgfr3^{P244R}$ , disturbed 418 temporomandibular joint developmental processes [83,84]. A mouse model for 419 achondroplasia,  $Fgfr3^{G380R/G380R}$ , presented structural anomalies of primary (Meckel) and 420 secondary cartilages of the condylar region, demonstrating the implication of *FGFR3* in both 421 membranous and endochondral ossification [28].

422 FGFR2 and FGFR3 code for a tyrosine kinase receptor. FGF receptors have an extracellular 423 ligand-binding portion composed of immunoglobulin-like domains (IgI, IgII, and IgIII), a 424 transmembrane region, and intracellular tyrosine kinase domains (TK1 and TK2) [8,12]. The 425 FGF family, composed of at least 22 known FGF ligands, binds an FGFR, resulting in 426 receptor monomer dimerization, activation of kinase domains and phosphorylation of the 427 receptor [8]. The impact of the mutations within the FGF receptor extracellular ligand binding 428 domain may also be an explanation for the variability of the mandibular phenotype: while 429 mutations within the IgII-IgIII linker region result in altered ligand-binding specificity and/or 430 affinity as in Apert and Muenke syndrome [85-89], mutations within the IgIII linker region 431 result in aberrant intermolecular disulfide bonds between unpaired cysteine residues leading 432 to the constitutive activation of the receptor, as in Crouzon syndrome [89-91]. 433 Crouzonodermoskeletal syndrome is due to a single point mutation (pAla391Glu) localized in 434 transmembrane domain of FGFR3 resulting in an increase of the phosphorylation level of 435 FGFR3 independently of the presence of ligand [11,92]. Further molecular and biological 436 studies are required to better clarify the affected signalling pathways downstream FGF 437 receptors involved in the craniofacial phenotype of *FGFR*-related craniosynostoses.

438 Mandibular development is affected in various malformation syndromes without439 craniosynostosis such as in Robin sequence, Treacher Collins or Nager syndromes, due to

440 mutations in distinct genes, in which mandibular hypoplasia and retrognathia are observed 441 within various degrees of severity [93,94]. Comparing mandibular shapes between 442 craniosynostosis and non-craniosynostosis craniofacial syndromes may help in the 443 understanding of the intrinsic effects of the different mutations on mandibular development 444 and morphology. In our series, we aimed to analyse mandibular shapes at early ages to avoid 445 potential confounding factors related to the effects of surgery on mandibular shape. As 446 mandibular shape varies all along growth, especially the condylar region which is a major 447 center of post-natal growth, analysing mandibular shape at the end of the growth when the 448 condyle achieved a complete ossification, would be required to confirm changes in 449 mandibular vertical height, as we observed in FGFR2-craniosynostosis. In fact, mandibular 450 vertical height may be underestimated in the youngest patients, before complete ossification 451 of the secondary condylar cartilages occurs [95].

452 To conclude, our results demonstrated distinct mandibular shapes at early ages in different 453 subtypes of FGFR2- (Crouzon and Apert syndromes) and FGFR3-related syndromic 454 craniosynostoses (Muenke and Crouzonodermoskeletal syndromes) and reinforce the 455 hypothesis of genotype-phenotype correspondence concerning mandibular morphology. 456 Studies on larger cohorts and integration of skull base and midface morphological data in the 457 analysis of mandibular shape are required to determine whether the mandible is less subjected 458 to interindividual variations than other craniofacial features. Mandibular shape would 459 therefore be a valid clinical parameter in the characterization of FGFR-related 460 craniosynostoses.

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## 471 Table I. Patient's characteristics: number per group, sex ratio, type of mutation and of

472 craniosynostosis, and age at CT-scan

Patient	Patient's	Mutations	Types of craniosynostosis	Median age
group	number		(n)	(IQR) at CT-
	Sex ratio			scan
	(F/M)			(months)
Crouzon	12 (7/5)	Cys342Tyr	BCS + MS + SS + BLS	11 (16.9)
FGFR2		(n=4)	(2),	
		Tyr105Cys	BCS + MS + SS(1),	
		(n=2)	BCS + SS + BLS (1),	
		Cys278Phe	BCS + SS (1), BCS (1),	
		(n=2)	UCS (1), UCS + partial	
		Gly338Glu	BLS $(1)$ , UCS + MCS $(1)$ ,	
		(n=2)	SS (1), BLS (1), None (1)	
		Ser267Pro		
		(n=1)		
		Arg347Cys		
		(n=1)		
Apert	12 (6/6)	Ser252Trp	BCS (9), BCS + SS (1),	8.9 (9.2)
FGFR2		(n=11)	UCS $(1)$ , MS + SS $(1)$	
		Pro253Arg		
		(n=1)		
Muenke	12 (9/3)	Pro250Arg	BCS (10), UCS (2)	6.8 (4.8)
FGFR3		(n=12)		
CAN	4 (3/1)	Ala391Glu	BCS (2), BCS + SS (1),	22.7 (12.6)
FGFR3		(n=4)	UCS + BLS(1)	

- 474 CAN: Crouzon with acanthosis nigricans; IQR: Inter Quartile Range
- 475 BCS: bilateral coronal synostosis, UCS: unilateral coronal synostosis, SS: sagittal synostosis,
- 476 MS: metopic synostosis; BLS: bilateral lambdoid synostosis.

477	* Frontofacial or frontoorbital advancement (performed after the studied CT-scan for all
478	patients).
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485	Figure 1. Landmarks on the buccal and lingual sides digitized on hemi mandibles surface
486	three-dimensional models: a: anatomical landmarks (31); b: curves (11) (based on previous

487 studies [41,42]).

