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COMMENTARY

BCOR mutations and unstoppable root growth: a commentary on oculofaciocardiodental syndrome: novel *BCOR* mutations and expression in dental cells

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OFCD SYNDROME AND ROBERT J GORLIN

Dr Robert J Gorlin, a dental geneticist, had a key role in delineating oculofaciocardiodental (OFCD) syndrome (OFCD, MCOPS2; OMIM 300166), a rare disorder with some very interesting things to teach us about the regulation of development. This is an X-linked dominant disorder with male lethality. Female patients show: (1) ocular abnormalities: congenital cataract (80%), microphthalmia or secondary glaucoma, (2) facial abnormalities: long narrow facies, pointed nose with cartilages separated at the tip, high-nasal bridge and cleft palate, (3) cardiac abnormalities: atrial septal defect, ventricular septal defect and mitral valve prolapse, and (4) dental abnormalities: radiculomegaly, delayed dentition, hypodontia, delayed exfoliation of primary teeth, root dilaceration and hyperdontia.[1] The consistent characteristic feature of this syndrome appears to be radiculomegaly of permanent teeth, especially the maxillary and mandibular permanent canines.

I was a student of Professor Gorlin from 1989 to 1991. I still remember how excited he was about the long-rooted permanent canines he saw in patients with OFCD syndrome. He told me that in a number of cases the roots of the mandibular permanent canines were reaching the lower border of mandible and the roots of the maxillary permanent ones were reaching the floors of the orbits (Figures 1a and b). This striking feature appears to be unique to the best of my knowledge, it has not been found in any genetic or nongenetic disorders. Dr Gorlin later coined the name Oculo-Facio-Cardio-Dental syndrome emphasizing the major affected organs of the syndrome,[1] and always thought that it was telling us something important about development, a belief that is being amply confirmed.

OFCD SYNDROME AND *BCOR* MUTATIONS

Since then, we have learned much more about this condition. OFCD syndrome is caused by mutations in the BCL-6 corepressor (*BCOR*) gene.[2] *BCOR* is ubiquitously expressed in human tissues during early development and the encoded BCOR protein functions as a transcriptional corepressor. It lacks a DNA-binding domain and interacts with the DNA-binding transcriptional repressor BCL-6 via Poxvirus and zinc finger domain[3] and the transcriptional regulator AF9.[4] It is not surprising that *BCOR* mutations are associated with dental anomalies, including hypodontia, hyperdontia and radiculomegaly since *Bcor* is expressed in both dental epithelium and the mesenchyme during the early stages of tooth development.[5] Knockdown experiments of *Bcor* expression in dental mesenchymal cells using the lentivirus-mediated RNA interference approach have demonstrated that during early tooth development *Bcor* in mesenchyme has crucial roles in cellular events, such as apoptosis and cellular differentiation.[5,6] It is important to note that *BCL-6* is not expressed in tooth primordium in early embryogenesis.[7] This implies that the role of *Bcor* in tooth development is *BCL-6*-independent[5,8] and the dental phenotypes found in patients affected with *BCOR* mutations are not associated with *BCL-6*. It is possible that BCOR is likely to be targeted to DNA by other transcription repressors.[3] In addition, the ankylin repeats of BCOR, which are involved in protein–protein interactions support the possibility that BCOR interacts with other proteins not associated with Bcl-6. *Bcor* is highly expressed in the tongue during early development but, interestingly, patients with *BCOR* mutations have never been reported to have tongue anomalies.[5] Besides radiculomegaly and hyperdontia, all other defects in OFCD syndrome appear to be 'deficiency' defects such as cardiac septal defects, hypodontia and cleft palate. Continuous root growth and hyperdontia appear to be the opposite. There must be mechanisms that control human organ sizes, including the closure of anterior and posterior neuropores. This control mechanism appears to malfunction in the teeth of patients with OFCD syndrome. That is why the tooth roots do not close and keep growing. In the normal situation *BCOR* might have roles in regulating the root to stop growing; thus its mutations lead to continuously growing roots, imitating rodent incisors.

