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ABSTRACT OF DISSERTATION

Title	Novel PAX9 Mutations Cause Non-syndromic Tooth Agenesis 非症候群性歯牙欠損症における新規 PAX9 遺伝子変異
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Introducction

PAX9 is a transcription factor expressed in the tooth mesenchyme during tooth morphogenesis. In *Pax9*-null mice, tooth development is arrested at the bud stage and cleft palate and other skeletal abnormalities in the head and limbs are observed. In humans, heterozygous mutations in *PAX9* have been associated with non-syndromic tooth agenesis, affecting predominantly molars. The purpose of this study is to identify tooth agenesis causing mutations in two unrelated patients with oligodontia and to study the characteristic of the mutated proteins.

Matherial and Methods

Pedigrees of the family were made by clinical examinations and interviews, and the diagnosis was verified by panoramic radiographs. Genomic DNA was extracted from saliva, and *MSX1, PAX9, AXIN2*, and *WNT10A* were selected for sequence analysis. Sequencing results were compared with online databases, and the putative functional consequences of all identified mutations were analyzed *in silico* with PolyPhen-2 and MutationTaster. Next, full coding sequence of mutant *PAX9* expression vectors obtained by *in vitro* site-directed mutagenesis was clone in-frame into pCMV-3Tag-2A, and then transfected in COS7 cells to analyse the expression and the nuclear localization of the mutant proteins 48 hours after transfection. Luciferase reporter assay was performed to verify the transactivation of the BMP4 promoter. This study was approved by the Ethical Committee of Tokushima University Hospital (H23-8).

Results

Novel mutations in the paired domain of PAX9, a three-nucleotide deletion and a missense mutation, were identified. The individual with the c.73-75 delATC mutation was missing all maxillary molars and mandibular second and third molars. The individual with the c.C146T mutation was missing the mandibular central incisors, maxillary second premolars, and first molars, along with all second and third molars. *In silico* analyses predicted that both mutations are disease-causing.

Fluorescent immunocytochemistry showed nuclear localization of wild-type and mutant PAX9 proteins. Western blot analysis revealed lower protein expression in cell lysates of mutated PAX9-transfected COS7 cells compared with the wild-type PAX9-transfected cells. In addition, no transcriptional activities of the target BMP4 promoter were observed in mutant PAX9.

Discussion and Conclusion

Novel c.73-75delATC and c.C146T mutations in *PAX9* were identified in two unrelated patients with non-syndromic tooth agenesis. The patients showed agenesis affecting preferentially molars, as described in previous *PAX9* mutation reports. Both mutations affected amino acids that are highly conserved among different species and are critical for DNA binding. Although nuclear localization of the mutant PAX9 proteins were not affected, the expression of both proteins were reduced. Our *in vitro* study supported the prediction of the *in silico* analysis, as a disease-causing mutation. On the basis of our results, we suggested that the two identified *PAX9* mutations affect DNA binding and BMP4 promoter transactivation, supporting the mechanism of functional habloinsufficiency as the underlying cause of tooth agenesis.