

COORDINATING CENTER and DATA COORDINATING CENTER PROTOCOL

The Center for Oral Health Research in Appalachia (COHRA), coordinated by Dr. Mary Marazita and Dr. Robert Weyant at the School of Dental Medicine, University of Pittsburgh, will characterize the oral health of approximately 700 families living in underserved populations in West Virginia and Western Pennsylvania. COHRA will evaluate the genetics of oral health by performing a variety of genetic linkage and association studies and dissecting gene-environment interactions, ultimately developing a DNA chip that will assess an individual's genetic predisposition to oral health problems. The University of Pittsburgh will serve both as the Coordinating Center and the Data Coordinating Center for COHRA.

SPECIFIC AIMS, BACKGROUND, AND SIGNIFICANCE

1. Specific Aims:

- (1) To ascertain a representative cohort of approximately 700 nuclear families from rural West Virginia, rural Bradford and Burgettstown, PA, and urban Braddock, PA, who have at least one child between the ages of 1 and 18, and who are underserved with regards to their dental care. A seven-year longitudinal assessment of oral health and health behaviors will be conducted on these families. Families will be assessed three times, every other year from their year of enrollment. To allow for attrition, COHRA will over-sample by 10% (ie., COHRA will enroll a projected 3850 individuals from 770 families).
- (2) To characterize the families clinically and genetically at each visit by performing: 1) several demographic, general-health, and oral-health interviews and questionnaires; 2) a standard dental screening (does **NOT** include x-rays), along with an oral microbiological examination in which tissue samples will be taken for bacteriological assessment; 3) venipuncture on all individuals, to obtain blood samples for DNA analyses (mouthwash samples, saliva samples, or cheek swabs will be an option for those unwilling to provide blood. Blood samples will only be taken once, unless additional blood is needed to obtain sufficient DNA for genotyping); and 5) collection of drinking water for analysis of fluoride content.
- (3) a comprehensive clinical genetics examination with emphasis on family structure and oral-facial dysmorphology. Blood samples were taken by venipuncture from individuals during visit 1 for chromosome analyses.
- (4) To perform genotyping of all family members for molecular genetic markers within 25-30 functional genetic loci known to play a role in oral health and behavior. Molecular markers will be developed for this purpose.
- (5) To perform several statistical genetic analyses, including 1) model-free linkage disequilibrium methods, e.g., the TDT and the quantitative TDT tests; 2) segregation analysis in the high-risk families; 3) both model-free and parametric linkage analysis; and 4) statistical analysis of gene-environment interactions.
- (6) To begin the development of a DNA chip, which will eventually include several hundred genes that are known or hypothesized to play a role in the growth of oral, dental, or craniofacial structures, and in behaviors that put individuals at risk for poor oral health.

2. Background

There are genetic, environmental and behavioral components to the expression of oral health status in all populations. For example, Sofaer (1993), Mandel (1994), and Townsend et al. (1998) have reviewed the genetic basis of dental caries. Furthermore, it is clear that there are interactions between these components and that each of these components may be transmissible (in a broad sense) within families.

Advances from the Human Genome Project and related projects have significantly changed the approach to studying the genetic basis of human disease. The genetic basis for differences in oral health, as for any complex disease susceptibility, is variation at the DNA level -- specifically, differences in the nucleotides at both coding and regulatory regions of genes (Chakravarti, 1999). These genetic variants are collectively termed "genetic polymorphisms." A rapidly growing body of data is emerging to identify and characterize specific genetic polymorphisms with variation in transcription, translation, and protein function (Mathew, 1999), and this is directly applicable to variation in oral health (Hart, Marazita, and Wright, 2000).

Significant advances have occurred in the basic methodologies available to acquire genetic information on an individual basis. Molecular genetic techniques now exist for rapid and efficient genotyping of hundreds of polymorphisms using small amounts of DNA (Beutler and Gelbart, 2000). However, such high-throughput genotyping methods must be selectively employed in order to minimize costs and maximize efficiency. The ultimate goal of this project is to develop an array of functional genetic polymorphisms that are related to various aspects of oral health, and to use these genetic polymorphisms to characterize individuals as to their genetic risk factors. To begin to develop such an array, we will focus on approximately 25-30 genes that are known to be important in oral health in the broad categories of growth factors and receptors, homeodomain genes, signaling and transcription factors, xenobiotic detoxification and metabolism, inflammation, and behavior. This array could ultimately be implemented via a high-throughput platform such as a DNA chip technology, or other emerging technology, for rapid and cost-effective genotyping.

3. Significance

The Appalachian population has never been characterized with respect to these categories of genes. Thus, successful completion of this project will: 1) provide the first systematic genetic characterization of an Appalachian population with respect to genes of known significance to oral health; 2) allow testing of specific genetic hypotheses regarding those genes and caries, as well as several other oral health outcomes; 3) allow comparisons between average- and high-risk families; and 4) lay the groundwork for development of a DNA chip that can allow quick and efficient tests of the genes determined to be important risk factors for oral health problems in Appalachia.

ORGANIZATIONAL STRUCTURE OF COHRA

The organizational structure of COHRA is diagrammed in Figure 1. COHRA is coordinated by Dr. Mary Marazita, Principal Investigator, and Dr. Robert Weyant, COHRA Director, at the University of Pittsburgh, School of Dental Medicine. Together, they assume overall responsibility for all aspects of the project. Dr. Marazita is the P.I. of the NIH research grant (1 R01 DE 14899 NIH/NIDCR) that funds this study. In addition to general administration

of COHRA, she employs a research staff that carries out numerous aspects of this project. These tasks include: 1) questionnaire and interview development; 2) overseeing shipment of blood, saliva, cotton cheek swab, and mouthwash samples for DNA analysis; 3) management of manual and automated data acquisition and entry processes; 4) maintenance of the computer local area network (LAN) to ensure data integrity and confidentiality; and 5) data analysis.

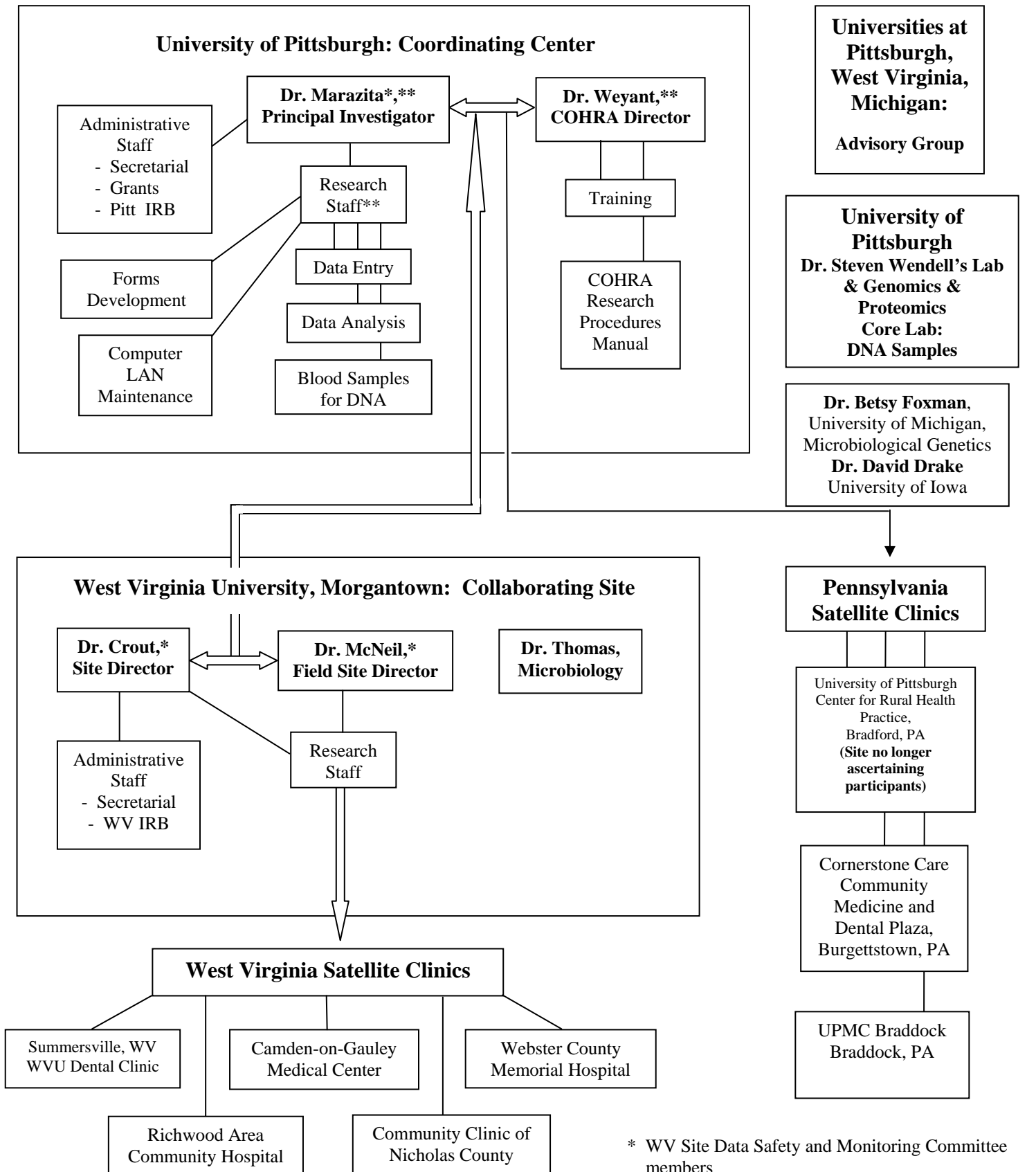
Dr. Weyant is responsible for data acquisition at all field sites and for training research personnel. In conjunction with Dr. Marazita, his staff has developed the research protocol, which is described in detail in the COHRA Research Procedures Manual (Appendix 1). Dr. Weyant also ensures that the protocol is carried out uniformly at the various field sites.