In this issue of *Journal of Human Genetics* the group led by Professor Keiji Moriyama

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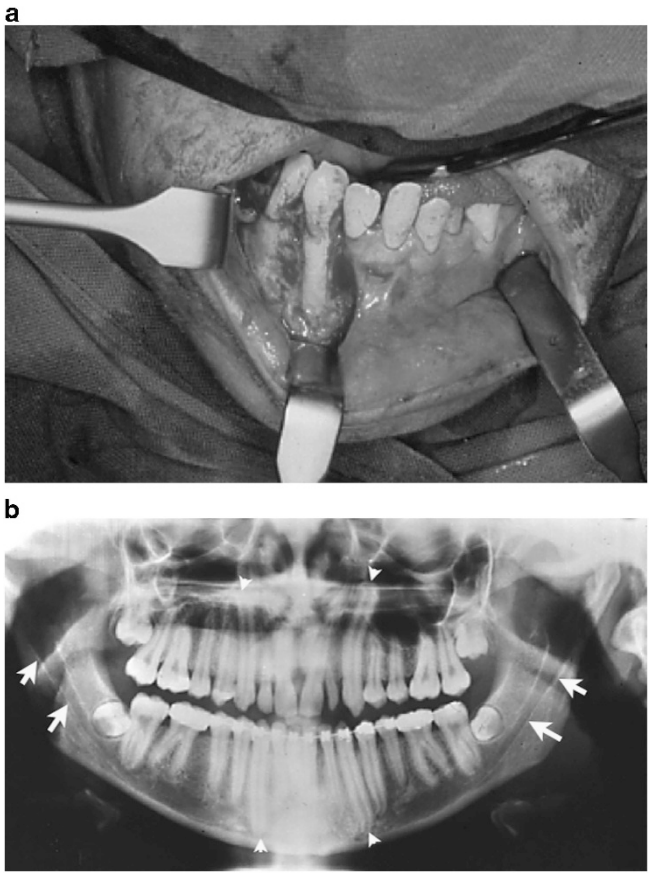

Figure 1 (**a**) Very long root of mandibular permanent canine. (**b**) Panoramic radiograph of a patient affected with OFCD. The roots of maxillary permanent canines reach the floors of orbits. The roots of the mandibular permanent canine reach the lower border of the mandible (arrow heads). Note very large mandibular canals (arrows). (Figures from Gorlin Slide Collection.) A full color version of this figure is available at the *Journal of Human Genetics* journal online.

reports two novel *BCOR* mutations, a nonsense and a frameshift mutation in two Japanese patients affected with OFCD syndrome along with studies of expression aimed at understanding why the tooth roots in patients with OFCD syndrome kept growing.[9] They studied the periodontal ligament (PDL) cells surrounding the apical 1/3 and apical pulp cells, which are tissues in the area of the growing root. They found that *BCOR* is highly expressed in the mesenchyme and the expression of the mutant PDL cells was higher than that of the mutant dental pulp cells. The mutant PDL appeared to have unstable transcripts and proliferated faster than wild-type cells. In addition they also demonstrated that the higher rate of nonsense-mediated mRNA decay in PDL led to insufficient function of BCOR to repress target genes, resulting in the promotion of cell proliferation and subsequently led to 'nonstop' root growth. This phenotype appears to be the result of haploinsufficiency.[9]

BCOR MUTATIONS AND UNSTOPPABLE ROOT GROWTH

There are exciting studies that add to our understanding of the mechanisms behind unstoppable root growth in OFCD, and I would like to review some developmental background to place them within a broader context.

Development of the tooth root requires the proliferation of the Hertwig epithelial root sheath (HERS), a transient epithelial double layer of flat, cuboidal cells which grows from the cervical loop of the dental organ between the dental follicle and the dental pulp, outlining the shape of the future tooth root.[10,11] HERS elongates and subsequently breaks up into epithelial cords at the very beginning of cementogenesis to allow the ingression of mesenchymal cells of the dental follicles to trespass the epithelial barrier and secrete cementum on to the dentinal surface of the root. Finally HERS collapses into the epithelial rests of Malassez.[11] The epithelial diaphragm at the most apical portion of HERS stays intact as it is not involved in the ingression of mesenchymal cells.[10]

Bcor is strongly expressed in the dental papilla cells and dental follicle cells that are attached to the cervical loops at the bell stage. This pattern of *Bcor* expression indicates the crucial role of *Bcor* in root development.[5] What controls the length of the root is still a mystery. In the normal situation the stem cell niche disappears, HERS invaginates and apical root closure commences.[11] In the permanent teeth, apical root closure takes place ~3 years after the teeth erupt into the oral cavity. In the primary teeth the interval is approximately 1.5 years. The stem cell population at the base of the tooth is the source of cells to replace all mesenchymal and epithelium-derived tooth cells.[12] In patients with *BCOR* mutations this does not appear to happen, indicating that the regulation in reducing the number of stem cells does not function properly.

