AAO Foundation Award Final Report

Principal Investigator	Luciana M. Van Westen
Co-Investigator	Andrew C. Lidral (mentor)
Secondary Investigators	
Award Type	Orthodontic Faculty Development Fellowship Award
Project Title	X chromosome Inactivation Patterns in Patients Diagnosed with a Syndromic Form of Cleft Lip and Palate, Craniofrontonasal Dysplasia
Project Year	2006
Institution	University of Iowa
Summary/Abstract (approximately 250 words)	The goal of this study was to determine the X chromosome inactivation status of females diagnosed with a syndromic form of orofacial clefting referred to as Craniofrontonasal Dysplasia (CFND). The methods used during this study and knowledge obtained on X chromosome inactivation will be applied in future similar research in females diagnosed with nonsyndromic cleft lip and/or palate. Females diagnosed with CFND are often more severely affected than hemizygous males, which is inconsistent with an X-linked mode of inheritance. Females show a range of phenotypic abnormalities described, from mildly to severely affected, but males show hypertelorism as their most consistent sign. Our hypothesis is that differences in phenotypic severity observed in heterozygous females diagnosed with CFND may be due to non-random X-inactivation patterns. In such cases, skewed X inactivation in mildly affected females is expected to correlate with the inactivation of the mutant allele. On the other hand, skewed inactivation in severely affected females is expected to correlate with the inactivation of the normal allele. The study subjects have been recruited from collaborators across the United States. Patients diagnosed with CFND were clinically examined and interviewed using a clinical survey to gather information regarding medical history, family history, and gestational environmental exposures. This study was approved for the use of human subjects by the internal review board at the University of Iowa and written informed consent was obtained from all participants. Blood samples were obtained and DNA extraction was performed using a commercial kit from Puregene, Gentra Systems (Minneapolis, MN). A recent study of 1,005 phenotypically normal females has shown that X chromosome inactivation ratios in blood samples using HUMARA follow a bell shaped, normal distribution (Amos-Landgraf et al. 2006). In particular, the majority of normal females (63.6%) show what is considered random X-inactivation (50:50 to <40:60). The other 36.4% of phenotypically normal females show the following X-inactivation ratio distributions: 25% show a ratio of <30:70/>70:30, 8.8% a ratio of <20:80/>80:20, 1.8% show a ratio of <10:90/>90:10, and 0.8% show a ratio of <5:95/>95:5. This

	categorical approach was applied in this study in order to make fair comparisons between affected patients and unaffected females. X chromosome inactivation analysis using the Human Androgen Receptor Assay (HUMARA) was completed for all females affected with CFND and mothers of probands if they were available, for a total of 14 females. According to the categorical approach applied in this study, females affected with CFND and their mothers were found to have random or mild skewed X-inactivation (50:50 to <70:30) in their lymphocytes. Actually, 64.3% of probands or mothers of probands showed random X inactivation ratios compared to 63.6% of phenotypically normal females. In contrast, 35.7% of probands or mothers of probands experienced mild skewed X-inactivation ratios (<30:70/>70:30) compared to 25% of normal females. None of the affected females with CFND or the mothers of probands showed moderate or extreme X-inactivation ratios in their lymphocytes, which in this study is defined as ratios between <20:80/>80:20 and <5:95/>95:5. It is interesting to note that the only mothers who showed mild skewed X-inactivation (<30:70/>70:30) were those who were themselves affected and had an affected male child. Even though, no conclusive evidence can be reported by this study due to a low number of subjects, mild skewed X-inactivation in affected mothers and knowledge about the gender of their offspring may be of future importance for genetic counseling.