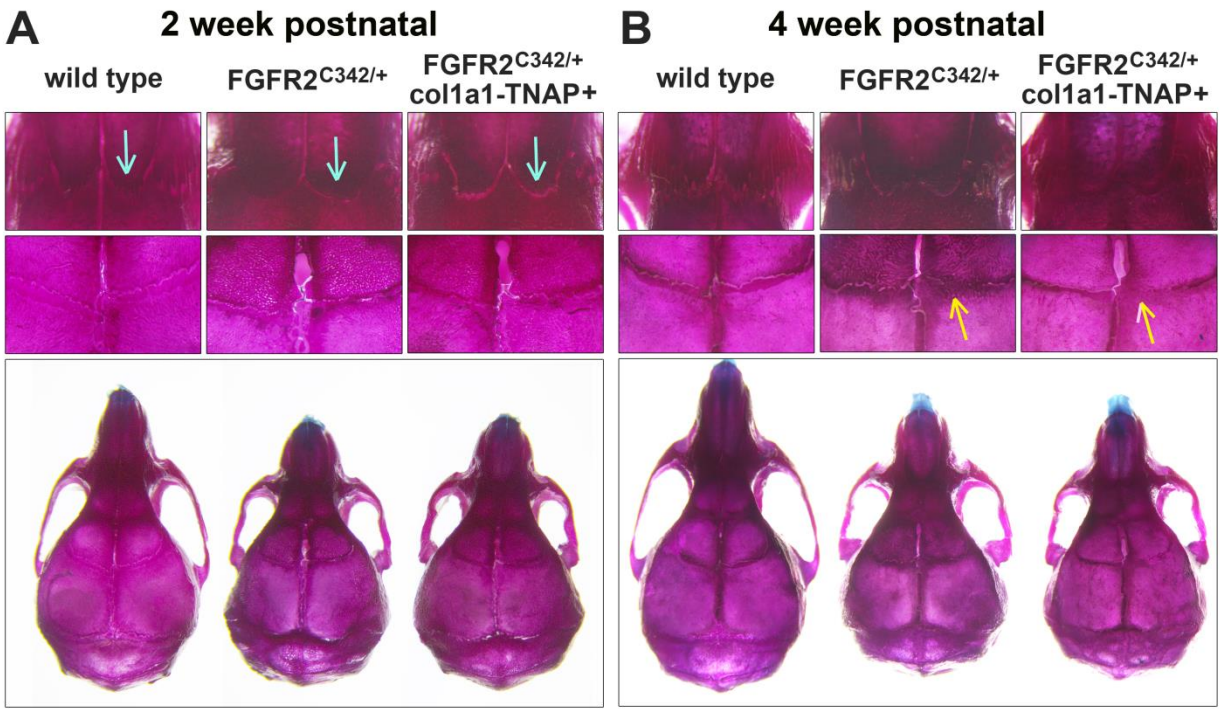
AAO Foundation Award Final Report

Principal Investigator	Nan Hatch
Co-Investigator	
Secondary Investigators	
Award Type	Biomedical Research Award
Project Title	Central Biologic Mechanisms of FGFR-Associated Craniosynostosis
Project Year	07/01/2012 – 06/30/2014 (no cost extension granted in 2013)
Institution	University of Michigan
Summary/Abstract (250 word maximum)	Craniosynostosis is a debilitating condition in which cranial bones prematurely fuse together. Even with an appropriately early diagnosis, craniosynostosis carries high morbidity. Untreated craniosynostosis can cause blindness, mental retardation, seizures and death. For over a decade, scientists have known that craniosynostosis occurs in association with mutations in the genes for fibroblast growth factor receptors (FGFR's; FGF receptors), yet the biologic process by which these mutations lead to craniosynostosis remains unknown. One mechanism by which mutations in FGF receptors may lead to abnormal cranial mineralization and craniosynostosis involves tissue non-specific alkaline phosphatase (TNAP), an enzyme whose activity is essential for the growth of hydroxyapatite crystals (the mineral component of bone). Evidence in support of this mechanism is provided by studies from our laboratory and others showing that: (1) FGF receptor activity inhibits TNAP enzyme expression; (2) TNAP controls bone and soft tissue mineralization by converting inorganic pyrophosphate to phosphate, and (3) craniosynostosis also occurs at high rates in children with inactivating mutations in the gene for TNAP. Together these findings suggest a model for FGFR-associated craniosynostosis in which activating mutations in FGF receptors inhibit TNAP enzyme expression, leading to diminished cranial bone mineralization with increased calcification of normally non-mineralized tissues, including the cranial sutures. The goal of this project was to define the precise contribution of TNAP in the overall effects of mutant FGF receptor activity on craniofacial tissue mineralization and craniosynostosis.