The field sites fall into two groups: 1) a major effort in collaboration with West Virginia University (WVU) at Morgantown, WV; and 2) supplemental field sites in Western Pennsylvania. The research effort in West Virginia is headed by Dr. Richard Crout, WV Site Director, and Dr. Daniel McNeil, Field Site Director, at the Dental School, WVU, Morgantown, WV. The other key member of the research team at WVU is Dr. John Thomas, who processes the microbiology samples. They will collect the bulk of the data (about 2200 individuals) from five field sites in Nicolas and Webster Counties in West Virginia—Camden-on-Gauley Medical Center, Community Clinic of Nicholas County, Richwood Area Community Hospital, West Virginia University Dental Health in Families Program Dental Clinic in Summersville, and Webster County Memorial Hospital.

Working closely with Drs. Marazita and Weyant, Drs. Crout and McNeil have hired and trained a COHRA research staff, consisting of a project coordinator, dentist/dental hygienist, and one dental assistant. A second staff member assists the rest of the research team as needed, and is also responsible for data entry in the field. Both Dr. Crout and Dr. McNeil supervise the staff in Morgantown, and closely monitor subject recruitment and the informed consent process. Dr. McNeil supervises the research staff in the field, ensuring that the protocol is followed correctly on site. Together, this research team is responsible for conducting the research protocol at five distinct satellite clinics in rural West Virginia.

Communication between Pittsburgh and West Virginia occurs on many levels. Drs. Marazita, Weyant, Crout, and McNeil communicate formally in a weekly teleconference, and informally as needed. The research staffs of both universities contact each other almost on a daily basis by both phone and email. Logs of all types of data samples are transferred between

Figure 1: Organizational Structure of COHRA



* WV Site Data Safety and Monitoring Committee members
 **Coordinating Center Data Safety and Monitoring Committee members (includes Mr. Lance Kennelly, University of Pittsburgh systems analyst)

sites continuously to track shipments. Computerized, coded questionnaires are sent securely via the internet, email, and mail.

The research effort in Pennsylvania is supervised directly by Dr. Weyant, and will ascertain about 1000 individuals. Together with Dr. Marazita, he has developed three satellite clinics in underserved areas of Appalachian Pennsylvania—the University of Pittsburgh Center for Rural Health Practice, Bradford, PA, the Cornerstone Care Community Medicine and Dental Plaza, Burgettstown, PA, and UPMC Braddock, Braddock, PA. Dr. Weyant has hired and trained part-time research personnel to conduct the protocol at each clinic. The staff at each site consists of a dentist and/or dental hygienist and at least one research coordinator. Dr. Weyant has trained the dentists to conduct the dental exam and take the appropriate samples for the oral microbiological exam. At least one of the research team is a trained phlebotomist, with experience in drawing blood from children.

Other key individuals include Dr. John Thomas at the West Virginia University who receives and stores all of the oral microbiological samples. Dr. Thomas also processes all of the samples except for the plaque samples. Dr. Betsy Foxman at the University of Michigan and Dr. David Drake at the University of Iowa, process the plaque samples. Dr. Steven Wendell at the University of Pittsburgh School of Dental Medicine processes the DNA samples.

Communication between the Pennsylvania sites and the University of Pittsburgh also occurs on many levels. Drs. Marazita and Weyant communicate formally with the research staff at each site in a monthly teleconference, and informally as needed. The research staffs at each site contact each other and the staff at the University of Pittsburgh as needed by both phone and email. Logs of all types of data samples are transferred from the sites to the University of Pittsburgh on a regular basis. Computerized, coded questionnaires are sent securely via the internet and email. Members of each research team send regular shipments via FedEx or UPS or make regular trips to Pittsburgh to hand-deliver hard copies of data and other relevant documents.

COHRA maintains an advisory group that has been instrumental in developing the overall research plan, and that monitors progress in data acquisition and analysis. In addition to Drs. Marazita, Weyant, Crout, McNeil, and Thomas, the advisory group includes Dr. Ralph Tartar, Dr. Brion Maher, Dr. Kathy Neiswanger, and Dr. Steven Wendell at the University of Pittsburgh, and Dr. Betsy Foxman, and Dr. David Drake. Ad hoc members of the COHRA advisory group include Dr. Debra Polk and Dr. Alexandre Vieira.

The organization to address data safety and monitoring issues will be discussed in detail in the Data Safety and Monitoring Plan.

SITES FOR SUBJECT ENROLLMENT/DATA COLLECTION

1. West Virginia

The majority of the families (80% or over 2000 people) are being collected at the five satellite clinics in rural West Virginia, under the auspices of the Dental School at West Virginia University in Morgantown (Federal Wide Assurance: FWA00005078). The five satellite clinics are located at the West Virginia University Dental Health in Families Program Dental Clinic in Summersville, the Webster County Memorial Hospital, the Camden-on-Gauley Medical Center, the Community Clinic of Nicholas County, and the Richwood Area Community Hospital (Figure 1). Each of these health care facilities is providing sufficient space for the research protocol,

including a room equipped with a dental chair and the equipment necessary for the dental screening, and access to computer ports for data entry and transmission. All research personnel are employees of WVU. The IRB at West Virginia University in Morgantown is responsible for the review of the protocol as it pertains to the West Virginia sites.

On clinic days, the research team from WVU drives to the appropriate clinic. Following the COHRA Research Procedures Manual (Appendix 1) the team operates the clinic. First they bring in scheduled family members for informed consent. Then they rotate family members through the dental, phlebotomy, and medical evaluation stations, and help them fill out the questionnaires and complete the interviews. All necessary research supplies are provided to each clinic by WVU. Questionnaire data is recorded onto Teleforms questionnaires for direct scanning into computer files or entered directly by subjects using PC Tablets, and then transferred to the computer system at the University of Pittsburgh.

The research team delivers coded oral microbiology samples to Dr. John Thomas at WVU, who analyzes and stores the samples. In some cases, these samples may also be shipped to Dr. Drake or Dr. Foxman, who are examining a small portion of these samples. The research team ships coded blood, mouthwash, saliva, and cotton swab samples for DNA analyses in biohazard bags at room temperature via overnight carrier to Dr. Wendell's lab, which is part of the Center for Craniofacial and Dental Genetics at the University of Pittsburgh, School of Dental Medicine. Alternatively, these specimens may also be shipped to Dr. Marazita at the University of Pittsburgh, who will then transfer them to the University of Pittsburgh Genomics & Proteomics Core Lab for processing and storage.

2. Pennsylvania

Approximately 20% of the families are being collected at the *two* satellite clinics in Pennsylvania, located at the Cornerstone Care Community Medicine and Dental Plaza in Burgettstown, PA and UPMC Braddock, Braddock, PA. (The University of Pittsburgh Center for Rural Health Practice in Bradford, PA, an original Pennsylvania satellite site, had also been ascertaining families until November 2007.) Each of these health care facilities is providing sufficient space for the research protocol, a room equipped with a dental chair and the equipment necessary for the dental screening, and access to computer ports for data entry and transmission. The research staff at each site are hired by Dr. Weyant and are employees of the University of Pittsburgh. They are employed part-time for COHRA, and work only at a single clinic. The IRB at the University of Pittsburgh is responsible for the review of the protocol for the three Pennsylvania sites.

