Evaluation of the Quality and Quantity of DNA from Buccal Samples in the National Birth Defects Prevention Study

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Analysis of polymorphisms in genes encoding proteins involved in metabolism of tobacco smoke is planned as part of a study to identify gene–environment interactions in the etiology of gastroschisis and anorectal atresia. The planned analysis will use data from a multisite, population-based case-control study of major birth defects that includes a maternal interview and self-collection of buccal cells using cytobrushes for each mother, father, and infant. Thus far, we have performed pilot studies to better understand the quality and quantity of DNA from buccal samples collected for the multisite study of major birth defects. An initial pilot study included 41 DNA samples from the Atlanta study site. Genotyping was completed in duplicate for 20 variants from 6 *CYP* and 2 *NAT* genes using Pyrosequencing® technology. 11 of 41 samples (27%) had low DNA concentrations (<0.1ng/µl), as determined by a real-time PCR assay specific for human gDNA. Among these samples, unsuccessful PCR amplification and evidence of allele drop-out (ADO) were observed. Three variants were selected for a subsequent pilot study completed on 65 Atlanta samples. The same methodology was used except that samples were tested in quadruplicate. 25 of 65 samples (38%) had low DNA concentrations. Unsuccessful amplification and discordance between replicate results, consistent with the occurrence of ADO, were again seen almost exclusively in the low DNA concentration samples. DNA quantitation is complete on all 1,667 parent and infant samples selected for the study of anorectal atresia and gastroschisis. 251 of the 1,667 samples (15%) had low DNA concentrations and will be excluded from further study to reduce genotyping errors. The preliminary studies have contributed to a better understanding of the quality and quantity of DNA obtained from buccal samples and the correlation between DNA concentration and ADO in NBDPS samples and may be of value to other genetic epidemiology studies.