Craniofacial morphologic alteration induced by bone-targeted mutants of FGFR2 causing Apert and Crouzon syndrome

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Objective: Activating mutations in the fibroblast growth factor receptor-2 (*FGFR2*) have been shown to cause syndromic craniosynostosis such as Apert and Crouzon syndromes. The purpose of this pilot study was to investigate the resultant phenotypes induced by the two distinctive bone-targeted gene constructs of *FGFR2*, Pro253Arg and Cys278Phe, corresponding to human Apert and Crouzon syndromes respectively. **Methods:** Wild type and a transgenic mouse model with normal *FGFR2* were used as controls to examine the validity of the microinjection. Micro-CT and morphometric analysis on the skull revealed the following results. **Results:** Both Apert and Crouzon mutants of *FGFR2* induced fusion of calvarial sutures and anteroposteriorly constricted facial dimension, with anterior crossbite present only in Apert mice. Apert mice differed from Crouzon mice and transgenic mice with normal *FGFR2* in the anterior cranial base flexure and calvarial flexure angle which implies a possible difference in the pathogenesis of the two mutations. In contrast, the transgenic mice with normal *FGFR2* displayed normal craniofacial phenotype. **Conclusion**: Apert and Crouzon mutations appear to lead to genotype-specific phenotypes, possibly causing the distinctive sites and sequence of synostosis in the calvaria and cranial base. The exact function of the altered FGFR2 at each suture needs further investigation. **(Korean J Orthod 2006;36(4):284-94)**

Key words: Craniosynostosis, Apert syndrome, Crouzon syndrome, FGFR2

INTRODUCTION

The heritability of malocclusion and craniofacial structures has been a theme of interest in clinical orthodontics.[1] Among the genetic aberrations causing craniofacial malformations, pathogenic mechanisms for various craniosynostosis syndromes have been investigated considering their clinical significance and high prevalence.[2-4] Syndromic craniosynostoses are primarily characterized by an altered shape of the calvaria and/or cranial base caused by the premature fusion of particular sutures; this in turn results in midfacial deficiency and relative mandibular prognathism.

In particular, Apert and Crouzon syndromes are two

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common syndromic craniosynostoses often faced in the orthodontic clinic. Both syndromes, named after their finders, Eugene Apert in 1906, and Octave Crouzon in 1912, respectively,[5] share a number of craniofacial findings including brachycephaly, underdevelopment of the maxilla, exorbitism, hypertelorism, and mandibular overjet. However, more serious phenotypes are common in Apert syndrome, such as cleft palate, bifid uvula, and syndactyly.

The relevance of these phenotypes to the genetic aberration was found much later in the 1990s.[6-9] Both syndromes have been found to be associated with mutations of the fibroblast growth factor receptor-2 (FGFR2) gene, which leads to altered function of the receptor protein, while normal FGFR2 is a key regulator of calvarial intramembranous bone formation.[10-12] Approximately, 99% of Apert patients have been associated with point mutations of *FGFR2*, i.e. either Ser252Trp or Pro253Arg.[9] Crouzon syndrome has been linked to over 20 different single amino acid substitutions in the extracellular domain of FGFR2 that are distinctive from those causing Apert syndrome.[13,14] Accordingly, patients with Apert or Crouzon syndromes display somewhat distinctive craniofacial phenotypes. However, despite the consistent correlation between phenotypes and the genetic alteration, it is difficult to understand the direct effects of the genetic alteration on the craniofacial morphogenesis due to the inherent individual variation in human subjects.[15] Therefore, appropriate transgenic animal models with minimal individual variation may be suitable for visualizing the primary phenotypic differences induced by specific genetic alteration.

Based on this inference, we speculated that each *FGFR2* mutation associated with each syndrome might lead to specific phenotypes which are distinct from each other in the animal models, with minimal individual variation. We have previously shown that the mutant *FGFR2* gene attached to the Col1A1 promoter inserted in the mouse germ cell induced bone-specific expression of the mutant gene while securing the viability of the animals, and that the premature fusion of calvarial sutures in transgenic mice model was reproducible.[16] One of the limitations of this transgenic approach was the possibility that the mutant gene may affect or deteriorate normal gene function by random insertion in the critical genes.[17] Therefore, it is essential to verify if the normal gene inserted into the embryo causes any alteration in the forms and functions during development, to validate the methodology.

The purpose of this pilot study was to examine, whether or not the two distinctive bone-targeted gene constructs of *FGFR2*, Pro253Arg and Cys278Phe *FGFR2*, corresponding to human Apert and Crouzon syndromes, would respectively lead to distinctive resultant phenotypes in the transgenic mice models. We generated three different strains of transgenic mice with normal *FGFR2*, Pro253Arg *FGFR2* and Cys278Phe *FGFR2*, to test the following hypotheses. First, both transgenic strains with either Apert or Crouzon mutation may exhibit premature fusion of the cranial sutures and distinctive phenotypes for each strain, representing the direct effect of each mutation. Second, transgenic mice with normal *FGFR2* may display normal phenotype if the added *FGFR2* is normal.

