Research Networking Programme

Final Report

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ESF/EUROCleftNet exchange visit (April 1st - September 30th, 2015)

Title: DNA Biobanking and Ecogenetics of Orofacial Clefts

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1. SCIENTIFIC BACKGROUND AND AIM OF THE VISIT

The bio-banks includes wide array of biological specimens in the form of DNA, Cells, tissues etc. which collectively can be considered as library of an individual human. These bio-banks play crucial role in bio-medical research and serve as essential resource for genotyping and next generation sequencing (NGS) based studies. In-line with this, the discovery of genetic factors associated with the development of cleft lip with or without palate (nsCL/P) has opened up opportunities of maintaining a Cleft DNA bank which preserves the opportunity for future research or genetic testing and may benefit the case-parents and cases community as a whole. Our effort was in building, managing and expanding the already set non-syndromic orofacial cleft (nsOFC) child-parent trio DNA harboured at University of Ferrara that meets the requirement of automation platforms and the newest genotyping technologies. The ability to seek out very specific collections of specimens is how DNA bank will serve as an essential resource for European scientists. This DNA bio-bank is an open access supplement to every partnered group/members under the aegis of EuroCleftNet network

The nsOFCs are considered multifactorial condition caused by interactions between genetic factors and the exposure to environmental risk factors. The environmental and nutritional factors [including exposure to folic acid, (Hernandez-Dias et al, 2000; Puh et al, 2007), tobacco smoke (Little et al, 2004), alcohol consumption (Romitti et al, 2007; Grewal et al, 2008) contribute to the development of cleft lip and palate during pregnancy.

Recent genome-wide association studies (GWAS) have identified the major loci associated with increased risk of nsCL/P in Europeans, but these alone can explain only a small fraction of the multifactorial etiology of nsOFCs. It is generally accepted that gene-environment interactions (GxE) could play a major role in the pathogenesis of nsOFCs, and the identification for these interactions could offer a benefit in clinical diagnostics and potentially provide new measures for primary prevention.

Concerning the pipeline, numerous candidate genes have been identified as genetic risk factor of nsCL/P one of which is TGFA reported long back by Ardinger et al, 1989 in Caucasian population. Although the association of TGFA polymorphism is well characterized and found to be associated with the development of CL/P, there exists an inconsistency in number of studies (Vieira, 2006; Lijia et al, 2015) which could be an outcome of both differences in the design of study and populations. In weigh, we explored an additional TGFA insertion/deletion variant along with the most commonly studied TGFA insertion/deletion variant to explore its association with nsCL/P in EUROCRAN samples. In order, we examined the pool of European trios for frequency of transmission between

the two TGFA insertion/deletion and susceptibility of developing nsCL/P. Additionally, the test of gene-environment interactions between the two insertion/deletion markers in TGFA and two common maternal exposures (smoking and folic acid supplementation) during pregnancy are factored.

2. WORK DESCRIPTION AND RESULTS

2.1. Project 1. DNA Bio-banking:

2.1.1. DNA Extraction and reconstitution:

Blood and DNA samples, collected in Scotland at the time of EUROCRAN project and previously stored in Edinburgh were transferred to University of Ferrara in order to expand the set of UK samples. Genomic DNA of case-parent trio was extracted from peripheral blood DNA was extracted using Nucleon BACC1 DNA extraction kit according to the manufactures protocol. This let to complete trios with missing samples or increase the quantity of gDNA in samples with low or insufficient amount.

2.1.2. DNA titration/quantification:

All EUROCRAN samples from west European countries, including UK, Italy, Netherlands and Spain, previously stored in multiple Eppendorf vials and with uncertain concentration, where pooled and re-titrated using fluorimetric technology, and stored in 2D-barcoded vials.