Were the original, specific aims of the proposal realized?	The original aim of this proposal was to determine if the abnormal craniofacial phenotype of $FGFR2^{C342Y}$ mice can be normalized when TNAP expression is increased in osteoblasts via mouse genetic technologies. To achieve this goal, $FGFR2^{C342Y/+}$ mice were crossed with mice that over-express TNAP specifically in osteoblasts. Towards this goal, we successfully obtained and genotyped an osteoblast specific, col1a1 promoter controlled, TNAP transgenic mouse (Col1a1-TNAP+ mouse) that expresses high levels of TNAP in osteoblasts, crossed this mouse with the $FGFR2^{C342Y/+}$ mouse, and analyzed the resultant craniofacial phenotype. Therefore, the original aims of the proposal were realized. This project took more time to complete than originally anticipated due to the fact that severity of the craniofacial phenotype was found to be variable on a mixed genetic background (this was revealed when we initially crossed the two mutant mouse lines). Therefore, we spent time and money back-crossing the osteoblast specific, TNAP transgenic mouse onto a clean genetic background and then repeated the cross of Col1a1-TNAP+ transgenic mice with $FGFR2^{C342Y/+}$ mice. Phenotyping of $FGFR2^{C342Y/+}$, Col1a1-TNAP+, double mutant $FGFR2^{C342Y/+}$/Col1a1-TNAP+, and wild type littermate mouse pups on a clean Balb/C genetic background has revealed that over-expression of TNAP in osteoblasts does not rescue craniosynostosis and craniofacial shape abnormalities in $FGFR2^{C342Y/+}$ mice (appendix, Fig. 1). This result negates our original hypothesis that osteoblastic TNAP activity mediates craniosynostosis. Importantly, this result is consistent with other recent findings from our laboratory which indicate that deletion of TNAP specifically in osteoblasts does not cause craniosynostosis (appendix, Fig. 2). These findings are significant and have led to modification of our initial hypothesis. Because global deletion of TNAP causes and systemic delivery of TNAP diminishes craniosynostosis, while osteoblast specific deletion or over-expression of TNAP does not alter craniosynostosis, these results suggest that expression and activity of TNAP in a cell type other than the osteoblast is essential for normal craniofacial skeletal development and craniosynostosis.

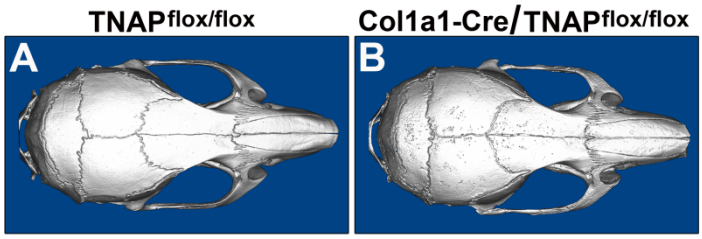
Were the results published? If not, are there plans to publish? If not, why not?	Results have not been published because results of this study were negative (over-expression of TNAP in osteoblasts did not rescue craniosynostosis or craniofacial shape abnormalities in Crouzon mice). That said, this data has been very important for guiding our ongoing project investigating molecular mechanisms that control craniosynostosis and craniofacial skeletal development. We are now investigating the alternative hypothesis that TNAP deficiency in cranial progenitor cells causes craniosynostosis. If these current studies (not funded by AAOF) are successful (if ablation of TNAP specifically in cranial skeletal progenitor cells causes craniosynostosis), results of the studies funded by this AAOF award will be included in that publication, to provide further evidence that TNAP activity in progenitor cells (as opposed to TNAP activity in differentiated osteoblasts) controls craniosynostosis.
Have the results of this proposal been presented? If so, when and where? If not, are there plans to do so? If not, why not?	Results of this proposal have not been presented. As described above, results of this proposal did not support our original hypothesis and there are no plans to present these results. Also as described above, results of these studies may be presented in the future, if combined with other data showing that TNAP ablation in cranial progenitor cells causes craniosynostosis.
To what extent have you used, or how do you intend to use, AAOF funding to further your career?	AAOF funding of this and prior research projects has been essential for my career as an orthodontic academic. Support of research projects by the AAOF has allowed me to generated strong in vivo data to support my overall investigation into molecular mechanisms behind craniosynostosis. This allows me to present at professional orthodontic and other scientific meetings, as well as submit manuscripts for publication. Finally, funding through the AAOF continues to be critical for generating the *in vivo* data required to achieve funding for this line of research from other foundations and the federal government, in the currently challenging federal funding climate. I cannot say enough good things about the AAOF and how important it has been for my career!

Fig. 1. Over-expression of TNAP in osteoblasts via the col1a1 gene promoter does not prevent craniosynostosis or skull shape abnormalities in Crouzon FGFR2-C342Y mutant mice.



A) Alizarin staining of 2 week skulls reveals diminished anterior-posterior length and increased width of both FGFR2-C342Y and FGFR2-C342Y/col1a1-TNAP mice, as compared to that seen in wild type mice. 2.5x magnification images reveal diminished interdigitation of the nasofrontal suture in both FGFR2-C342Y and FGFR2-C342Y/col1a1-TNAP mice, as compared to that seen in wild type mice (blue arrows point to nasofrontal suture). Coronal suture fusion is not evident in any mice at this age. B) Alizarin staining of 4 week skulls again demonstrates that both FGFR2-C342Y and FGFR2-C342Y/col1a1-TNAP mice have a brachycephalic skull shape, as compared to that seen in wild type mice. Fusion of the coronal suture is seen in FGFR2-C342Y and FGFR2-C342Y/col1a1-TNAP mice, but not in wild type mice (yellow arrows point to fusion).

Fig. 2. Ablation of TNAP in osteoblasts diminishes cranial bone mineralization but does not cause craniosynostosis



Micro CT isosurface images of young adult mice reveals a similar skull morphology and no craniosynostosis in mice in which TNAP was ablated downstream of the Col1a1 gene promoter. Significantly diminished cranial bone volume and mineral content as assessed by micro CT is present in Col1a1-Cre/TNAPflox mice (data not shown, $p<.05$, $n=3$ per genotype). These results support the idea that TNAP has functions distinct from and in addition to its accepted role in differentiated osteoblasts that regulate craniosyostosis.