

5

Data collection aimed at supporting research

5.1 The European Science Foundation Project

For CFA research, consistent protocols across populations are fundamentally important. The following summary contains a proposal for the use of the “European Science Foundation (ESF) Common Core Protocols Project – Minimum Data Sets” for ongoing GEI research, tailored towards a case triad study design. This will provide guidelines on the core information required in eight different areas and will provide some rationale for the recommendations. Apart from the core data, information on the development of further desirable and/or optional data will also be provided where applicable.

It is noteworthy that a complimentary and collaborative group of international scientists based in the US, the International Consortium for Oral Clefts Genetics, also produced a document entitled *Guidelines for the design and analysis of studies on non-syndromic cleft lip and cleft palate in humans*. The report of this was published in the *Cleft Palate Craniofacial Journal* (Mitchell et al, 2002).

The following is a summary of the deliberations of the ESF Special Interest Group on Cleft Lip and Palate.

5.1.1 Case ascertainment: recommendations for core information

Orofacial clefting (OC) is a heterogeneous group of defects with a considerable range of severity so there is, inevitably, variability in the ascertainment rates. The information collected should be divided into essential, desirable and optional.

Core information recommended for case ascertainment

Essential information:

- Base ascertainment of congenital anomalies and precise diagnosis on multiple sources of information.
- Make it clear if terminations and fetal deaths are included and, if so, describe the inclusion criteria and methods used.
- Include multiple anomalies and syndromes.
- Present all epidemiological and genetic data by specific cleft type.
- Differentiate between CP and CL/P and, where possible, subdivide CL and CLP.
- Subdivide each cleft type by the presence or absence of associated congenital malformations.
- Separate syndromic cleft cases from non-syndromic cases.

Desirable information:

- Record the type of classification and how this was done for syndromic cleft cases that are separated from non-syndromic ones, for example, where classified by a dysmorphologist.
- Tally birth prevalence statistics for clefts separately for familial and sporadic cases; this will further benefit risk-factor studies.
- Record late-diagnosed cases.
- Code congenital anomalies, minor anomalies, and give precise diagnoses.

Optional information:

- Diagnose all degrees of cleft expression (including sub-mucous clefts) to prevent under-ascertainment.
- Where possible, present data within countries by ethnic group.
- (Ideally) collect data sets containing core information agreed by consensus; additional information can be collected for studies in suspected high-risk population subgroups.
- In preparing incidence data to support genetic and other etiologic studies, include all terminated pregnancies and stillbirths or make appropriate adjustments.
- Make diagnoses more specific as further investigation is performed.

5.1.2 Clinical assessment of oral clefting: recommendations for core information

- 1) Record basic demographic information, including basic lifestyle data.
- 2) Follow guidelines for recording of baseline (neonatal) minimal-record data sets.
- 3) Take photographic records; if possible standardized extra-oral and intra-oral views.
- 4) Have access to clinical dysmorphology expertise, if possible a clinical geneticist/dysmorphologist.
- 5) Use an internationally recognized system of coding and subsetting for CFA.
- 6) Use an internationally recognized system for cleft classification.
- 7) Record pre-natal diagnosis, ultrasound or maternal serum screening.
- 8) Diagnose isolated CP subsets, e.g., 22q11 deletions; and, where applicable, cleft lip and palate subsets. (*See also Box 17 above.*)

5.1.3 Nutritional factors and food frequency questionnaires: recommendations for core information

Nutrition remains one of the most eligible aspects of orofacial clefting research.

- 1) For core nutritional data, compile a food-frequency questionnaire to assess total energy intake.
- 2) Report nutrient intake.
- 3) Make the questions population-specific.
- 4) Validate data by comparing it with relative ranking obtained by another method, such as diet, diary or weight record.
- 5) Include vitamin supplements and food fortification.
- 6) Consider whether food-frequency questionnaires are the optimum method to obtain nutritional data. Minimum requirements might include food fortification, and multivitamin supplements.
- 7) Use food-frequency questionnaires only for relative ranking of reported intake and not as a measure of absolute intake.

5.1.4 Lifestyle and environmental factors: recommendations for core information

- 1) Collect data on lifestyle exposures, such as smoking and alcohol, during pregnancy (first trimester); regard these as core data.
- 2) Include occupational exposure and recreational drugs when examining congenital abnormalities; these are desirable and optional additional data but are difficult to collect and analyse consistently.
- 3) If socioeconomic status is to be examined, consider what the most consistent measures of this would be – education, housing, postal code, occupation, other lifestyle factors, a combination of these or something else?