## 488 A. Anatomical landmarks (n=31)

AntGo	Antegonial
CoAnt	Anterior border of the articular surface of the condyle
CoExt	External border of the articular surface of the condyle
CoInt	Medial border of the articular surface of the condyle
CoPost	Posterior border of the articular surface of the condyle
Cor	Coronoid process (highest point)
Col	Condylar neck (highest concavity)
Gn	Gnathion
Go	Gonion
InfDe	Infradentale (most superior point of the alveolar bone at the inter incisive
	midline)
Me	Mental point
MeSp	Mental spine
Pog	Pogonion

RetroMolExt	Retromolar external
DatuaMallut	Determeden internel
Retroivionnt	Retromotar internal
Sig	Sigmoid notch (highest concavity)
Spix	Spix spine
All the landmarks were positioned bilaterally except midline landmarks (InfDe, Pog, MeSp).	

#### B. Curves (n=11)

Course	Number of
	sliding
	semi-
	landmarks
	per curve
from 'Cor' to the most inferior point of the	7
anterior edge of the ramus	
from 'CoPost', all along the posterior	20
border of the neck, ramus, corpus, to the	
most inferior point of the symphysis	
from 'CoAnt' to 'Cor'	10
from the most posterior point of the alveolar	15
bone, running along the lingual side of the	
alveolar curve, to the inter incisive point	
from the most posterior point of the alveolar	15
bone, running along the vestibular side of	
the alveolar curve, to the point 'InfDe'	
from 'InfDe', surrounds the basilar border,	20
and ends at the inter incisive point at lingual	
side	
	Course from 'Cor' to the most inferior point of the anterior edge of the ramus from 'CoPost', all along the posterior border of the neck, ramus, corpus, to the most inferior point of the symphysis from 'CoAnt' to 'Cor' from the most posterior point of the alveolar bone, running along the lingual side of the alveolar curve, to the inter incisive point from the most posterior point of the alveolar bone, running along the vestibular side of the alveolar curve, to the point 'InfDe' from 'InfDe', surrounds the basilar border, and ends at the inter incisive point at lingual side



All curves were positioned bilaterally except Symphysis curve (midline)

Figure 2. Plots of Principal Components (PC) scores for PC1 and PC2 with corresponding
variances (in %) (A), and (B) 3D shape changes associated with PC1 and PC2 by morphing
the extreme mandibular surface model (blue: negative values; red: positive values). A:
patients (square box); controls (round box): AP: Apert Patients ; AC: Controls for Apert
Patients; CP: Crouzon Patients; CC: Controls for Crouzon Patients; CANP: CAN
(Crouzonodermoskeletal) patients; CANC: Controls for CAN Patients; MP: Muenke Patients;
MC: Controls for Muenke Patients.

Figure 3. Plots of Canonical Variate (CV) scores (A) and 3D mandibular shape associated to
CV1 and CV2 (B) (n=40 patients: 12 with Crouzon syndrome, 12 with Apert syndrome, 12
with Muenke syndrome, 4 with Crouzonodermoskeletal syndrome and n=40 age and sex-

505 matched controls).

- A: patients (square box); controls (round box): AP: Apert Patients ; AC: Controls for Apert
  Patients; CP: Crouzon Patients; CC: Controls for Crouzon Patients; CANP: CAN
  (Crouzonodermoskeletal) patients; CANC: Controls for CAN Patients; MP: Muenke Patients;
  MC: Controls for Muenke Patients.
- 510 B: The dots represent the extreme values (green: negative values; red: positive values) of each511 anatomical landmark and sliding curve semi-landmark.
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Figure 4. Comparison of mandibular shape between patients with *FGFR2*-craniosynostoses
and controls: plots of Principal Components (PC) scores for PC1 and PC2 with corresponding
variances (in %) (patients (magenta), controls (black)), and 3D shape changes associated with
PC2 by morphing the extreme mandibular surface model (blue: negative values; red: positive
values) for A: Crouzon group (n=12 patients; n=12 controls); B: Apert group (n=12 patients;
n=12 controls).

520 Figure 5. Comparison of mandibular shape between patients with *FGFR3*-craniosynostoses 521 and controls: plots of Principal Components (PC) scores for PC1 and PC2 with corresponding 522 variances (in %) (patients (magenta), controls (black)), and 3D shape changes associated with 523 PC2 by morphing the extreme mandibular surface model (blue: negative values; red: positive 524 values) for A: Muenke group (n=12) patients; n=12 controls); B: CAN 525 (Crouzonodermoskeletal) group (n=4 patients; n=4 controls).

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Figure 6. 3D representations showing mandibular shape variations between patient groups
using Canonical Variate Analysis, for A: Crouzon vs Apert group; B: Crouzon vs Muenke
group; C: Apert vs Muenke group. The dots represent the extreme values (green: negative
values; red: positive values) of each anatomical landmark and sliding curve semi-landmark
(n=12 patients; n=12 controls for each group).

532 All comparisons were statistically significant (p < 0.05).

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843	Dr. Anne Morice contributed to the conception, design, acquisition, analysis, and
844	interpretation of the data, writing and revision of the manuscript.
845	Dr. Raphael Cornette contributed to the acquisition and analyses of the data and critical
846	analysis of the manuscript.
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848	Dr Corinne Collet contributed to the acquisition of the genetic diagnoses.
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851	Drs Laurence Legeai-Mallet and Roman Hossein Khonsari contributed to the conception,
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A: Crouzon vs controls

B: Apert vs controls



A: Muenke vs controls

B: CAN vs controls