MATERIAL AND METHODS

Generation of transgenic mice expressing normal or mutant FGFR2

Transgenic constructs consisting of specific *FGFR2* transgene, hemagglutinin epitope, and poly-A chain were prepared through a multi-step subcloning strategy, as previously described.[16] A bone-specific 3.6 kb rat type I collagen promoter conjugated with the first exon and intron was attached on the 5' end of the transgene construct. The linear transgenic constructs were randomly inserted into the mouse embryos of the B6SJL strain. Predominant expression of the transgene in the calvarial bone tissue has previously been demonstrated.[16] The constructs used in this study included the following transgenes: 1) normal *FGFR2* with hemagglutinin epitope; 2) Pro253Arg *FGFR2*: An Apert mutation in *FGFR2* where the 253rd proline has been substituted by arginine for generation of Apert mice; 3) Cys278Phe *FGFR2*: A Crouzon mutation in *FGFR2* where the 278th cystein has been substituted by

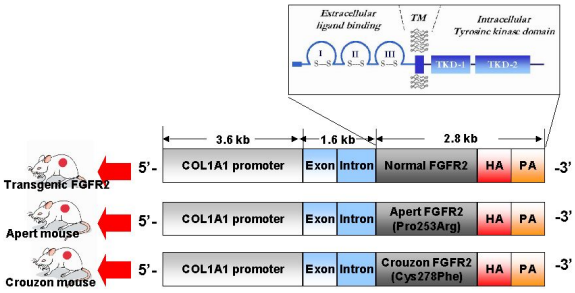

Fig 1. A schematic figure of the transgene constructs and type of mutation. Each heterozygous transgenic mouse was paired with the wild type littermates for breeding.

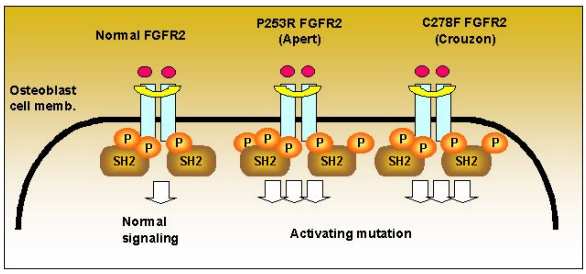

Fig 2. Altered FGFR2 function induced by mutants in *FGFR2*. Both Apert and Crouzon mutation have been shown to enhance the receptor function.

phenylalanine for generation of Crouzon mice.

According to the breeding strategy described previously,[16] heterozygous transgenic mice, paired with a group of wild type littermates, were produced (Figs 1 and 2).

Genomic DNA Isolation and Polymerase Chain Reaction (PCR)

The tails from a postnatal day 1 mice were obtained and digested with 100 g/ml of Proteinase K in a 500 µl buffer containing 100 mM of EDTA, 50 mM of Tris pH 8 and 0.5% SDS at 55℃ for 1-2 hours. This was followed with RNAase (40 g/ml) treatment for 30 minutes at 37℃. The digest was micro-centrifuged for 10 minutes at 4℃ and the 400 µl supernatant was removed. 200 µl of 7.5 M ammonium acetate (NH4OAc) was added to the supernatant and micro-centrifuged for 5 minutes at 4℃. 600 µl of isopropanol was added to the supernatant to precipitate the DNA. The DNA pellet was washed with 70%-ethanol (Pharmaco, Brookfield, CT, USA), air-dried and resuspended in 50 µl of dH2O. To completely dissolve the DNA pellet, the DNA solution was incubated at 37℃ for 30 minutes.

PCR was carried out using Tag polymerase (5 µl) (Promega., Madison, WI, USA) and each of the specific primers described below, for 40 PCR cycles, with each cycle consisting of 1 minute at 94℃, 45 seconds at 60 ℃, and 2 minutes at 72℃ (Hybaid PCRexpress, UK) (Fig 3).

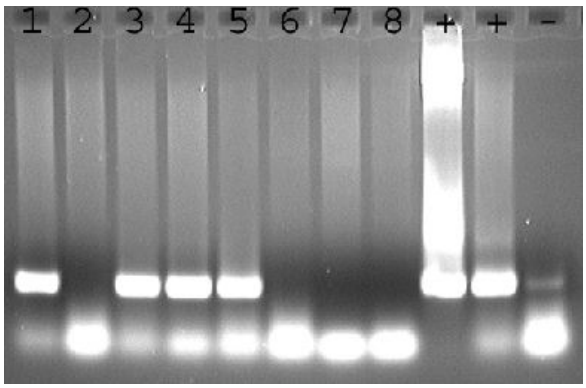

Fig 3. PCR from tail DNA for genotyping. Tail DNA of the offspring from the heterozygous transgenic mouse and the wild type spouse went through 40 PCR cycles with specific primers. The PCR products were run on agarose gel. Intense bands at lanes *1*, *3*, *4*, and *5* indicate that these mice are heterozygous transgenic, while lanes *2*, *6*, *7*, and 8 represent wild type littermates (+: positive control, -: negative control).