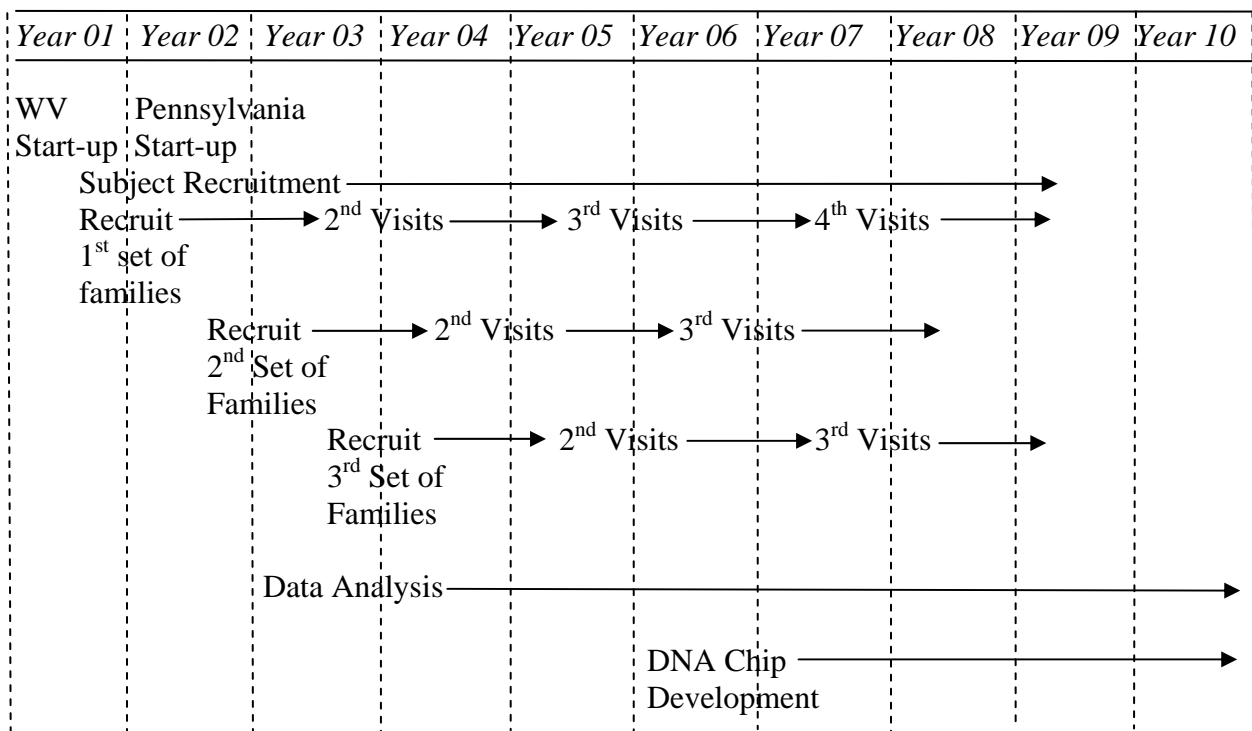
Clinics are held once a week at each site. The staff operates the clinics, bringing in scheduled family members first for informed consent, and then rotating them through the research stations. All necessary research supplies are provided to each clinic by the University of Pittsburgh. During the data collection protocols, questionnaire data are recorded onto Teleforms questionnaires for direct scanning into computer files or entered directly by subjects using PC Tablets, and transferred to the computers at the University of Pittsburgh.

The research teams ship the coded oral microbiology samples to Dr. Thomas at WVU, who logs them in and then ships them to Dr. Betsy Foxman at the University of Michigan or Dr. Drake at the University of Iowa for analysis. Finally, the research team ships coded blood, mouthwash, saliva, and cotton swab samples for DNA analyses in biohazard bags at room temperature via overnight carrier to Dr. Wendell's lab, which is part of the Center for Craniofacial and Dental Genetics at the University of Pittsburgh, School of Dental Medicine.

Alternatively, these specimens may also be shipped to Dr. Marazita at the University of Pittsburgh, who will then transfer them to the University of Pittsburgh Genomics & Proteomics Core Lab for processing and storage.

TIME LINE FOR STUDY

Below is the anticipated timeline for completion of the study. COHRA is a major initiative, which is currently funded for seven years. We anticipate renewal of funding, so as to complete the project as defined in the time frame given below. After the initial start-up period, we plan to bring in families every two years over a span of seven to eight and a half years. Data analysis and DNA chip development will begin during the period of family visits, and continue for two years after the data collection phase is completed, for a total study duration of ten years.



START-UP PROCEDURES

COHRA has been recruiting subjects in both West Virginia and Pennsylvania for well over a year, so that training and start-up procedures have already occurred. To summarize, research staff were hired first for the West Virginia initiative, and an extensive training/calibration protocol was followed. The COHRA Research Procedures Manual (Appendix 1) was developed by Dr. Weyant’s research staff. At the Dental School of WVU, Drs. Weyant and Crout trained the dental personnel on the Dental Screening Protocol. Control subjects had the dental screening performed by multiple research dental personnel, and the results were calibrated across clinicians. Dr. Weyant made initial visits to the field sites with Dr. McNeil until the protocol was completed several times. At that point Dr. McNeil accompanied the research staff to all field sites, and communicated continuously with Dr. Weyant on

procedural issues. Dr. McNeil continues to monitor the West Virginia field sites on a monthly basis. Calibration sessions for both the caries and periodontal examinations are being performed periodically during the two years COHRA has been collecting data.

Questionnaires were developed by the COHRA advisory group and implemented by Dr. Marazita's research staff. Many of the questionnaires are formatted using the Teleforms system, which allows data from the forms to be scanned directly into the computer. In addition, all of the self-report questionnaires have been programmed to be used on PC Tablets. This facilitates their completion by subjects with poorer reading skills, as well as simplifying data entry. During this period of training and forms development, Drs. Crout and McNeil developed the details of the recruitment procedures, trained the non-dental research staff, and worked extensively with the satellite clinics to ready them for the research effort.

The Pennsylvania satellite clinics were identified soon after the initial training was completed in West Virginia, and subject recruitment began in earnest. Start-up procedures followed those used in West Virginia. After the research staff for each site were hired, a multi-day training/calibration session was held in Pittsburgh. This session included the West Virginia team, so that the newer staff could be trained to the same standards as the WV staff. At the University of Pittsburgh, School of Dental Medicine, the dental screening was performed multiple times on control subjects, to insure that it was being consistently administered. Dr. Weyant made numerous trips to each Pennsylvania site to insure correct administration of the protocol. COHRA will follow these procedures to add the newest site—UPMC Braddock, an underserved urban site in Braddock, PA.

SUBJECT RECRUITMENT AND INCLUSION/EXCLUSION CRITERIA

1. Subject Recruitment

Households are being recruited from the 14,000 households in Nicholas and Webster counties, the 8,000 households in Burgettstown, PA and the area served by UPMC Braddock in Braddock, PA. Approximately 700 nuclear families will be recruited all together, over-sampled by 10% to allow for attrition. In addition, about 80 of these families will be expanded to include all available relatives. Participants will be both male and female, and will be aged 1 year and older. The justification for the inclusion of children in this study is provided in the COHRA site protocol (IRB #020773). The racial, gender, and ethnic characteristics of the proposed sample will reflect the demographics of Nicholas and Webster counties, West Virginia, and rural Washington/Greene counties and Bradford, PA, which are over 99.5% Caucasian, 0.0% African American, and less than 0.5% Other. The subjects recruited at UPMC Braddock will be over 70% African American, reflecting the demographics of urban Braddock, PA.

In West Virginia, there is a phased recruitment strategy, designed to evenly and easily recruit participants over the course of the study. Families who participated in the initial pilot projects have been contacted by mail to invite their participation. A variety of advertising methods are being utilized. IRB-approved flyers have been posted throughout the two counties, talks are being given to schools and community organizations, radio and television interviews are planned. Flyers and talks will also be used in similar fashion to recruit in Pennsylvania.

Family participation is voluntary and self-referred. When an individual calls to express an interest in the study, the primary caregiver in the family is designated the index case for the family. COHRA monitors these recruitment strategies by asking the individual where he/she heard about the study. The study is described to him/her, and he/she then determines if other

members of the family are interested in participating. If a family is interested, they return a phone call to the research team, to discuss the study in more detail and schedule an appointment. During this phone call, the index case is asked a series of questions (See Screening Interview for details). In the last section of the Screening Interview, the index is asked if anyone in the family has a pre-existing condition that requires taking prophylactic antibiotics before dental exams, has a reduced capacity to fight infection, or has a reduced ability to form blood clots (see Screening Interview, Part 4).

