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Gene/environment interactions

6.1 Introduction

The role of genes, genetic susceptibility and gene/environment interactions (GEI) in the etiology of orofacial clefts remains largely unknown. However, with the availability of the human genome sequence, researchers have increasing opportunities to study the role of genes and gene/environment interactions in human health and disease (Schutte and Murray, 1999). Discussions, led by Lorenzo Botto, sought to examine these opportunities and the major accompanying challenges in three main areas:

- **The first area relates to data:** to identify and, if possible, rank the major data gaps separating our current knowledge from that needed for clinical and public health action.
- **The second area relates to methods:** how to conduct, analyse and present studies of multiple genetic and environmental factors in ways that efficiently fill the data gaps.
- **The third area relates to people and institutions:** how to learn more and more quickly, using the unique opportunities inherent in international collaboration.

Gaps and challenges in the study of GEI in orofacial clefting

Data challenges

- Representative populations
- Focus on common exposures and gene variants

Methodology challenges

- Improved assessment of environmental exposures
- Careful design, complete presentation
- Systematic assessment of risks and impact

Collaboration challenges

- Use, share, pool data
- Sample size – more people, more countries
- Standardized methodologies

6.2 Data challenges

6.2.1 *Representative populations*

Because the ultimate goal is population-based action (prevention, intervention), scientists need data that is representative of populations. For example, the frequency of gene variants and exposures should come from population-based surveys, the risk estimates from population-based case-control studies, and so on. Such requirements for population-based studies can be a major constraint to study design and conduct; ultimately, however, there is no known alternative for gathering population-based data. Some measures of risk (e.g., the effect of genes alone, departure from multiplicative interaction) could be provided by family studies or case-only studies that are not population based. Such studies can be very useful. However, the full spectrum of gene effects and gene/environment interactions and estimates of attributable fraction require, for identification or confirmation, population-based studies such as population-based case-control studies, as discussed below.

6.2.2 Focus on common exposures and gene variants

There are many genes and exposures that one could study. Indeed only a handful of gene variants and exposures have been studied in relation to orofacial clefting, leaving options virtually limitless. From the preventive perspective that underlies this discussion, it is natural to suggest an initial focus on factors that might contribute to the greatest fraction of cases in the population, i.e., factors with the highest attributable fraction. The latter is a function of the factor's relative risk and its frequency in the population. Because the relative risk is difficult to gauge in advance, frequency of exposures might be a reasonable factor to consider in ranking the potential interest of exposures. This concept is put into numbers in Table 11 (below) which summarizes the population-attributable fraction of a hypothetical exposure, given a range of associated relative risks and exposure frequencies.

Table 11: Population-attributable fraction in relation to frequency of exposure and relative risk

Frequency of exposure	Relative risk (RR)						
	1.2	1.5	2	3	5	10	20
0.0001	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.001	0.00	0.00	0.00	0.00	0.00	0.01	0.02
0.01	0.00	0.00	0.01	0.02	0.04	0.08	0.16
Fever	0.05	0.01	0.02	0.05	0.09	0.17	0.31
Obesity	0.1	0.02	0.05	0.09	0.17	0.29	0.47
	0.3	0.06	0.13	0.23	0.38	0.55	0.73
Smoking	0.5	0.09	0.20	0.33	0.50	0.67	0.82
	0.7	0.12	0.26	0.41	0.58	0.74	0.86
No supplement	0.9	0.15	0.31	0.47	0.64	0.78	0.89
	0.9	0.15	0.31	0.47	0.64	0.78	0.89

Source: Dr Lorenzo Botto (unpublished data)

Studying small relative risks is, however, challenging as it requires large sample sizes and careful assessment of bias and confounding. Multi-centre and international collaboration with common protocols might be a useful strategy to overcome some of these difficulties. Finding GEI that involve common exposures might also be useful in confirming the role of such exposures in the etiology of orofacial clefting, particularly when the exposure alone is associated with low increased risk (e.g. smoking) that might be due entirely to unrecognized bias or confounding.

Finally, because of the potential impact of these common factors, negative studies become very important. Their replication and publication should therefore be encouraged.

6.3 Methodology challenges

The problems in gene/environment interaction research reside mainly with the *a priori* specification of the interaction model and with the statistical power required. It is also felt that there are difficulties in measurement of the environmental exposure.

It should also be noted, however, that genotype may effect the level of a biomarker and this is particularly important when examining nutrient status.

6.3.1 *Improved assessment of environmental exposures*

The problems in gene/environment interaction are mainly with the environmental aspect. With genes it is possible to carry out more analyses in shorter time periods with good reliability, but better assessment methods are urgently needed for assessment of environmental factors, as well as issues such as measuring versus reporting – the former being more objective while the latter is easier and less expensive.

Environmental exposures are now usually based on maternal reports, often taken months or years after the relevant exposure period. Objective biomarkers of exposure and effect are, for the most part, lacking. Biologic samples for measurement of environmental exposures (urine, hair, serum, whole blood) are difficult to obtain – more so than DNA sources – as are environmental samples (air, water, soil). The precision and validity of GEI studies is a function of the validity and precision of both the genetic and the environmental component, making improvements of environmental measurements a priority in GEI studies.