The genomic DNA was titrated using 2.0 Qubit Fluorometer and dsDNA BR Assay Kit (Life Technologies) according to the manufactures protocol. The general pictorial representation of the assay procedure :

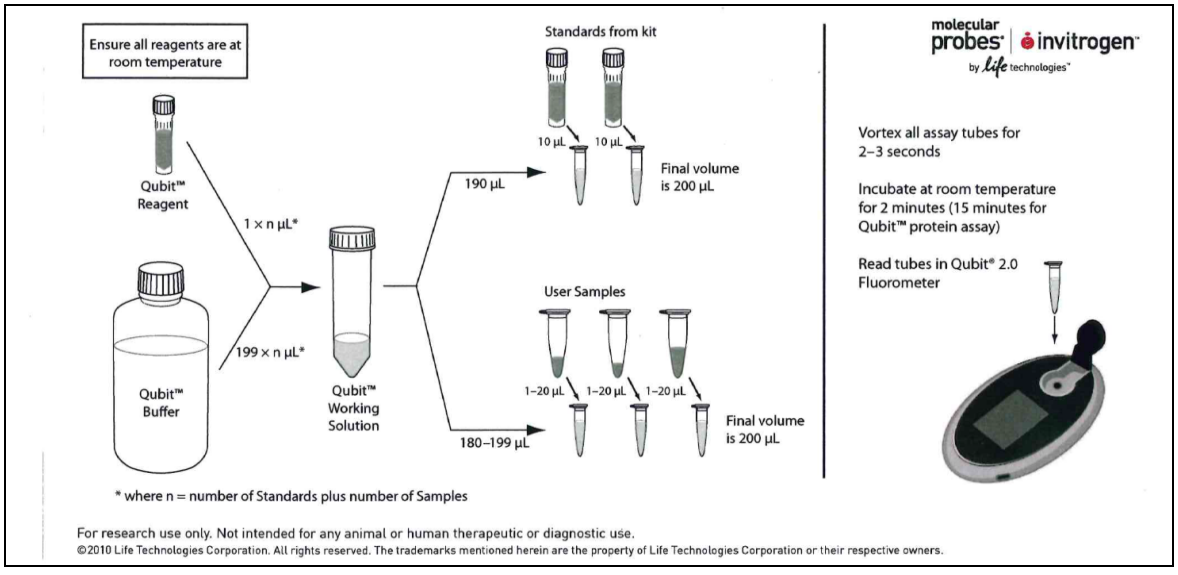

Figure 1. Qubit DNA assay procedure

2.1.3. DNA storage:

The extracted DNA is stored in the 2D bar coded matrix vials which are not labelled with individual patients' or family names, but rather unique barcode identifiers. Samples are delinked: Biographical data of the case-parent trios is removed and the sample is identifiable by only a unique bar code.

2.2. Project 2. GxE association study

2.2.1. Method

The DNA samples from EUROCRAN nsCL/P trios were genotyped for two TGFA insertion/deletion variants located in introns 5 and 6 of the TGFA gene. Genetic association between genotypes and nCL/P or nsCP was assayed using the transmission disequilibrium test (TDT) or relative risk analysis by logistic regression using STATA package. The relative risks associated with interaction between genotypes and environmental factors was evaluated with log-linear model using STATA software. Moreover, the parent-of-origin effect was evaluated for each variant.

2.2.2. Result.

The outcome of the study exhibit little evidence of interaction between child genotypes at TGFA insertion/deletion variants and the two maternal exposures (smoking and folic acid) with nsOFC in EUROCRAN population, with the possible exception of child's gender.

3. FUTURE PLANS AND PUBLICATIONS

3.1. Future plan.

We plan to augment the DNA bio-bank with the Eastern European samples which can provide a further increase in the statistical power of the present EUROCRAN DNA-bank, and possibly lead to successful gene-environment interaction studies.

Moreover, the EUROCRAN samples will be used to explore gene-environment interactions and gene-gene interactions involving variants located in GWAS-loci, to further delineate the role of exposure to environment and epistasis in the development of cleft.

Furthermore, we have plan to set a lip tissue bank of different Cleft sub-types obtained at the time of primary surgery, providing additional support to the EuroCleftNet network to unravel the tissue specific markers associated with etiology of Cleft.

2. Future publication.

The work is accepted as poster presentation in the Italian Society of Human Genetics (SIGU) - Congress, Rimini-Italy, 21st-24th October, 2015. The manuscript defining the association of TGFA insertion/deletion variants and environmental factors in European decent to the risk of non-syndromic cleft lip with or without palate (nsCL/P) is under process.

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