2. Inclusion/Exclusion Criteria

Families with at least one child between the ages of 1 and 18 who respond to flyers and talks are invited to participate. No exclusion criteria are based on race, ethnicity, or gender. Individuals with a neurological impairment, a severe physical or intellectual handicap, or psychosis will be excluded from the study. If it is determined that someone in the family may need prophylactic antibiotics, or may not be able to complete all portions of the dental exam due to a reduced capacity to fight infection or a reduced ability to form blood clots, a decision is made whether or not to exclude the family (See Screening Interview, Part 4). Households will be excluded if they contain an adult family member (parent) who needs to take a prophylactic antibiotic prior to a dental exam, but who refuses to do so. Children who are “at risk” and need antibiotic pre-medication for dental work will be excluded from any procedure that may cause bleeding. Households may also be excluded if they contain an adult or child who has a reduced capacity to resist infection (these include those individuals who have leukemia, cancer, are taking corticosteroids or immunosuppressive therapy, are HIV+, have unstable diabetes, or who have had a transplant) or the reduced ability to form blood clots (due to a blood clotting disorder or taking blood thinners). Adults and children who have a reduced capacity to resist infection or a reduced ability to form blood clots will be excluded from any dental exam procedures that may cause bleeding. Decisions will be made on a case-by-case basis before an ultimate decision is made to exclude the entire family. This decision is based on the premise that for the particular genetic analyses which COHRA will perform, each family needs to include at least one biologically related parent and child who can complete all age-appropriate portions of the dental exam.

Females of reproductive age are included in this study, and some of these women could be pregnant. The standard dental and oral microbiology exams and the small blood sample for the DNA analyses do not pose any extra risks for pregnant women.

PROTOCOL AND CONSENT FORMS

COHRA site protocols and consent forms have already been approved for use in West Virginia and Pennsylvania by the IRBs of WVU and the University of Pittsburgh. See Appendix 3 for a copy of the currently approved protocols and consent forms for West Virginia and Pennsylvania, along with the current IRB approval letters from both IRBs.

A template for the consent forms to be used at the Pennsylvania sites is provided in Appendix 4. This template follows the format and requirements of the University of Pittsburgh IRB. This template will be followed in the future at Pennsylvania sites, after it has been approved by the University of Pittsburgh IRB for each site.

The template provided in Appendix 4 cannot be followed precisely at the West Virginia sites, because of the requirements of the WV IRB. The WV IRB requires separate assent forms

for children aged 7 – 13 and 14 – 17, as well as a separate Parental Consent form. All consent and assent forms must be written in the first person, and simplified as much as possible. Adults are required to sign the consent form for adults prior to data collection. Parents who agree to the participation of their children aged 1 – 17 also sign a separate parental consent form. After the parental consent form is signed, children aged 7 - 17 discuss the study with the investigators, and sign one of the separate assent forms for children. After children give their assent, their parents then sign the assent form as well. Thus, the consent form for adults, the two assent forms for children aged 7 – 17, and parental consent form for children aged 1 – 17 must be kept separate at the West Virginia sites. This structure is required by the West Virginia IRB, and is also in keeping with West Virginia state law, which mandates that individuals under 18 may not sign consent forms.

DATA COLLECTION PROTOCOL: FORMS AND BLOOD/TISSUE SAMPLES

The research protocol is designed to take place during one visit (two may sometimes be necessary) to one of the five satellite clinics in Nicholas and Webster counties in West Virginia or in Bradford, Burgettstown, or Pittsburgh, PA. It should take about four hours or less for each participant. The dental and oral microbiology exams, phenotypic tests, and saliva and blood samples will be obtained specifically for the described research study, and will not be part of the participants' medical records. The protocol is described in detail in the COHRA Research Procedures Manual (Appendix 1). Refer to this manual for the complete set of data collection forms, as well as detailed descriptions of the protocols for collecting blood and tissue samples, instructions on how samples are stored and shipped, and various definitions of study parameters and lab values. To summarize, the protocol has four parts:

1. Interviews and Questionnaires

A series of interviews and questionnaires, currently approved by the University of Pittsburgh and WVU IRBs, is administered to family members, in order to obtain information about general demographics, oral health, medical and dental history, and habits related to oral health. The interviews for adult subjects (18 years and older) include a Demographics and Health History Interview (including an assessment of blood pressure, height, weight, and abdominal girth), an Oral Health History Interview, and several other Oral Health Questionnaires. Self-report questionnaires include the DUSI-R for alcohol and drug use and related behaviors, the Fagerstrom tests for smoking and smokeless tobacco use, the Fatalism Scale, the Health Locus of Control Scale, the Parental Supervision and Involvement Survey, the Family Assessment Measure, the ISEL, the West Virginia Identity Scale (West Virginia sites only), the Dental Fear and Anxiety Survey, the SF-36 Health Survey, the Perceived Stress Scale, and the Oral Health Impact Profile – 14. A childhood behavioral scale for parents to fill out about their children is included. Additionally, in a small random subset of adult subjects at the West Virginia sites (up to 100), we will assess the satisfaction and utility associated with computerized versus paper-and-pencil versions of the above described self-report instruments. Half of this subset will receive the computerized version of the self-report instruments and the other half will receive the paper-and-pencil version. The time taken to complete the questionnaires will be recorded with a stop-watch. Upon completion of the questionnaires, subjects will be asked to complete a brief Semantic Differential Scale, a brief Likert-type Satisfaction Scale, and the Wide Range Achievement Test (see Procedures Manual for

evaluation instruments). These additional evaluation instruments will take about 20 minutes to administer.

Children aged 14 – 17 essentially follow the adult protocol, except that parents or researchers help children answer the self-report questionnaires as needed, and the DUSI-R and ISEL for youth, as well as the Parental Supervision, Involvement and Discipline Survey for children is used. Children aged 11 – 13 use simplified forms, with the DUSI-R and the ISEL for youth omitted, and receive help with the self-report questionnaires as needed. Children aged 7 – 10 have a greatly reduced set of questionnaires, identical to the one for children aged 1 – 6, except that they answer some of the questions themselves. For children aged 1 – 6, parental informants answer the interviews for their children. (Time for interviews and questionnaires: 1 – 2 hours)

2. Dental and Oral Microbial Screening

The dentists and/or hygienists are trained to perform standard periodontal and caries assessments; the assessment is similar to a standard dental examination except that no x-rays are taken. The evaluation includes assessment of problems with dentition (tooth loss, caries, restorations, dentures, sealants, plaque, calculus, malocclusion, orthodontic appliances, traumatic injury, erosion, pain), the supporting structures (gingivitis, periodontal destruction, periodontal microbiota, bleeding on probing, strep mutans, pain), and soft tissue (oral mucosal lesions, malformations, salivary gland function, pain). Patients will not have eaten or brushed their teeth for at least two hours before this screening. The periodontal exam is only performed on adults (ages 18 and up). After obtaining informed consent and prior to conducting the Dental and Oral Microbial Screening, each participant is administered the COHRA Antibiotic Screening Interview (adult or child version). This interview consists of questions that the index case was originally asked to answer about the entire family during the above mentioned Screening Interview, Part 4. During the COHRA Antibiotic Screening Interview (adult or child version), each individual is asked about any pre-existing conditions they may have requiring them to take antibiotics before dental exams, reduced capacity to resist infection, or reduced ability to form blood clots. This extensive protocol has been developed to identify these individuals and either offer them prophylactic antibiotics (when appropriate) or exclude them from those parts of the dental screening that can cause bleeding (see section Procedures to Minimize Potential Risks and Antibiotic Screening Interview (adult & child versions)).