6.3.2 *Careful design, complete presentation*

Currently, several approaches are being used. Some classic published studies of GEI in OFC were conducted using the population-based case-control design (Denmark and the United States (Iowa and California)). In recognition of the genetic predisposition and GEI, a study design in the United Kingdom adopted a strategy using both case triads and control triads (ITSMAGIC Consortium) and a large ongoing study in the United States is based on a similar design. Some ongoing studies from Europe and the United States are based on case-triad designs. At least one large ongoing study in the United States is based on a mixed case-control design, using both case triads and control triads. These designs were carefully chosen as being the best for the objectives of the studies, given practical constraints; the hope is that the cumulative knowledge so obtained can be integrated to completely characterize, in the sense discussed above, the population-based indices of GEI in orofacial clefting.

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It is important to look not only at genes alone, or at environmental factors alone, but also at their interaction. A simple and effective way of looking at gene/environment interaction is exemplified by the 2 x 4 table approach using a case-control model. This approach allows for the study of the effects of each factor or gene alone, joint effects, and the assessment of interaction in terms of departure from any specified model, be it additive or multiplicative (or other).

6.3.3 Systematic assessment of risks and impact

In addition to the summary measure of interaction (be it additive or multiplicative), it is useful to derive and present the component factors, i.e., the effect of the genotype alone, the exposure alone, and the joint effect of both genotype and exposure. For each of these factors, it is useful to present three numbers: the frequency among controls, the relative-risk estimate, and the attributable fraction. These numbers (the frequency, risk and impact for the three components of interaction and the summary measure) neatly summarize many important aspects of a GEI.

6.4 Collaboration challenges

6.4.1 Use, share, pool data

Like most research, results from studies of OFC carried out independently are often difficult to compare because the studies are relatively small and often use different classifications of exposures and outcomes. Indeed, one of the most common sentences in published reports may be variations of “comparison with other studies is difficult because of methodologic differences”. Such comparisons, however, might still be possible if one reverts to the original, individual-level data. Thus collaborative, primary-pooled analyses might be an efficient strategy to maximize the information yield of already-conducted studies. In addition, international collaboration might benefit from the sharing of unpublished data from studies that may have been published in part, perhaps using a common repository of unpublished tables. Pooling data from such tables might be appropriate in some cases, provided there is an awareness of differences in data-collection methodology, biases and confounders, and that any subsequent evaluation or analysis recognizes these factors.

6.4.2 *Sample size*

BOX L

More people, more countries

Sample size is a fundamental issue in GEI studies. In the case of orofacial clefting studies, the challenge of sample size is evident in the published literature where the expected number of cases in the relevant exposure category is usually very small, often less than 10 and sometimes less than 3. Carefully conducted multi-centre and international collaboration might provide a useful strategy to study larger numbers of people, provided there is adequate control of confounding and elimination of biases.

Most data on GEI in orofacial clefting derives from studies of small, wealthy populations (e.g., Denmark and the United States (Iowa, California)). Whilst this is to some extent unavoidable, it underscores the need for similar data in populations that are geographically and ethnically diverse. Orofacial clefting occurs more frequently and causes more morbidity and mortality in the less wealthy countries (Schutte and Murray, 1999; Rosano et al., 2000). Finding GEI that are relevant to these populations (and simple, inexpensive, low-tech prevention strategies) would satisfy elementary requirements for social justice.

Also, broadening the range of exposure probably makes misclassification have a smaller impact than improving the precision of exposure assessment would.

6.4.3 *Standardized methodology*

In disorders that are thought to have a polygenic multi-factorial etiology, as is the case for non-syndromic orofacial clefting, there is a compelling need for researchers to be able to compare their data on putative environmental and genetic factors. The fundamental principle on which multi-centre collaborative research works is that there is a consistency in the methodology of data collection, thus enabling combined analysis.

A multidisciplinary multi-centre European initiative, supported by the European Science Foundation (ESF) has, as one of its main objectives, sought to define in a number of key areas the important data and accompanying methodology of this data collection. The common factor which brought this body of expertise together was a research interest in orofacial clefts and, because of the polygenic multi-factorial etiology and evidence of heterogeneity, this group sought to develop consistent protocols across populations with variable genetic backgrounds, lifestyles, diets and environmental exposures. The parallel development of global networks in CFA research, through funding from the European Union, the NIH and WHO, will enable researchers throughout the world to benefit from these “common core protocols”.

While these have been developed in the context of orofacial clefting, they may provide useful information in the wider context of reproductive outcome – in particular, for other birth defects also suspected of having a polygenic multi-factorial etiology.

6.5 Conclusions

The study of GEI in orofacial clefting has achieved some remarkable successes, and developments in genetic technology promise that such successes are only the beginning (Schutte and Murray, 1999). The eight challenges presented here might stimulate discussions that could lead to useful collaboration. The task ahead is still enormous. There are thousands of gene – gene/environment interactions possible and 99.96% of genes in the population remain untested. In those that are tested, genotype frequencies vary in different populations. Shared priorities, clear planning and international collaboration are likely to be key factors in progressing from basic science to population-based opportunities for primary prevention worldwide.