Pro253Arg (Apert) *FGFR2*
5'-primer: 5'-CGATGTCGTTGAACGGTCACGACACCGGCCCATCCTCCAA-3'
3'-primer: 5'-GCTTGGAGGATGGGCCGGTGTCGTGACCGTTCAACGACAT-3'
Cys278Phe (Crouzon) *FGFR2*
5'-primer: 5'-TAGAGGCATGGAGTACTTGGCTTC-3'
3'-primer: 5'-AGATGGCTGGCAACTAGAAGGCAC-3'

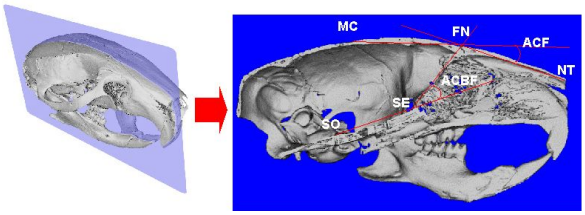

Fig 4. Illustration of measurements taken at the midline section on the three-dimensional reconstruction of the micro-CT scanned image. *ACF,* Anterior calvarial flexure angle; *ACBF,* anterior cranial base flexure angle*; MC*, midline coronal suture; *FN*, frontonasal suture; *NT*, bony nose tip; *SO,* spheno-occipital synchondrosis; *SE*, spheno-ethmoidal junction.

Transgenic mice with normal *FGFR2* were discriminated using western blot analysis using anti-hemagglutinin(HA) antibody.[16]

Phenotypic analysis of each transgenic strain using micro-computed tomography (CT) scanning

Each set of the offspring consisting of heterozygous transgenic mice and wild type littermates was carefully examined, in order to find any morphological alteration in the craniofacial region.

Transgenic mice that survived up to 8 weeks of age and their wild type littermates were decapitated and the skulls were scanned using micro-computed tomography CT20 (Scanco, Southeastern, PA, USA). Three mice for each strain were selected for comparison. Each slice was taken at increments of 13 micron. A CT Tomography V4.6 G program (Scanco, Southeastern, PA, USA)) was used for the 3-dimensional reconstructions of the heads. Sagittal cross-sectional images were obtained from the reconstructed 3-dimensional images to review the deformation in the neurocranium.

The following measurements were defined at the midsagittal section to assess the deformity particularly in the anterior cranial region (Fig 4): anterior calvarial flexure (ACF), an acute angle formed at the intersection between lines connecting MC-FN and FN-NT; anterior cranial base flexure (ACBF), an acute angle formed at the intersection between lines SO-SE and SE-FN; MC, midline coronal suture (intersection between coronal suture-interparietal suture); FN, frontonasal suture; NT, bony nose tip; SO, spheno-occipital synchondrosis; SE, spheno-ethmoidal junction.

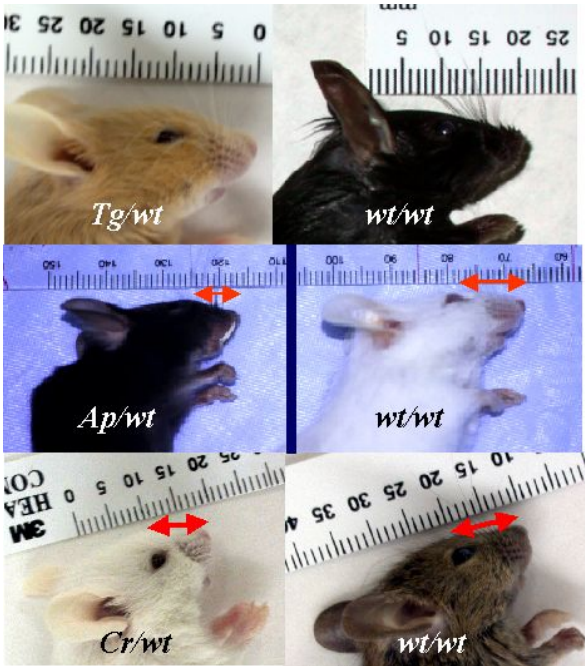

Fig 5. Phenotypic comparison between wild type and transgenic mice. Each transgenic mouse was compared to their own wild type littermates. *Tg/wt*, Transgenic mouse with normal *FGFR2*; Ap/wt, heterozygous transgenic mouse with Apert(P253R) *FGFR2*; *Cr/wt*, heterozygous transgenic mouse with Crouzon(C278F) *FGFR2*.