For the oral microbial exam, the dentist collects interproximal plaque using stimudent, subgingival and supragingival plaque by scaling the teeth, and scrapes the tongue and takes a throat swab. Saliva is collected by a standard spatule assay. To collect additional saliva, subjects aged 4 and older are asked to spit in a vial for salivary flow rate determinations. Tobacco levels are determined utilizing the level of salivary cotinine, a major metabolite of nicotine. For children aged 1 – 3, an abbreviated lift-the-lip exam is conducted in lieu of the dental exam. Any significant dental health problems are reported back to the family members, along with appropriate referrals. (Time for dental screening: 1½ hours)

3. Sample Collection for DNA Analysis

The purpose of the intended genetic research is to identify genetic factors involved in oral health. Supplies for the phlebotomy, mouthwash, saliva collection, or cotton swabs are purchased by the COHRA research staff at the University of Pittsburgh. They are shipped via overnight carrier to West Virginia University as needed. The research coordinator at WVU hand

delivers these supplies to each field site in West Virginia. Supplies are shipped directly to each Pennsylvania site when they are purchased. To collect cells for the DNA analyses, trained and experienced phlebotomists draw peripheral venous blood samples (approximately 1½ teaspoons or 7.5 ml) by venipuncture from all family members aged 1 and older. For family members who are unwilling to provide a blood sample, saliva samples, mouthwash samples or cotton swabs (a total of 6 cotton swabs per person) are taken. This part of the protocol will be performed only once, unless the first sample fails to yield sufficient DNA for the genetic analyses. Thus, a total of 7.5 ml blood for DNA analysis is taken from all willing participants. For very small children, a formula of 2 ml blood per kg weight is applied to determine the amount of blood to draw, and if this amount is less than 10 ml blood, the smaller amount of blood will be taken. (Time for sample collection: 5 minutes)

4.) Water Sample Collection for Fluoride Content Analysis

Prior to the study visit, subjects will receive a small vial to collect drinking water via mail. Subjects will be asked to provide a sample of their drinking water at their upcoming clinic visit. See the instruction sheet provided in the COHRA Research Procedures Manual (Appendix 1). Water samples will be shipped to Dr. Steven Wendell's lab where analysis for fluoride content will be performed. Water samples will then be discarded.

STUDY RISKS

1. Potential Risks

For most subjects, the clinical examinations and questionnaires create no risks beyond brief, mild discomfort and tedium, respectively. However, one risk unique to an oral periodontal examination is the possible induction of a bacteremia during periodontal probing, subsequently resulting in a systemic infection. Two types of infection are of particular concern. The first is bacterial (infective) endocarditis for patients with a history of previous bacterial (infective) endocarditis, certain specific and serious congenital heart conditions, or artificial heart valves (Dajani et al., 1997 & Prevention of Infective Endocarditis – Guidelines from the American Heart Association, 2007). The second is infections associated with artificial joint replacement (American Dental Association, 1997), as well as those subjects with a reduced capacity to resist infection (including individuals who have leukemia, cancer, are taking corticosteroids or immunosuppressive therapy, are HIV +, have unstable diabetes, or who have had a transplant). Also, subjects with blood clotting disorders in which they have reduced ability to form blood clots and subjects who are taking blood thinners could be at risk for mild, but prolonged bleeding during dental exam probing procedures. If at-risk adult subjects choose not to have the periodontal exam, they and their families may be excluded from the study. Children are not participating in the periodontal exam.

There are no risks associated with the collection of a saliva sample. Risk of local bruising or pain and rarely, fainting or infection, is sometimes associated with the venipuncture required to obtain blood samples, as is psychological distress. There is also the rare, potential risk of the inappropriate release of confidential information. In the unlikely event of a breach of confidentiality, there is a very remote possibility that the genetic information could affect a subject's ability to be insured, employed, their family relationships. However, the data collected by this study only consist of oral health indicators present in all individuals to some degree and random genetic marker data that are not known to cause disease. Thus, even if the genetic and

phenotypic data from this study were to be made generally known, the risks delineated above are so low as to be negligible. There are no risks associated with collection of drinking water for analysis of fluoride content.

2. Procedures to Minimize Potential Risks

To minimize the risk for infection resulting from the periodontal exam, care will be taken with all subjects to perform the periodontal segments of the exam with as little tissue trauma as possible. Only trained dentists or dental hygienists will perform the periodontal exam. Furthermore, the periodontal exam will only be performed on adults aged 18 and older. Subjects will be excluded from those parts of the dental screening that can cause bleeding if they have a reduced capacity to resist infection (these include those individuals who have leukemia, cancer, are taking corticosteroids or immunosuppressive therapy, are HIV +, have unstable diabetes, or who have had a transplant) or the reduced ability to form blood clots (due to blood clotting disorders or taking blood thinners). The literature suggests that less than 4-5% of the population will present with indications for antibiotic prophylaxis. The Prophylactic Antibiotic Screening Interview will ensure that at-risk subjects are provided antibiotic coverage. Adult patients who have a history of previous bacterial (infective) endocarditis, certain specific and serious congenital heart conditions, or artificial heart valves (Dajani et al., 1997 & Prevention of Infective Endocarditis – Guidelines from the American Heart Association, 2007) will be offered antibiotic premedication or they will rescheduled until a letter can be sent to their physician after the patient provides authorization to determine the need for premedication. After they sign informed consent for the study, adults will be screened initially to see if they are in this “at-risk” group. At-risk subjects will be asked to obtain or offered the American Heart Association's recommended oral prophylaxis regimen of amoxicillin (2g) or--for those who are allergic to penicillin-based antibiotics--clindamycin (600mg), azithromycin (500mg), or clarithromycin (500mg) one hour before their dental exam (Dajani et al., 1997 & Prevention of Infective Endocarditis – Guidelines from the American Heart Association, 2007), or they will be excused from the periodontal probing exam. They will be informed of possible medical risks associated with taking these antibiotics (Guggenheimer et al., 1998). The possible side effects associated with amoxicillin include headaches, mild nausea, vomiting, oral or vaginal candidiasis (> 10%), skin rashes, dermatitis, wheezing, allergic reactions (1% to 10%), seizures, blood disorders, jaundice, liver or kidney problems (< 1%). The possible side effects associated with Clindamycin include nausea, vomiting, diarrhea, abdominal pain (< 10%), and decreased blood pressure, rashes, skin reactions, blood disorders, joint or muscle inflammation (1% to 10%). The primary adverse effects observed with azithromycin in clinical studies to date have been wheezing (17%), diarrhea (5%), nausea, vomiting, & abdominal pain (3%). Other possible, but more rare side effects associated with azithromycin include: central nervous system effects (vertigo, somnolence, hyperkinesia, agitation, nervousness, & insomnia (1.3%)); cardiac dysrhythmia, chest pain & palpitations (<1%); and there have been a few reports of elevations in liver enzymes and pruritis, rash, urticaria, & rarely, serious skin reactions (Micromedex – Drugdex evaluation for azithromycin). The primary adverse effects observed with clarithromycin include: taste disturbances (13%); epigastritis, nausea, and vomiting (5.8%); diarrhea (3%); dyspepsia, abdominal pain, & headache (2%); increased prothrombin time (1%); elevations in liver function tests and decrease in white blood cells (<1%); and rare cases of anaphylaxis, Stevens-Johnson syndrome, QT prolongation & ventricular arrhythmias, leucopenia

& neutropenia, and severe, but rarely fatal, hepatocellular and cholestatic hepatitis, with or without jaundice, have been infrequently reported. (Micromedex – Drugdex evaluation of clarithromycin). Amoxicillin and clindamycin will continue to be the first choice of medications for those requiring antibiotics, with azithromycin and clarithromycin as alternatives for prophylactic antibiotics. The above mentioned antibiotics are FDA approved and commercially available. At-risk children will not be subject to any part of the dental or microbiological exam that might cause bleeding.

Only qualified medical personnel will draw blood samples. All personnel coming into contact with participants in this study will be from West Virginia or Pennsylvania, and will have previous experience in contacting subjects and in protecting confidentiality. If a dental problem is discovered, appropriate referrals will be made.

DATA SAFETY AND MONITORING PLAN

In order to assess the safety of the research subjects and the confidentiality of the data on an ongoing basis, COHRA has implemented the following Data Safety and Monitoring Plan. It is designed to monitor data safety for the entire project, as well as the specific Pennsylvania sites; West Virginia University has a site-specific Data Safety and Monitoring Plan that has been approved by the WV IRB. This plan also covers the specific needs of the Data Coordinating Center. The Data Safety and Monitoring Plan is as follows:

1. Composition of the Data and Safety Monitoring Committees

There are two Data Safety and Monitoring Committees: 1) a WV Site Committee to monitor data safety and confidentiality issues at the West Virginia field sites; and 2) a Coordinating Center/Site Committee, which directly monitors data safety and confidentiality at the Pennsylvania sites, as well as coordinates data safety and confidentiality issues for the entire project. The West Virginia Site Committee consists of Drs. Mary Marazita and Robert Weyant at the University of Pittsburgh, and Drs. Richard Crout and Daniel McNeil at WVU. They directly monitor data safety and confidentiality at the West Virginia sites. The Coordinating Center/Site Committee consists of Drs. Mary Marazita and Robert Weyant; Mr. Lance Kennelty, a systems analyst at the University of Pittsburgh; and Judith Resick, a Research Program Manager at the University of Pittsburgh. The Coordinating Center Committee directly monitors data safety and confidentiality at the Pennsylvania sites, as well as oversees the West Virginia DSM Committee. Based on a continuing assessment of the protocol as it is being conducted at all field sites, it also oversees the study design safeguards and protocol modifications or changes in the research risk level, if necessary.