BCOR MUTATIONS AND DISRUPTION OF EPIGENETIC MECHANISMS

The abnormal root growth in patients with OFCD syndrome has been demonstrated to be caused by the increase of the osteo–dentinogenic potential of mesenchymal stem cells (MSCs) secondary to *BCOR* mutations.[13] *BCOR* mutations result in abnormal activation of AP-2α, a repressive target of BCOR which is a key factor that mediates the increased osteo–dentinogenic capacity of MSCs. In the normal situation BCOR complex works as a negative regulator of osteo–dentinogenic capacity of MSCs. It maintains tissue homeostasis and gene silencing through epigenetic mechanisms. *BCOR* mutations have been demonstrated to increase histone H3K4 and H3K36 methylation in MSCs, and subsequently reactivating transcription of silenced target genes.[13]

BCOR AND POLYCOMB REPRESSIVE COMPLEX-1

Besides the nonsense-mediated mRNA decay mechanism, the polycomb repressive complex (PRC) might have a role in nonstop root growth in patients with OFCD syndrome. It has been demonstrated that polycomb complex group (PcG) proteins have roles in the maintenance of embryonic and adult stem cells, control of cell proliferation, cancer development, genomic imprinting and X-chromosome inactivation. The role of PcG complexes is to maintain the transcriptional repression of target genes by binding to the chromatin and inducing higher-order chromatin structures.[14] It is hypothesized that Bcor, which forms a complex with PRC1,[6] has an important role in root formation, especially in controlling the final

root length. PRC1 complex regulates the transit-amplifying cells of the dental MSC niche and cell differentiation in developing mouse incisors, and also has roles in molar root formation in mice.[12] The genes encoding members of the PRC1 complex, which is crucial for stable maintenance of gene repression by preventing nucleosome remodeling, are expressed in the incisor apical mesenchyme in an area that has transit-amplifying cells, the cells with the highest proliferation potential. Fibroblast growth factor signaling from the mesenchyme and the downstream targets are regulated by Ring1a/b, the core PRC1 components. Fibroblast growth factor signaling from the mesenchyme and the downstream targets are important in the maintenance of the dental epithelial stem cell compartment in the cervical loop, and are downregulated in *Ring1a*$^{-/-}$; *Ring1b*$^{cko/cko}$ incisors.[12] In *Ring1a/b* double-knockout mice the incisors have been demonstrated to lose the ability to grow continuously, indicating that in the normal situation Ring1a/b have crucial roles in the regulation of continuous root growth.[12]

The question of why the roots of the permanent canines are the most severely affected in teeth of patients with OFCD syndrome cannot be answered. It is obvious that 'all teeth are not created equal' and there is intertissue and interindividual variation for every mechanism that runs in our bodies. In addition there is not much known about the permanent canines. Besides *BCOR*, *WNT10A* appears to be the only gene that is known to be involved in the maxillary permanent canines, as mutations in *WNT10A* have been reported to cause agenesis of the maxillary permanent canines.[15]

CONCLUDING REMARKS

Unstoppable root growth in patients with OFCD syndrome appears to be caused by a few mechanisms. *BCOR* mutations causes abnormal activation of AP-2α, a repressive target of BCOR, leading to increased osteo–dentinogenic potential of MSCs. *BCOR* mutations also cause disruption of epigenetic mechanisms, thereby reactivating transcription of silenced target genes.[13] Surapornsawasd *et al.*[9] have beautifully demonstrated that the 'unstoppable' root growth in patients with OFCD syndrome is also the result of premature termination codon-induced nonsense-mediated mRNA decay mechanism in PDL cells which leads to unstable mutant transcripts and increased cell proliferation. It is hypothesized that BCOR, which forms complex with PRC1 might have important role in root formation especially in controlling the final root length. Its mutations are believed to cause 'unstoppable' root growth as a result of abnormal PRC1 complex, which in the normal situation regulates the transit-amplifying cells of the dental MSC niche and cell differentiation. Hopefully, these studies will be extended to the tissue of the apical papilla, which is highly involved in the growing root, and should tell us even more about the processes involved. In the meantime, this and the other work on OFCD is a lovely tribute to the memory of Dr Gorlin, who always believed that rare syndromes offered important insights into basic developmental processes.

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