RESULTS

General assessment and morphological comparison (Fig 5)

Most of the transgenic mice were viable until 7-8 weeks after birth. In the first one to two weeks, craniofacial morphological characteristics were not remarkable compared to the wild type mice. However, the differences became more noticeable as they grew older to 3 to 4 weeks, in cases of Apert and Crouzon mice. Described below are the major findings in the head structure of 2 month old mice in each strain.

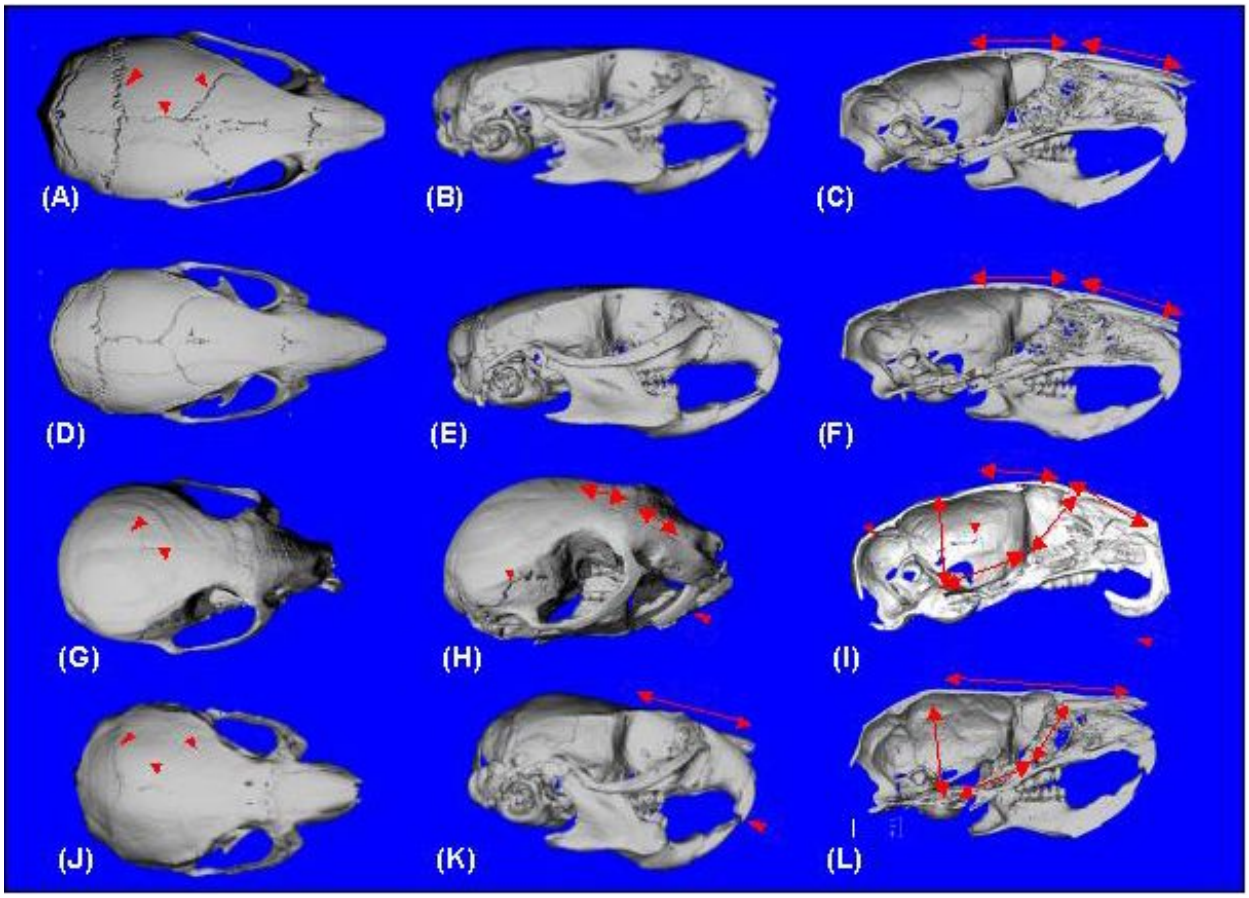

Fig 6. Three-dimensional reconstruction of micro-CT scanned images and midline section viewing the patterns of deformity in the calvaria and cranial base. *(A-C)* wild type *(D-F)* transgenic mice with normal *FGFR2 (G-I)* Apert mouse *(J-L)* Crouzon mouse aged 56 days. In contrast to the wild type and transgenic mice with normal *FGFR2*, both Apert and Crouzon mice displayed significant deformation in the anterior calvaria and cranial base. Apparently more flexure in the nasofrontal contour in the Apert mice was noted *(I)*, while flattened contour was typical in the Crouzon mice *(L)*. Incisal elongation and incomplete suture formation in the suture between the parietal bone and temporal bone and in the interparietal suture are indicated with arrows in the Apert mice *(H, I)*. Scale bar represents 1 mm length on *(L)*.