2. What Is Monitored?

Each committee monitors subject safety and data confidentiality issues at their respective sites. They review the status of the outcomes and any adverse events. (See #3 below for more detail on adverse events.) The security status of the database and participant files is reviewed, including random checks to be sure files are locked, databases are secure, and backup procedures are operational. The committee members convey any new developments in the genetics of oral health that may impact the safety or ethics of the study. Each committee completes the DSM Sheet for their respective sites at their regularly scheduled meetings (See Appendix 5 for the DSM sheets). Through Dr. Marazita, the Coordinating Center DSM Committee is kept abreast of any data safety or security issues in West Virginia.

3. Adverse Events

In West Virginia, adverse events will be handled initially by Drs. Crout and McNeil. They will determine the circumstances of the event and make sure that the COHRA Adverse Event Form is completed within 24 hours. They will also contact Dr. Marazita or Weyant as soon as is feasible, and fax a copy of the Adverse Event Form to the University of Pittsburgh. They will report serious or unexpected adverse events occurring in West Virginia to the WV IRB, following the guidelines of the West Virginia IRB. For the Pennsylvania field sites, adverse events will be handled directly by Drs. Marazita and Weyant, who will make sure that the Adverse Event Form is completed within 24 hours. The COHRA Coordinating Center DSM Committee will report serious or unexpected adverse events occurring at any site that are deemed to be related to the research intervention, including major disputes between a research subject and the researchers, to the University of Pittsburgh IRB following the guidelines detailed in Sections 3.4 and 3.5 of the IRB Reference Manual. (See Appendix 5 for the COHRA Adverse Event Form.)

4. Monitoring Procedures

Data safety and monitoring issues are specifically addressed and updated during scheduled teleconferences. To insure that the standards and protocols are being followed properly on site, Dr. McNeil accompanies the research staff to each satellite clinic in West Virginia at least monthly, while Dr. Weyant visits the clinics in Pennsylvania. During these visits, Drs. McNeil and Weyant observe the dental screenings, make suggestions as needed, and answer staff questions pertaining to any yearly. Judith Resick, Research Program Manager will visit the Pennsylvania sites one to two times per year, as well. Dr. Marazita monitors the quality of the oral microbial samples with regular calls to Drs. Thomas', Foxman's, and Drake's labs, and periodic on-site audits. Hard-copy data are transferred from all field sites by hand, and personally delivered to the data management teams at each university. Electronic transfer of data includes only identifier codes, and is monitored for completeness by the systems analysts at the University of Pittsburgh. The quality and completeness of the interview and questionnaire data is monitored on an ongoing basis when the data are entered at the University of Pittsburgh; problems are handled by phone and email, using only subject identifier codes.

5. Frequency of Monitoring

Each committee communicates quarterly via conference call or face-to-face meeting to formally monitor and update the safety of the research subjects. However, teleconference calls are scheduled on a weekly and bi-weekly basis, so that if any issues arise relating to data safety and monitoring, they are handled informally as needed. If an adverse event occurs at any West Virginia or Pennsylvania site, Dr. Marazita will be notified at once.

6. Reporting to the IRB

At COHRA's annual renewal, Dr. Marazita will generate a report that summarizes the following information for both committees: 1) dates of phone conferences and site visits; 2) the number of subjects who were recruited and completed the protocol; 3) the number of adverse events. For any adverse event, a discussion of the how the experimental intervention caused the event will be included; 4) a list of current scientific literature impacting the safety and ethics of the research, and whether changes should be implemented based on this literature; 5) a summary of the procedures used to ensure subject privacy and research data confidentiality; 6) a conclusion as to whether the risk/benefit ratio has changed, and if there is a need to change the study or terminate it.

STUDY BENEFITS

In this study, all participants receive a dental screening examination. This qualifies as a direct benefit because a large proportion of the Appalachian population (about 20%) have either never seen a dentist or have not seen a dentist for more than five years. Although we do not provide clinical services as a part of the study, we do provide a direct referral to community based providers who have agreed to see our subjects, and we indicate the degree of urgency in seeking such care. In the years that this study has been taking place, we have had a handful of subjects who were “urgently” referred due to soft tissue changes consistent with oral cancer that did indeed turn out to be oral cancer (those subjects reported to us, grateful for our referral that led to clinical detection of the cancer). Furthermore, the research protocol may well have a direct impact on participants’ long-term oral health, since it raises their awareness about the importance of regular oral health care.

Other potential benefits that might arise from characterizing loci for oral health problems are many and far-reaching. Understanding the mechanisms underlying poor oral health may eventually lead to improved methods of treatment and ultimately to its prevention. The proposed study presents minimal risk to the human subjects involved, and the potential risks are far outweighed by the potential benefits that might arise from a better understanding of the genetics of oral health problems.

COST AND PAYMENTS TO STUDY SUBJECTS

1. Costs

There are no costs for participating in COHRA, except in the case that the subject requires a prophylactic antibiotic. In this case, subjects who chose to participate in the study and obtain the prophylactic antibiotic will pay for this out of pocket or have it covered by their insurance, if applicable. However, any out-of-pocket expense that the subject incurs will be reimbursed.

2. Payments

Participants are reimbursed \$25 for each visit. A visit occurs every 2 years for a total of 3 or 4 visits. If the protocol can not be completed at one appointment, each subject will receive an extra \$25 compensation when they return to complete the protocol. If all members of the household complete all phases of the protocol, an extra \$100 will be provided to the parent who originated the contact (index case). To reduce the possibility of parents coercing their children to participate, children will be paid via a gift certificate (e.g., WalMart) made out in the child’s name. Adults will also have the option of receiving an immediate certificate or a check to be received at a later time. These payments will be repeated at each visit over the seven-year study. Thus, payments could potentially total over \$400 dollars per family over the course of the study, depending on the total number of visits. Subjects will also be given the opportunity to gain an additional \$25 for each family referral that leads to participation by a new household. At the subjects’ request, subjects will be reimbursed for any personal expenses they accrue for during their participation in the study, including out of pocket expenses for the prophylactic antibiotic, parking, transportation, or a meal.

DATA COORDINATING CENTER RESPONSIBILITIES

Dr. Marazita is responsible for data management and analysis. The Data Coordinating Center will be kept in her extensive research suite located at the Cellomics Building/Bridgeside Point. Here she will store the hard-copy data, maintain a secure computer local area network (LAN), and supervise a research staff that designs data entry forms and databases, enters and cleans up data, and coordinates data transfer and shipment of blood samples among sites. All statistical and genetic analyses will be performed here as well. The responsibilities of the Data Coordinating Center include:

1. Quality of Interview/Self-Report Questionnaire Data

In general, the interviews and self-report questionnaires used by COHRA are standard instruments that have been extensively validated and used in different research contexts. Thus, they are already known to yield consistent results in the hands of multiple interviewers. Training on the administration of these questionnaires was provided at the early start-up sessions for both the West Virginia group and the field staff at the Pennsylvania sites.

Interviews and questionnaires have been developed in three formats: 1) Microsoft Word or Excel documents filled out by research staff or subjects that are manually entered into computer databases maintained by Dr. Marazita at the University of Pittsburgh. This format is being phased out of the study, and will be used only for accessory forms, e.g., tracking logs; 2) Interviews formatted with a Teleforms system that are completed by research staff at each site and scanned directly into the computer LAN at the University of Pittsburgh with a dedicated scanner; and 3) Self-report questionnaires programmed to run on PC tablets, in which the data are entered directly by research subjects into files that are uploaded to the Pittsburgh LAN on a daily basis, with electronic copies provided to WVU.

Unless a subject requests otherwise, questionnaire and interview data will be stored indefinitely, for at least five years after the completion of the study.

2. Quality of Tissue/Blood Samples

Plaque samples are coded and delivered via overnight mail to the microbiological laboratory of Dr. John Thomas at WVU, Dr. David Drake at the University of Iowa, or to the Center for Molecular and Clinical Epidemiology of Infectious Disease at the University of Michigan, Dr. Betsy Foxman, Director, for microbiological analysis. Blood, saliva, mouthwash, and cotton swab samples for DNA analyses are coded, placed in biohazard bags at room temperature, and shipped via overnight carrier to Dr. Wendell's lab, which is part of the Center for Craniofacial and Dental Genetics at the University of Pittsburgh, School of Dental Medicine. Alternatively, these specimens may also be shipped to Dr. Marazita at the University of Pittsburgh, who will then transfer them to the University of Pittsburgh Genomics & Proteomics Core Lab for processing and storage.