5.1.5 Obstetric and medical history: recommendations for core information

- 1) Include illnesses and medications in the first trimester as minimum data.
- 2) Record the obstetric history.
- 3) Enter date of conception.
- 4) Describe birth-control methods.
- 5) Record timing of awareness of pregnancy.
- 6) Ask if the mother suffered from morning sickness.
- 7) Record medical history of illnesses, including common ailments such as colds and influenza, as well as any specific medical conditions that may have implications for birth defects.
- 8) Note any drug therapy as this would be related to the medical conditions.
- 9) Tailor the questions on drug therapy to the hypothesis, such as anti-convulsants; also record epilepsy/anti-epileptics, radiotherapy or X-ray exposure.
- 10) Tailor hypothesis e.g., folate and the folate antagonist drugs, such as methotrexate, anti-malarials etc.
- 11) Record other aspects of medical history specifically related to the hypothesis being tested, e.g. vitamin A teratogenesis, accutane, etc.
- 12) Record previous obstetric history in terms of number of siblings, previous stillbirths or other related congenital abnormalities.

5.1.6 *Biochemical assays: recommendations for core information*

There are four main issues that relate to the methods of **sample collection, processing, storage, and analysis**. These are dependent on the hypothesis under test and/or the purpose for which blood or other tissue samples are being collected. As an example, where the study proposes to investigate nutritional biochemistry, the core data set in OC should include:

- full blood count,
- red cell folate,
- plasma folate, plasma vitamin B 12, plasma homocysteine,
- other assays, plasma vitamin B2 and B6,
- methylmalonic acid,
- genetic analysis,
- vitamin A and other nutrients,
- immortalized cell lines obtained from lymphocytes.

5.1.7 *Genetic protocols and assays: recommendations for core information*

Molecular genetic factors in OC, DNA, polymorphisms, adjacent to or within the candidate genes aim at identification of etiologic genetic loci. Case-control triads remain the “gold standard”, case-only design has limited usefulness, but nuclear triads have several advantages (and a few drawbacks).

- 1) For congenital birth defects such as OC, a common core protocol should pursue case triads and the subsequent genetic analysis protocols should include:
 - transmission disequilibrium test (TDT),
 - parent of origin, effects and imprinting,
 - chromosomal deletions,
 - uni-parental disomy.

Information should also be included on:

- 2) Method of collection of samples, alternative methods:
 - buccal cells via saliva samples, cytology brushes or cotton swabs,
 - dried-blood spots (Guthrie cards),
 - blood samples.
- 3) Candidate genetic loci for OC – five overlapping categories:
 - genes expressed during palatogenesis with temporal and spatial specificity to clefting,

- chromosomal deletions, duplications or translocations causing OC,
- genes or loci identified in animal models,
- genes that possess or control specific biological activities that may explain orofacial clefting,
- genes at genome locations identified by genetic linkage.

5.1.8 Family history: recommendations for core information

Core information could be subdivided as follows:

- 1) **Minimal information (compulsory):**
 - for the family history include immediate family as first-degree relatives, i.e. grandfather and grandmother on both sides; all fathers' siblings and paternal first cousins; all mothers' siblings and maternal first cousins;
 - record malformations in the family;
 - design the questionnaire so that, to maintain confidentiality, nominal information will be collected but not computerized.
- 2) **Complete family history:**
 - a desirable option is to employ an interviewer, trained in family investigation, to obtain greater detail using a more complete, in-depth family-history questionnaire on both maternal and paternal sides, plus information on other CA and familial diseases.
- 3) **Blood samples:** Collect blood samples from relatives to enable genetic analysis to be performed, including:
 - all siblings, whether affected or not;
 - affected relative/s (other than parent or sibling);
 - blood sample/s from any relative/s in the affected branch of the family.

5.1.9 Bio-ethical issues: recommendations for core information

- 1) Include minimum data on legal requirements and guidelines with respect to informed consent, confidentiality and the principles of medical research espoused in the Declaration of Helsinki.
- 2) For multi-centre international collaborative research that involves genetics, specific areas of ethics and confidentiality need to be applied.