Transgenic mice with normal FGFR2

Apparent differences in the craniofacial phenotype and fertility were not found in any of the transgenic mice with normal *FGFR2*, compared to the wild type littermates. The body weight measured 19.8 ± 1.1 grams at 38 days, with no statistical difference from the wild type mice (19.2 ± 1.2 gram, $p > 0.5$, n = 3).

Apert mice

With some variations in individual phenotypes, Apert mice generally displayed reduced anteroposterior dimension of the head compared to the wild type littermates. As a result, anterior crossbite between the upper and lower incisors was a consistent finding in the transgenic participants. Apert mice had no significant variations in body weight (18.5 ± 2.5 gram) compared to the wild type littermates (19.3 ± 0.9 gram, $p > 0.5$, n = 3) and the fertility of Apert mice appeared reduced.

Crouzon mice

Similar to the Apert mice, overall reduced anteroposterior facial length was an apparent phenotype

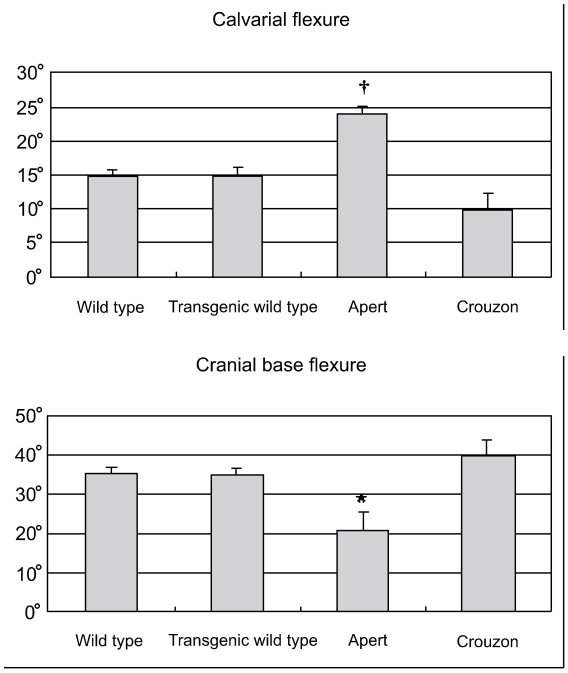

Fig 7. Comparison of anterior calvarial flexure and anterior cranial base flexure angle. Significant differences are noted in the Apert mice, compared to the wild type mice (*p < 0.5; †p < 0.001).

in the Crouzon mice. However, unlike the Apert mice, the anterior crossbite was not present. Body weight of Crouzon mice (16.3 ± 1.2 gram) was significantly lower than wild type littermates (20.2 ± 1.3 gram) at 38 days after the active growth phase (p < 0.001, n = 3).

Micro-CT analysis (Fig 6)

Transgenic mice with normal FGFR2

There was no apparent morphological difference between the wild type and transgenic mice with normal *FGFR2*. Their head structures and shapes were all normal. There was no evidence of fusion of particular sutures in any of the participants. Major calvarial sutures are demonstrated as demarcation lines on the calvaria, implicating the patency of the sutures even in the fully grown participants (Fig 6, *D-F*).

Apert mice

A smooth calvarial surface with no demarcation lines indicated premature fusion of sutures in the Apert mice. In contrast to the anterior calvarium, sutural defects around the temporal and occipital bone were found in the posterior cranial region. Anteroposterior constriction was remarkable in the midsagittal cross-sectional view, together with the exaggerated inward flexure of the bony nose. A flat cranial base in the Apert mice compared to the wild type littermates was evident. Elongation of the incisors appeared because of the loss of a vertical stop, possibly caused by severe restriction of anterior growth of the nasomaxillary complex (Fig 6, *G-I*).

Crouzon mice

Similar to the Apert mice, generalized sutural fusion on the calvaria was a primary finding. However, the fusion occurred in a more uniform fashion and there was no evidence of a bony defect on the entire calvarial surface. Anteroposterior constriction of the face was noted similar to the Apert mice. However, in the midsagittal cross-section, an upward lifting of the bony nasal bridge was observed in the Crouzon mice, unlike in the Apert mice. In spite of the anteroposterior constriction of the face, crossbite was not present in any of the affected participants. Increased height of the calvarial vault with dome-shaped calvaria was also demonstrated (Fig 6, *J-L*).

Apert mice displayed a significantly higher calvarial flexure angle, followed by two controls, i.e. wild type and transgenic with normal *FGFR2*. Flexure of the anterior cranial base was seen in the order of highest to lowest in Crouzon, wild type or transgenic *FGFR2*, and Apert, with significance in the Apert mice compared to wild type littermates (Fig 7).