Dr. Marazita will have control over the blood, saliva, mouthwash, and cotton swab samples. DNA samples will be stored with coded laboratory ID's in the freezers of Dr. Steven Wendell's Lab and the University of Pittsburgh Genomics & Proteomics Core Lab. If a subject provides their additional consent, their biological samples and DNA will be saved indefinitely for future testing of newly identified factors involved in oral health. Any remaining biological samples and DNA will be destroyed at such time that all of Dr. Marazita's research studies of the genetics of oral health are completed. However, if adult subjects provide a second additional consent to be recontacted, their DNA, with coded lab IDs, will be kept available for secondary

researchers. If subjects do NOT provide these two additional consents, their biological samples and DNA will be discarded at the end of this specific research study. No information permitting personal identification of family members will be made public or published.

Subjects will be free to drop out of the study at any time. Non-participation will not affect their ability to obtain other medical or dental treatment. Should a subject withdraw from the study, the data collected up to that point in time will be kept with identifier codes, while the blood samples and other specimens will be destroyed (See IRB approved consent forms, Appendix 3).

3. Procedures to Ensure Subject and Data Confidentiality

Data integrity for the entire project is monitored by Drs. Marazita and Weyant, while Drs. Crout and McNeil monitor data integrity in WV. At both universities, hard copies are kept in locked file cabinets, and computerized data are kept in secure databases. Physical specimens and data sheets are coded during the clinics, so that only coded identifiers are present on samples delivered to labs for analysis. Data safety and monitoring is accomplished as described in the Data Safety and Monitoring Plans for each site, including a section on prompt review and resolution of adverse events.

Drs. Marazita supervises two systems analysts who maintain a secure computer LAN at her research site containing the COHRA databases. The LAN has multiple security precautions, e.g. firewalls, anti-virus programs, and password protections—that are continuously updated to maximally protect the data. Further, there are confidentiality agreements signed by all individuals with access to the databases. Data are transferred to Dr. Marazita's site either directly by hand, or sent via the internet in files with only coded identifiers. Data are scanned for completeness and accuracy at the time they are entered. The automated data entry procedures for the Teleforms data and the PC Tablets contain checks for data completeness; note fields are reviewed manually.

COHRA employs an extensive system of identifier codes to keep track of paper, electronic, and physical samples (See COHRA Research Procedures Manual). In West Virginia, paper questionnaires that have already been completed are stored at West Virginia University in locked file cabinets, with subject identifiers secured by Dr. Crout. Each of the paper data forms has a front page with subject identifiers. Before sending the data to Dr. Marazita, the front page is removed, leaving only coded identifiers on the data for the research staff at the University of Pittsburgh. For the five West Virginia satellite clinics, WVU maintains the key between COHRA identifier codes and individual identities. Only staff who interact directly with research subjects have access to both participant names and codes. All other personnel who use the data access subjects only through their identifier codes. Interviews and self-report questionnaires in either the Teleforms or the PC Tablet formats are stored, with coded identifiers, on the computer LAN at the University of Pittsburgh; the actual subject identifiers are maintained at WVU.

For the Pennsylvania field sites, questionnaire data, and data from the dental and oral microbiology exams and the medical genetics evaluations are stored by Dr. Marazita at the University of Pittsburgh. Subject identifiers and the identifier codes are kept in locked file cabinets at each field site. Hard copy data with identifiers and identifier codes are transported by hand from the field sites to Dr. Marazita's research suite at the University of Pittsburgh, where they are stored in locked file cabinets. Electronic data are stored with identifiers and coded identifiers on Dr. Marazita's computer LAN at the University of Pittsburgh. Participants' names are protected by assigning anonymous identification numbers; participants' addresses are protected by assigning anonymous geo-codes.

Together, these procedures are design to minimize the risk of a breach of confidentiality. To date, there have been no breaches of confidentiality at any of the research sites; thus, with continuous monitoring, the risk to research subjects from a breach of confidentiality is considered very low.

4. Data Analysis

With input from the COHRA Advisory Group, Dr. Marazita will analyze the data statistically. In addition to standard, statistical summaries and GIS (geographical information system) analysis of the data, she will use parametric and non-parametric linkage analyses, segregation analysis of familial transmission patterns, association analyses (e.g., TDT) between genetic markers and detailed oral health phenotypes, and statistical analyses of gene-gene and gene-environment interactions. One major orofacial disease outcome of interest is caries, and caries will therefore provide a major focus for the genetic studies outlined in this proposal. However, similar strategies will pertain for the other orofacial phenotypes assessed on the study subjects.

Either Dr. Marazita, Dr. Alexandre Vieira, or Dr. Steven Wendell will perform the molecular genetic analyses for this project. They will analyze markers from about 25-30 genes in the broad categories of growth factors and receptors, homeodomain genes, signaling and transcription factors, xenobiotic detoxification and metabolism, inflammation, and behavior, many of which may play a role in oral health. Dr. Marazita, Dr. Alexandre Vieira, and Dr. Wendell will analyze all of the molecular genetic data. Any positive results will serve to identify specific genetic polymorphisms associated with risk for poor oral health, as the first step in generating a DNA chip for genetic risk factors involved in oral health. None of this genetic information will be reported to family members, since Dr. Marazita's, Dr. Vieira's, and Dr. Wendell's labs are research labs, and not authorized by CLIA to provide genetic information to research subjects or family members.

QUALIFICATIONS OF INVESTIGATORS

Lindsey Cohen, Ph.D., is an Assistant Professor in the Department of Psychology at Georgia State University. He is an expert in pediatric pain and will collaborate with Dr. McNeil in analyzing psychological data.

Richard Crout, D.M.D., Ph.D., is the Associate Dean for Research and Professor of Periodontics at West Virginia University. Dr. Crout provides the executive and administrative oversight of the subject recruitment efforts. He will also assist Dr. McNeil with the field implementation of the research protocol, maintaining staff and subject safety and IRB compliance, and will specifically oversee the clinical dental protocols.

Geri Dino, Ph.D., is Associate Professor, Director of the Center for Healthy Communities, and Director of the Prevention Research Center at the Department of Community Medicine at West Virginia University. **Kimberly Horn**, Ed.D. is a Robert C. Byrd Associate Professor in the Department of Community Medicine at West Virginia University They will be helping to develop a tentative multi-level "ecological" model. This model will include the analysis by predictor level-individual, family, community to help guide analytic procedures. These include hierarchical modeling, mediator-mediator analyses as it particularly pertains to obesity. Smoking will also be a main area of focus with the high rates of use in WV.

David Drake, Ph.D., is a Professor of Microbiology Research at the Dows Institute for Dental Research at the University of Iowa. He will perform analyses of the oral bacterial samples and advise COHRA on the microbiological aspects of the study.

Thomas Elliott, Ph.D., is a Professor in the Department of Microbiology, Immunology & Cell Biology at West Virginia University. **Slawomir Lukomski**, Ph.D., is an Associate Professor, Department of Microbiology, Immunology, and Cell Biology at West Virginia University. They have extensive experience in molecular microbiology, and will work with Drs. Olson and Cuff in the design/development of methodologies and analysis approaches for a microbiology sub-study of oral biofilms using selected COHRA microbial samples.

Robert Ferrell, Ph.D., is Professor, Department of Human Genetics and has more than 25 years experience studying the role of genes in common diseases, including infectious diseases. He is expert in determining genetic factors that influence interindividual differences in disease susceptibility.

Betsy Foxman, Ph.D., is the Director of the Center for Molecular and Clinical Epidemiology of Infectious Diseases, and Professor of Epidemiology at the University of Michigan in Ann Arbor. She is a molecular epidemiologist with extensive experience in molecular bacteriological genetics. She will perform the genetic analyses of the oral bacterial samples and advise COHRA on the microbiological aspects of the study.

Stephanie Frisbee, M.Sc., is a research instructor in the Department of Community Medicine with affiliations in the Center for Interdisciplinary Research in Cardiovascular Sciences, and the Center for Lung Biology and Respiratory Disease at West Virginia University. She has extensive experience coordinating national multi-site studies, health services and clinical outcomes research, and in outcomes research methods and evaluation. Ms. Frisbee will be responsible for statistical analyses.

Elodie Ghedin, Ph.D. is an Assistant Professor in the Division of Infectious Diseases. The underlying theme in her research is the genomics of infectious agents, particularly parasitic nematodes, parasitic protozoa, and RNA viruses. During her time at The Institute for Genomic Research (TIGR) Dr. Ghedin led the Viral Genomics group, working on the design of high throughput pipelines for virus discovery and characterization. Her laboratory was the first to produce an efficient and rapid workflow for the systematic decoding of complete influenza genomes. Ongoing projects in her laboratory presently include virus detection in chronic diseases such as juvenile-onset diabetes using a metagenomics approach, gene structure characterization in a filarial nematode by motif recognition, and the study of intra-host diversity and evolutionary dynamics in influenza virus. In this project, Dr. Ghedin plans to use her expertise in molecular biology and genomics to process oral samples for sequencing using a metagenomics approach. Viral and bacterial fractions will be separated by filtering and centrifugation. Sequencing data from viral whole genome shotgun and bacterial ribosomal RNA will be assembled and analyzed by Dr. Ghedin's bioinformatics team.

