Report of "Research Award of Oral Sciences"

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Title: *In vivo* study of Msx1 gene using CRISPR/Cas system in mice
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Among many signaling molecules and their receptors involved in epithelial-mesenchymal interactions in the tooth mophogenesis, mutations in the homeobox gene *MSX1* have been associated with non-syndromic tooth agenesis. *Msx1*-null mice exhibit failure of tooth development, secondary cleft palate and other craniofacial anomalies. To date, most of mutations identified in this transcription factor are located in the homeodomain or upstream this domain, affecting a highly conserved sequence that encodes DNA binding domain.

We identified a novel heterozygous G deletion (c.844_844delG) located in a highly conserved domain among mammalians named Msx homology domain 6 (MH6), near the carboxyl terminus of *MSX1*, in a Japanese family with non-syndromic oligodontia. To corroborate the importance of the MH6 domain in craniofacial development *in vivo* and to verify the genotype/phenotype correlation, *Msx*1 gene was targeted using CRISPR (Clustered regularly interspaced short palindromic repeats)/Cas system in mice.

Targeted mutagenesis was mediated by CRIPR/Cas system through RNA microinjection into one-cell embryos. Mosaicism was detected in embryos obteined with CRISPR/Cas-mediated targeted mutagenesis. E16.5 mutant specimens showed cleft palate and failure of tooth development. The present study demonstrated that the novel *MSX1* mutation causing non-syndromic oligodontia might be responsible of the oligodontia phenotype. Further *in vivo* study in homozygous MH6 deficient mice is required.

I would like to express my appreciation and gratitude to the Tokushima University Graduate School of Oral Sciences for having selected me as one of the "Research Award of Oral Sciences" winner as well as the research collaborators for their constant support and encouragement.

Meeting presentation:

- In vivo study of Msx1 gene using CRISPR/Cas system in mice. The 10th Tokushima Bioscience Retreat. Shodoshima, Japan. September 18-20, 2014. Silvia Naomi Mitsui, Akihiro Yasue, Kiyoshi Masuda, Issei Imoto, Sumihare Noji and Eiji Tanaka. (Oral)
- Sequence analysis and *in vitro* study of novel mutations identified in patients with tooth agenesis. The 3rd ASEAN plus and TOKUSHIMA Joint International Conference on "Strategic Achievement of Oral Sciences and Promotion of Quality of Life. Makassar, Indonesia. December 4-5, 2014. Silvia Naomi Mitsui, Akihiro Yasue, Kiyoshi Masuda, Keiichiro Watanabe, Shinya Horiuchi, Issei Imoto, Eiji Tanaka. (Oral)

- Mutation affecting the C-terminal of MSX1 causes non-syndromic tooth agenesis. IADR General Session & Exhibition. Boston, USA. March 10-14, 2014. (Poster) Silvia Naomi Mitsui, Akihiro Yasue, Kiyoshi Masuda, Issei Imoto, Sumihare Noji and Eiji Tanaka. (Poster)

Journal publication:

1-) Akihiro Yasue, Silvia Naomi Mitsui, Takahito Watanabe, Tetsushi Sakuma, Seiichi Oyadomari, Takashi Yamamoto, Sumihare Noji, Taro Mito & Eiji Tanaka (2014). Highly efficient targeted mutagenesis inone-cell mouse embryos mediated by the TALEN and CRISPR/Cas systems. *Sci Rep.*, 16,4:5705.

2-) Erica Hattori-Hara, Silvia N. Mitsui, Hiroyo Mori, Keiji Arafurue, Takuji Kawaoka, Kanji Ueda, Akihiro Yasue, Shingo Kuroda, Jan Harm Koolstra, Eiji Tanakaa (2014). The influence of unilateral disc displacement on stress in the contralateral joint with a normally positioned disc in a human temporomandibular joint: an analytic approach using the finite element method. *J Craniomaxillofac Surg.*; 42:2018-24.