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Possible Role for IRF6 in the Development of Neural Crest-Derived Palate

M. Weider[1], M. Wagner[1], G. Rodrian[1], T. Schmid[1, 2], M. Wegner[2], L. Gölz[1]
[1]Department of Orthodontics, Universitätsklinikum Erlangen, Erlangen, Germany, [2]Chair of Biochemistry and Pathobiochemistry, Friedrich-Alexander-Universität, Erlangen, Germany

Objectives The fact that TFAP2A activates IRF6 in orofacial epithelial tissue via the enhancer MCS-9.7 containing a risk single nucleotide polymorphism (SNP) for orofacial clefting has long been known (Rahimov et al. 2008). Given that TFAP2A is a bona fide marker for neural crest cells, we speculated that the TFAP2A – IRF6 axis is also important for proper development of neural crest-derived orofacial tissue.
Methods For luciferase assays, the neural crest-derived cell line Neuro-2A was transfected in triplicates with a reporter plasmid containing MCS-9.7 upstream of a minimal promoter and expression plasmids for effectors under control of CMV enhancer/promoter. 24 h after transfection, cells were harvested and luciferase activity was measured. Controls with empty expression vector were set to 1. Mouse embryos were fixed in 4% paraformaldehyde, dehydrated and embedded in tissue freezing medium at -80°C. 10 µm sections were stained by immunofluorescence with antiserum directed against Irf6. A mouse cranial neural crest cell line was used for quantification of Irf6 transcripts.
Results Transcription factors expressed in neural crest tissue could activate the Irf6 enhancer MCS-9.7. Antiserum directed against Irf6 could detect Irf6 protein in mouse embryonic neural crest-derived palate tissue. Irf6 transcripts were detected in a cranial neural crest cell line.
Conclusions Irf6 is also expressed in neural crest-derived palate tissue. Therefore, neural crest-related phenomena should be considered as possible disease-causing mechanisms of risk SNPs for orofacial clefts related to IRF6.