Marybeth Hummel, M.D., is an Associate Professor of Pediatrics at WVU, and a board-certified pediatrician and clinical geneticist with over 14 years experience in counseling families with genetic disorders. She had been responsible for supervising the genetic counselor, regarding cytogenic analyses results, on this study in years 1 and 2 of the project, but will now only be involved in the publications resulting from the study.

Suresh Madhavan, Ph.D., is a Professor of Pharmaceutical Systems and Policy at the WVU School of Pharmacy and Professor of Community Medicine at the WVU School of Medicine. Dr.

Madhavan has a great deal of experience in analyzing large databases and as a co-investigator on several research studies. He will be providing input as we look at dental utilization.

Brion Maher, Ph.D., is an Assistant Professor at the Medical College of Virginia. He is a statistical geneticist with extensive experience in genetic analysis of complex disease, in particular, psychiatric and behavioral genetics. He will perform the genetic/family analyses of both the medical and the behavioral data.

Mary L. Marazita, Ph.D., F.A.C.M.G., is the Associate Dean of Research and Head of the Division of Oral Biology at the University of Pittsburgh, School of Dental Medicine. She is board-certified in Medical Genetics and is a Founding Fellow of the American College of Medical Genetics. She has been involved in research on clefting and other craniofacial anomalies since 1980, and has many publications in the area. As Principal Investigator, Dr. Marazita will assume overall responsibility for the management of this study, be responsible for maintaining the data from the Pennsylvania sites, and will also perform the statistical genetic analyses of the data generated in West Virginia and Pennsylvania.

Furthermore, she will supervise and coordinate the molecular genetic aspects of the project (extracting DNA and candidate gene genotyping).

Chris Martin, DDS, MS, is an Assistant Professor in the Department of Orthodontics at West Virginia University. Dr. Martin will continue to evaluate orthodontic changes in young children. He has helped in the initial analysis (paper under review by the Journal of the American Dental Association) and the development of the technique of analysis. As children are seen every two years, a continuation of the needs and desires of this Appalachian population for orthodontic care will be expanded and continued.

Suzanne McGeary, D.M.D is a research dentist at the Burgettstown, PA COHRA site.

Daniel McNeil, Ph.D., is an Associate Professor of Psychology at West Virginia University. He will oversee and implement the research protocols in the field. He will be responsible for the integrity of all protocols and data collection.

Kathy Neiswanger, Ph.D. is a full-time Research Assistant Professor in the Center for Craniofacial and Dental Genetics at the School of Dental Medicine, as well as a board-certified Ph.D. medical geneticist. She will assist Dr. Marazita in managing the day-to-day aspects of the study for the Pennsylvania sites.

Joan Olson, Ph.D., is an Associate Professor in the Department of Microbiology, Immunology & Cell Biology at West Virginia University. **Christopher Cuff**, Ph.D., is an Associate Professor and Director of the Flow Cytometry Core Facility at the West Virginia University School of Medicine. Together, they will coordinate a microbiology sub-study of oral biofilms using selected COHRA microbial samples.

Louise Platt-Schulhof, RDH, MHPE, CHES is a research dental hygienist and coordinator at the Burgettstown, PA and Braddock, PA COHRA sites.

Deborah E. Polk, Ph.D. is an Assistant Professor of Behavioral Sciences in the School of Dental Medicine at the University of Pittsburgh School of Dental Medicine. She will perform some analysis of demographic data and participate in advisory group planning.

Usha Srinivasan, Ph.D., is a research investigator in epidemiology at the University of Michigan School of Public Health. She has extensive experience in microbiology and epidemiology and will work with Dr. Foxman in analyzing some of the project's microbiological samples.

Ralph Tarter, Ph.D., M.P.A., is a Professor of Pharmaceutical Sciences and Psychiatry at the University of Pittsburgh. As Director of the University of Pittsburgh Center for Education and Drug Abuse Research, he has extensive experience in assessing, evaluating and analyzing health

behaviors, especially under longitudinal designs. He will help develop methods of incorporating the health behavior phenotypes into the genetic analyses.

John Thomas, Ph.D., H.C.L.D. (ABB), is a Professor in the Department of Pathology of the School of Medicine at West Virginia University. As Director of the WVU Hospital and the Oral-Facial Microbiology Laboratories, he has extensive experience in research and clinical analysis of microbiological samples. He will store and analyze the plaque, throat swab, and saliva samples for microbiological organisms.

Stephen Thomas, Ph.D. is the Director of the Center for Minority Health at the University of Pittsburgh. He is the Philip Hallen Professor of Community Health and Social Justice, and is one of the nation's leading advocates in the effort to eliminate health disparities based on race. He will serve as an advisor and co-investigator in the research project.

Alexandre Vieira, D.D.S., Ph.D., is a geneticist and an assistant professor in the Department of Oral Medicine and Pathology at the University of Pittsburgh. He will collaborate in the project with the molecular genetic analyses.

Steven Wendell, Ph.D., is an Assistant Professor in Oral Medicine and Pathology at the School of Dental Medicine at the University of Pittsburgh. With a Ph.D. in Molecular Biology, he has extensive experience in genotyping and performing other molecular analyses of DNA, and will provide molecular genetic analyses for COHRA.

Sharon Wenger, Ph.D., F.A.C.M.G., is a Professor of Pathology at West Virginia University and a board-certified cytogeneticist with over 20 years experience in both clinical and research cytogenetics. She had been responsible for all clinical cytogenetic analyses as needed in years 1 and 2 of the project, but will now only be involved in the publications resulting from the study.

Robert Weyant, M.S., D.M.D., Dr. Ph., is an Associate Professor and the Chair of Dental Public Health, Head, Division of Pediatric and Developmental Dental Sciences at the University of Pittsburgh School of Dental Medicine, with over 15 years experience in designing and conducting field epidemiology research using large cohorts and longitudinal designs. He will be responsible for developing and overseeing the clinical dental examinations, and work with the field directors, Dr. McNeil and Dr. Ettaro, to insure successful subject recruitment and maintenance of data integrity.

Michael Wiener, D.M.D. and R. Constance Wiener, D.M.D. are Assistant Professors at the West Virginia University School of Dental Medicine. They are dentists and have clinical expertise in the diagnosis and treatment of native American populations who exhibit oral health disparities. They will utilize their dental expertise as we evaluate the dental variables in the hypothesis that higher periodontal disease scores will be more likely with lower education, income, socioeconomic status, and oral health values.

Xiaojing Wang, Ph.D., is an Assistant Professor in Oral Biology at the School of Dental Medicine at the University of Pittsburgh. With a Ph.D. in Human Genetics from University of Pittsburgh, he has his expertise in statistical genetics and genetic epidemiology. He will be responsible for designing and conducting genetic as well as statistical data analyses for COHRA.

Bei Wu, Ph.D., is an Assistant Professor for the Center on Aging and Department of Community Medicine in the School of Medicine, a Clinical Assistant Professor for the Department of Dental Practice and Rural Health in the School of Dentistry, and an Adjunct Assistant Professor for the Center for Women's Studies at West Virginia University. She will investigate the association between dental care utilization and oral health status.

Lixin Zhang, Ph.D., is an Assistant Research Scientist in the Department of Epidemiology, University of Michigan, School of Public Health. He has extensive experience in genetic

diversity and population structure of pathogenic bacterial species and implications of such diversity in disease patterns using molecular genetic, bioinformatic, and epidemiological approaches. He will work with Dr. Foxman in analyzing some of the project's microbiological samples.

Jayne Zovko, R.D.H., B.S. is currently a faculty member of the Dental Hygiene program at the University of Pittsburgh School of Dental Medicine. She will be a research hygienist on the COHRA project. She is a member of various professional organizations and has served as a research associate or clinical research coordinator on numerous NIDCR and industry sponsored research projects.

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APPENDICES

- 1. COHRA Research Procedures Manual**
- 2. Currently Approved Protocols, Consent Forms, and IRB approval letters**
 - a. West Virginia sites**
 - b. Pennsylvania sites**
- 3. Consent Form Template for future Use at Pennsylvania Sites**
- 4. DSM Sheets and Adverse Events Forms for West Virginia and Coordinating Center DSM Committees**
- 5. Site Visit and Teleconference Logs**

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