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Research Paper

Extensive phenotyping of the orofacial and dental complex in Crouzon syndrome

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ABSTRACT

Objectives: *Fibroblast growth factor receptor 2 (FGFR2)* $^{C342Y/+}$ mutation is a known cause of Crouzon syndrome that is characterised by craniosynostosis and midfacial hypoplasia. Our aim was to conduct extensive phenotyping of the maxillary, mandibular and dental morphology associated with this mutation.

Materials and methods: Morphometric data were obtained from 40 mice, representing two genotypes (Crouzon and wild-type) and two sexes (males and females) (n = 10 in each group). Dental analysis further categorised the first molars into the two jaws (maxillary and mandibular) (n = 20 in each group). Maxillary, mandibular and dental morphology was compared by analysing 23 linear landmark-based dimensions in three-dimensional micro-computed tomography reconstructions.

Results: Compared with wild-type, Crouzon ($FGFR2^{C342Y/+}$) maxillae were significantly shorter in maximum height, anterior and posterior lengths and middle width, but larger in posterior width ($p < 0.05$ for height; $p < 0.001$ for other comparisons). In the Crouzon mandible, the ascending and descending heights, effective and mandibular lengths, and intercoronoid and intercondylar widths were significantly shorter, whereas intergonial width was larger ($p < 0.01$ for intercondylar width; $p < 0.001$ for other comparisons). Crouzon teeth were significantly smaller mesiodistally, but larger in crown height ($p < 0.001$ for each comparison). All Crouzon mice presented with bifid mandibular condyles and a quarter presented with expansive bone lesions in the mandibular incisor alveolus.

Conclusions: Our findings of hypoplasia in all three planes in Crouzon maxillae and mandibles, together with the presence of bifid mandibular condyles and expansive bone lesions, may be relevant to maxillofacial surgery and orthodontics. Beyond skeletal effects, the $FGFR2^{C342Y/+}$ mutation is now implicated in affecting tooth development. This study's skeletal phenomics data also provides baseline data against which the effect of various treatments can now be assessed.

1. Introduction

Crouzon syndrome is a relatively common craniofacial syndrome in humans and has a birth prevalence of 16.5 in 1,000,000 (Cohen & Kreiborg, 1992). It is part of a family of autosomal dominant syndromes most commonly associated with fibroblast growth factor receptor 2 (FGFR2) mutations. This syndrome is characterised by craniosynostosis (the premature fusion of one or more cranial sutures), ocular disorders and midfacial hypoplasia (Johnson & Wilkie, 2011). Severe forms of craniofacial malformations, including morphological anomalies of the maxilla and mandible, have been associated with neurological, respiratory and psychosocial disorders as well as aesthetic problems (Helman, Badhey, Kadakia, & Myers, 2014; Inverso, Brustowicz, Katz, & Padwa, 2016; Staal et al., 2015). Abnormal dental manifestations in Crouzon syndrome maxillae and mandibles have been implicated in dental malocclusion (Helman et al., 2014). The underlying pathogenesis involved with development of various forms of craniosynostosis in not well understood, but recent advances in three-dimensional (3D) computed tomography (CT) imaging techniques have allowed formulation and evaluation of hypotheses about growth processes (Anderson, Yong, Surman, Rajion, & Ranjitkar, 2014; Guevara, Wallender, Steinberg, & Ranalli, 2016). Despite the potential that this technique holds in yielding extensive phenotypic data (i.e. on a large set of parameters) for characterisation of different types of craniosynostosis, little progress has been made in this field (Hermann et al., 2016).

Perlyn et al. (2006) have confirmed the value of the micro-computed tomography (micro-CT)-based murine model to study Crouzon

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syndrome. Their findings also supported the earlier observation that the *FGFR2*$^{C342Y/+}$ mutant mice commonly display features that are analogous to affected humans, such as bicoronal synostosis, increased skull height and width, increased interorbital distance and decreased skull length (Kreiborg, 1981). In relation to orofacial features in Crouzon syndrome, most studies reporting maxillary hypoplasia are restricted to the sagittal plane (Kreiborg, 1981; Reitsma, Ongkosuwito, Buschang, & Prahl-Andersen, 2012). Kreiborg and Aduss (1986) reported Crouzon maxillary hypoplasia in all three planes, but only on four patients and without specifying their method of assessment.

Class III malocclusion associated with mandibular prognathism is observed commonly in Crouzon patients (Helman et al., 2014), but it is unclear whether the Crouzon mandible is normal in size and shape. Perlyn et al. (2006) reported a significantly shorter mandibular length in Crouzon mice compared with wild-type littermates, reflecting human observations (Costaras-Volarich & Pruzansky, 1984). Reitsma, Ongkosuwito, Buschang, and Prahl-Andersen (2012) noted a longer lower face height in Crouzon patients, but unaltered mandibular ramus height. They attributed the causes of Class III malocclusion and increased lower face height to adaptation to a hypoplastic maxilla, with increased mandibular rotation and anteriorly-positioned mandibular condyles.

Stages of tooth development are well conserved through toothed vertebrates, and data from murine models are well established to provide clues in tooth development (Caton & Tucker, 2009). Crouzon syndrome exhibits a higher prevalence of agenesis of at least one tooth (12.8–42.3%) compared to the general population (3.2–6.7%) (Reitsma, Elmi, Ongkosuwito, Buschang, & Prahl-Andersen, 2013; Stavropoulos, Bartzela et al., 2011). Maxillary dental crowding is also common in Crouzon patients (Helman et al., 2014) and case reports have described macrodontia, peg-shaped and widely-spaced teeth in Crouzon syndrome (Boutros et al., 2007; Padmanabhan, Hegde, & Rai, 2011). Despite this, we are unaware of any previous study, besides case reports, that has assessed the dental phenotype of Crouzon syndrome.