DISCUSSION

This pilot study recruited transgenic mice models to test the hypotheses regarding the effects of *FGFR2* mutations on eventual craniofacial forms. The pronuclear injection method is considered a useful tool to investigate specific gene functions in particular

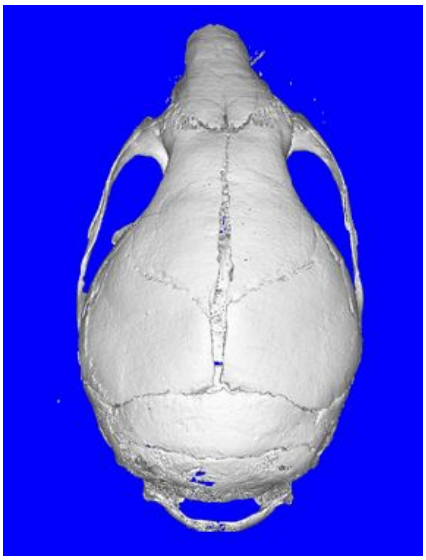

Fig 8. Midline defect in an Apert mouse aged 1 month.

tissues using appropriate promoters.[18] Micro-CT scanning enables a non-invasive analysis of the head structure at arbitrary sections. The accuracy and reproducibility of the measurements in the reconstructed images have proved to be more superior than the manual measurements on the actual structure.[19]

The first finding was that the phenotype of the transgenic mice with normal *FGFR2* was identical to that in wild type littermates. This indicates that the forced insertion of normal *FGFR2* would not alter the head shape or the sequence of suture development. Since these transgenic mice remained viable during the months after birth, it can be postulated that the endogenous gene function was not interrupted in this strain.[20] In our previous study, it has been shown that the *FGFR2* expression level was notably increased by the addition of the gene in the germ cells demonstrated in the immunoblot.[16] Therefore, this result may indicate that the increase in the normal FGFR2 expression may not necessarily lead to a craniofacial morphologic change. According to Kim et al., FGF4 treatment accelerated calvarial growth in tissue culture.[21] However, it was not clear if FGF treatment lead to a true fusion of osteogenic fronts. Because the FGFs have suppressive effects on the terminal differentiation of osteoblasts,[22,23] FGF treatment alone may not necessarily lead to premature fusion of the calvaria.

Both Apert and Crouzon mice exhibited premature fusion of calvarial sutures, resulting in deformation of the calvaria. This finding, along with that from the transgenic mice with normal *FGFR2*, confirmed that the alteration of the FGFR2 function by mutation, not the intensity of the normal FGFR2 expression, might be the determinant of craniosynostosis, like in the knock-in mice with Apert *FGFR2*.[24]

Due to the limited number of transgenic mice, a serial observation of the sequence and site of suture fusion during growth was not performed in this study. Instead, aged mice models were analyzed to demonstrate the accumulated effects of abnormal suture fusion on the eventual craniofacial forms, like in human studies where the morphological difference between normal and deformed patients became more distinct in the fully grown subjects.[25] Our Apert and Crouzon mice were mostly found viable until the end of active growth. Transgenic mice models showing alteration of critical molecules for normal development, including FGFs/FGFRs, tend to be lethal or infertile because of serious damage in the major physiologic functions, which is probably why the report regarding the eventual craniofacial shape following active growth period in the transgenic mice with mutant *FGFR2* is rare.[18,24,26,27] As previously demonstrated, type I collagen promoter efficiently induced the expression of transgene constructs within the bone tissue, which may have helped preserve the viability of transgenic mice in this study.

Obvious dissimilarities in craniofacial phenotypes between Apert and Crouzon mice were interesting findings. Although an exact comparison between mice and human was not feasible due to the differences in skeletal components,[28] specific features in each transgenic strain resembled those of human phenotypes.

First, in the Apert mice, sutural defects were still present in the posterior calvarial sutures, in contrast to the uniform approximation of overall calvarial sutures in the Crouzon mice. This resembles the human skeletal phenotype, as shown in Kreiborg's report.[29] In Apert patients, closed coronal sutures and midline calvarial defects have been shown to be common findings early

in their life. One of our Apert mice showed similar midline defects at 1 month after birth (Fig 8), implicating a similar pathogenic mechanism.

Second, the craniofacial phenotype was generally more serious in the Apert mice, including severe maxillary retrusion and anterior crossbite; this coincides with the observation in the human phenotype.[25,29-31] The reason why Crouzon patients exhibit a milder phenotype when overall sutures are nearly simultaneously fused is uncertain. However, it was notable that similar patterns in the severity of phenotype were reproducible in mice models as well.

Third, one of the major differences between the two transgenic strains was the shape of the anterior calvaria and cranial base, represented as calvarial flexure angle and cranial base angle as shown in Fig 7. It has been suggested that the cartilage abnormalities in the anterior cranial base (spheno-ethmoid and spheno-frontal suture) extending to the anterior calvaria (coronal suture) referred to as 'coronal rim' or 'coronal suture system' play a major role in the cranial development in Apert syndrome in the early intrauterine life,[29] or during the postnatal period.[32] The posterior cranial base synchondroses have also been found to fuse later during growth.[29,33] However, an increase in anterior cranial vault characterizing Apert syndrome was also reproduced in the mice model. This implicates that differences in the sequence and preferred sites of fusion in the two syndromes obviously exist, depending on the genotypes and are likely to be reproduced in the mice model as well.

Based on both the previous and present findings, we can hypothesize that the severe restriction of growth confined in the anterior cranial base may have caused the higher anterior calvarial flexure in the Apert mice, while simultaneous synostosis in the Crouzon mice may have helped maintain the incisor relation. The exclusive role of cranial base fusion in the flattening of the cranial base angle has been demonstrated in the rabbit model.[34] The characteristics of the cranial base in the human phenotype have been described as 'platibasia' in Apert syndrome and 'basilar kyphosis' in Crouzon syndrome, each representing increased and decreased cranial base angle, respectively.[35,36] However, significant individual variation has hindered the speculation of genotype-phenotype correlation in humans. In this study, the littermates with either Apert or Crouzon genotype exhibited minimal individual variation in the same strain, characterized as increased anterior calvarial flexture and flattened anterior cranial base in the Apert mice, and vice versa in Crouzon mice.

This study primarily focused on the skeletal shape of the anterior calvaria and cranial base, because these areas have been the sites of interest in the pathogenesis of craniosynostosis syndromes. It is not yet certain to say that the facial sutures are also involved in the pathogenesis. Some authors claimed that the facial sutures tend to remain patent until the later stages of life, unlike the cranial sutures, likely by the intervention of a capsular layer intersecting the bone segments.[2,3,37] The mandible in human patients, according to Kreiborg et al., exhibited a relatively normal downward and forward growth.[30] Changes in the mandibular shape is thought to be an adaptive phenomenon secondary to the deformation in the cranium.[25]

The severity of the phenotypes in Apert patients have been shown to be related to the type of mutation.[38] According to Gernet, Apert patients with Pro253Arg *FGFR2* displayed relatively mild craniofacial phenotype with a higher incidence of complete syndactyly, compared to those with Ser252Trp *FGFR2*. This variation in the phenotype has yet to be investigated to further formulate the effect of each normal/abnormal gene function. Both Pro253Arg and Cys278Phe mutations are regarded as activating mutations of *FGFR2*, meaning enhanced function of the normal receptors. The term 'activating mutations' have been evidenced by previous studies where the mutant receptors exhibited reduced specificity of the ligands or constitutive activation of the receptor regardless of the presence of ligands.[10,39,40] Hence it can be concluded that each type of mutation induces specific type of functional aberration, which eventually leads to unique morphological alterations.

It has been reported that early surgical reshaping to allow growth of the neurocranium involving Le Fort III osteotomy for the affected infants, did not facilitate the

post-surgical growth of the midface. Instead, surgical manipulation may totally block the post-surgical change of the midface possibly due to their inherent tendency to re-fuse, which demands repeated surgery during growth.[41] Therefore, it is essential to elucidate the molecular basis of etiology of abnormal bone formation, for which animal models could be very useful tools. Further research will include the altered FGFR2 functions and related sites and sequence of synostosis depending on the type of mutation.

CONCLUSION

This pilot study aimed to investigate the possible difference in the resultant phenotypes induced by the two distinctive bone-targeted gene constructs of *FGFR2*, Pro253Arg and Cys278Phe *FGFR2*, corresponding to human Apert and Crouzon syndromes, respectively, using transgenic mice models. Wild type and transgenic mice with normal FGFR2 were used as controls for comparison. Gross and detailed craniofacial phenotype with micro-CT, along with morphometric analysis on the anterior calvaria and cranial base revealed the following results:

1. Both Apert and Crouzon mutants of *FGFR2* induced fusion of calvarial sutures and anteroposteriorly constricted the facial dimension, with phenotypic difference including anterior crossbite in Apert mice. Apert mice exhibited a significant increase in the calvarial flexure and a decrease in the cranial base flexure angle, compared to the Crouzon mice and controls, implying a possible difference in the sites and sequence of sutural synostosis between Apert and Crouzon mice.
2. The transgenic mice with normal *FGFR2* displayed normal craniofacial phenotype, implicating that the altered function of FGFR2, not necessarily the intensity of FGFR2 expression, might be primarily responsible for the morphologic change.

According to the findings in this study, it could be inferred that each Apert and Crouzon mutation appears to lead to genotype-specific phenotypes possibly causing the distinctive sites and sequence of synostosis in the calvaria and cranial base. The exact function of the altered FGFR2 at each suture needs further investigation.