Our aim was to assess whether there were significant differences in maxillary, mandibular and dental morphology between Crouzon (*FGFR2*$^{C342Y/+}$) and wild-type mice. Our null hypothesis was that the size of Crouzon maxillae, mandibles and dentition would not differ significantly to wild-type.

2. Materials and methods

Animal experiments were performed according to the current guidelines for 'Australian code for the care and use of animals for scientific purposes' published by the National Health and Medical Research Council of Australia in 2013 and the 'South Australian Animal Welfare Act 1985'. Ethical approval was obtained from both the Animal Ethics Committee of The University of Adelaide (Adelaide, Australia) and Children, Youth and Women's Health Service Animal Ethics Committee of the Women's and Children's Hospital (Adelaide, Australia; Permit Number: AE884/6/15).

2.1. Animal husbandry

Crouzon (*FGFR2*$^{C342Y/+}$) mice were donated by Associate Professor Chad Perlyn (Florida International University, St Louis) (Perlyn et al., 2006). The mice were preserved on a mixed genetic background of 72% C57BL and 28% Swiss and were maintained at the Women's and Children's Hospital (Adelaide, Australia).

2.2. Samples

Forty four-month-old (young-adult) mice were used for this study. After sacrificed by CO_2 asphyxiation, the skulls were dissected and stored in 95% ethanol. The skulls were categorised into two genotypes (Crouzon and wild-type) and two sexes (males and females) (n = 10 in each group). Dental analysis further categorised the first molars into the two jaws (maxillary and mandibular) (n = 20 in each group).

2.3. Micro-CT imaging and 3D reconstruction

Micro-CT images were acquired on SkyScan 1076 small animal micro-CT scanner (Bruker; Kontich, Belgium) at a resolution of 8.65 μm using the follow parameters: source voltage = 60 kV, source current = 172 μA, rotation step = 0.5°, filter = 0.5 Al, exposure time = 5890 ms. The skulls were then reconstructed using the SkyScan NRecon software package (Bruker; Kontich, Belgium) with a ring artifact correction of 5, beam hardening correction of 30%, smoothing of 3 pixels, post-alignment correction of −1.5 and upper and lower threshold limits of 0.000 and 0.135, respectively. The bitmap image slices were resized by half with SkyScan TConv Version 2.0 (Bruker; Kontich, Belgium). Then, the resized bitmap slices were volume rendered using the Avizo Fire 9.0.0 software package (Visualization Sciences Group; Massachusetts, USA). Once volume rendered, the mandible was segmented from the craniofacial skeleton as a single unit. MeshLab: an Open-Source Mesh Processing Tool 64bit v1.3.3 (Cignoni et al., 2008) was utilised for 3D morphometric analysis.

2.4. Morphometric analysis

One of the authors (A.K.) was blinded for both assessment of suture patency and morphometric analysis of orofacial and dental features using micro-CT reconstructions. Multiple suture fusion was found in all Crouzon mice but appeared normal in control mice (i.e. all sutures were patent except the posterior frontal suture), which was consistent with observations made by Hermann et al. (2016). Murine craniofacial parameters similar to those described previously were used (de Carlos et al., 2011; Richtsmeier, Baxter, & Reeves, 2000). Sagittal maxillary measurements were obtained from landmarks described by Perlyn et al. (2006), and sagittal and coronal mandibular landmarks were based on those described by Mian, Ranjitkar, Townsend, and Anderson (2017). Although both the hemi-maxillae and hemi-mandibles in mice fuse in the midline they are morphologically different and, as such, it is not possible to use anatomic parameters that are entirely complementary. Nevertheless, we included additional landmarks to incorporate axial and coronal measurements (e.g. maxillary tuberosity) that are analogous to 3D human craniofacial measurements used in orthodontics (Karatas & Toy, 2014). A new set of 23 landmark-based orofacial and dental linear dimensions were calculated in all three planes in the 3D anatomical reconstructions (Table 1 and Figs. 1–3). Actual landmarks were located by orienteering these reconstructions in all three planes, as described by Chien et al. (2009).

Murine hemimandibles fuse anteriorly by a fibrous joint, hence they appear disarticulated in micro-CT scans. We did not separate the hemimandibles during the scanning process and, unlike studies conducted on disarticulated hemimandibles, manual re-orientation was not required when calculating mandibular dimensions.

Due to expansive bone lesions in the mandibular alveolus adjacent to the incisor, the anterior width of Crouzon males (n = 3), the effective and mandibular lengths of a Crouzon male (n = 1), and the effective and mandibular lengths of a Crouzon female (n = 1) could not be measured or used for morphometric analysis.

2.5. Statistical analyses

Statistical analyses were performed using the SPSS software package (version 24, IBM Software Group, Somers, United States). Two-way multivariate analysis of variance (MANOVA) and Bonferroni's post hoc tests were used to assess whether there were significant differences between mean maxillary and mandibular dimensions between the two genotypes (Crouzon and wild-type) and the two sexes (male and female). Three-way MANOVA and Bonferroni's post hoc tests were used

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