- 국문초록 -

어퍼트 및 크루즌 증후군을 유발하는 골조직 특이성 FGFR2 돌연변이에 의한 두개안면 형태의 변화

이기준 · 나현덕 · 스티븐 소아 · 박영철 · 백형선 · 윤태민 · 송진욱

유전적으로 결정되는 두개안면 기형의 발생 기전을 밝히기 위해 관련된 유전자의 기능 변화에 의한 효과를 이해하는 것이 필수적이다. 섬유아세포성장인자수용체-2 (*FGFR2*)의 활성형 돌연변이가 어퍼트 및 크루즌 증후군에서의 봉합의 조기유합의 원인이 된다고 알려져 있으나 인류에서는 다양한 개인차가 존재하므로 임상적으로 정의된 두 증후군에서의 유전형-표현형의 상관관계에 대해서는 의문이 제기되어 왔다. 본 연구의 목적은 어퍼트(Pro253Arg)및 크루즌(Cys278Phe) 돌연변이를 갖는 골특이성 *FGFR2*를 발현하도록 제작된 형질변환 쥐에서 결과적인 표현형의 차이를 분석하여 유전형에 의한 기형 형성의 인과관계를 추정하기 위한 것이다. 유전자 조작을 하지 않은 정상군과 정상 *FGFR2* 유전자를 가진 군을 대조군으로 하여 육안 관찰 및 micro-CT를 이용한 형태계측학적 방법으로 주로 전방두개 및 전두개저 부위의 이상을 분석하여 다음과 같은 결론을 얻었다. 첫째, 어퍼트 및 크루즌 돌연변이를 갖는 각각의 형질변환 쥐는 두개 봉합의 유합과 전후방적 두부 길이 감소를 공히 보였으나 어퍼트 형질변환 쥐에서만 전치부 반대교합이 나타났다. 또한 어퍼트 개체는 크루즌 개체 및 대조군에 비해 전방두개 및 전두개저 굴곡에 있어서 정상군과 비교해 차이를 나타냈으며 이는 유합을 보이는 봉합의 부위 및 순서에 있어서의 차이에 기인하는 것으로 사료된다. 둘째, 정상 *FGFR2* 유전자를 주입한 형질변환 쥐는 정상적인 두개안면 형태를 보였다. 이상의 결과를 토대로 어퍼트 및 크루즌 돌연변이는 각각의 유전형에 특이한 두개안면 기형을 유발할 것으로 보이며 정상 FGFR2의 발현 강도보다는 기능의 이상이 두개골 유합과 관련이 있는 것으로 사료된다. 변형된 FGFR2와 각 봉합에서의 기능과의 상관성은 추가 연구가 필요할 것으로 사료된다.

주요 단어: 두개골 유합증, 어퍼트 증후군, 크루즌 증후군, FGFR2

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COMMENTARY

The presented work is a rather complex treatise on possible mechanisms of inducing and also explaining the morphogenesis of two severe craniofacial anomalies, namely Apert and Crouzon Syndromes.

The work should be of a considerable interest to academia and to some clinicians who treat these complex cranio-facial anomalies. For this reviewer, who is both an academic and a clinician, the value of this contribution is measured in terms of how much better or how much faster will one be able to treat these anomalies. Even more importantly: will this work one day enable us to understand the involved mechanisms well enough to prevent *in toto,* or significantly decrease the prevalence of these anomalies.

The fibroblast growth factor receptors-2 (FGFR2) research should be viewed as one of the powerful, albeit somewhat fashionable methodology. Certainly it is not an easy tool to use or to understand its research potentials. Consequently, it is up to us, the teachers and researchers, to offer a credible explanation to an average reader. The average clinical orthodontist may not be prepared to fully grasp the meaning and the importance of this and other similarly high caliber reports. It is, therefore, appropriate to offer a "running commentary", as it were, for the purpose of providing a clear narrative of what this technology describes. This may be helping such a reader by giving him a cogent explanation.

First, it should be understood and accepted that much of the cellular and further magnified, of molecular detail of histo- or morpho-differentiation is not fully understood. The scientist have particularly been puzzled understanding where, at what molecular level something goes wrong, such that it will produce a particular clinical condition, for instance the Crouzon Syndrome. It is widely believed that several type of biological substances play either controlling or rate limiting factors in defining the outcome of the morpho-differentiation. Because of the ubiquitous nature and roles of the connective tissues in defining our phenotypic expressions or the appearance of many of our body parts, it is not surprising to look at the chief connective tissues forming cell, the fibroblast and, in turn, to the factors controlling the growth of these cells [thus: growth factor receptors]. Finally, the authors submit that studying the FGFR2 offers a "window" into a possible mechanism by which the morpho-differentiation of the face may be affected *en route* to expression of the Apert and Crouzon Syndromes.

It is hoped that this commentary will make the understanding of this highly sophisticated investigation somewhat easier. Before we go has the basic question of "how does this affect my patients" been answered? Probably not! Not many of the readers will be in a position to measure the minute deviations in function of the FGFR2. Consequently, we will not know if an individual is developing Crouzon before we actually see it clinically. We should hope and have enough faith in the scientific process to believe that what is today's understanding of a process will lead us to the tomorrow's cure of an undesirable anomaly. This in itself is well worth supporting and reading the basic scientific research.

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