
Personalized Orthodontics, The Future of Genetics in Practice

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“Personalized medicine” is a new buzz phrase, based initially on pharmacogenetics and now exploding as genome-wide association studies are undertaken. However, it still remains to be seen how much this will really affect daily practice. The same may be projected for the future of orthodontics. What would personalized orthodontics be based on, how would the studies be undertaken and then validated in practice? How will this be funded? The understanding of the combination and interaction of genetic and environmental (including treatment) factors (nature and nurture together) that influence the treatment response of our patients is fundamental to the evidence-based practice of orthodontics. (Semin Orthod 2008;14: 166-171.) © 2008 Elsevier Inc. All rights reserved.

Your first patient of the day comes in for initial evaluation. It is a Class I “borderline” crowding case with good positioning of the incisors. Evaluation of the polymorphic variations of the major and modifying susceptibility to external apical root resorption genes indicate that this patient has a relatively high risk of external apical root resorption. Along with the expected tooth movement you anticipate depending on if you prescribed extraction of permanent teeth, as well as other factors such as root shape and anticipated length of treatment, you use this diagnostic data to decide on a treatment plan.

Your next patient, a 7-year-old, comes in with a negative anterior overjet. Cephalometric analysis indicates a relative retrusion of the maxilla involving certain anatomical structures. Evaluation of the polymorphic variations of the major and modifying Class III malocclusion genes,

your examination and radiographic evaluation indicate a diagnosis of Class III malocclusion, type 3. Based on this you know what type of treatment, at what stage of development, will result in the greatest likelihood of successful treatment.

Your next young patient comes in with a Class II malocclusion. Cephalometric analysis indicates a relatively retruded mandible. Knowing that although Class II early treatment studies were equivocal about the average benefit of functional appliances, or head gear treatment versus nontreated control patients, you also know that there was notable variation in growth among all three of the experimental groups in those studies. Now you evaluate the polymorphic variations of the major and modifying genes to see if this patient might be one who will likely “catch up” in mandibular growth without treatment, or perhaps one who would respond more with a functional appliance. You use this diagnostic information to devise your treatment plan for the patient on an evidence-based foundation.

These are some of the possible scenarios that, although in the future, are now within our reach to work toward. The first is not at all improbable from where we are today, and the second is probably not far behind. However, care must be taken to manage the expectations of practitioners and the public that have already been set by promises, press release hyperbole, and the re-

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peated exposure to headlines about genetic discoveries in general.¹

These discoveries are often milestone events, and rightfully so. However, they are also just the beginning of a potentially long process of understanding how variation in the gene(s) and therefore the product protein(s) are associated with, lead to, or influence (ie, what is the mechanism of) the pathology or other type of trait of interest. This is particularly true when a gene that contributes some increased likelihood of pathology or other trait developing is only one of several that may be involved. These are often referred to as susceptibility genes, as particular polymorphic variations present in them may be associated with, but not guarantee, the likelihood that a pathology or other trait will be present or develop.

It is difficult to get too excited about the discovery that a particular polymorphic variation in a gene is associated with 15% of the variation in the pathology or other trait. Yet this type of analysis is the underpinning of understanding what and how susceptibility genes contribute. The association of genes with pathology or other type of trait has traditionally been analyzed by testing DNA polymorphic variants one or a few at a time. There are several shortcomings to this approach including the tedium of analyzing multiple polymorphic variants one or a few at a time, the difficulty in excluding a gene, or understanding the combination of a large number of polymorphic variants in an individual that may be contributory.

Rather than initially focusing only on “candidate” genes (which can still have use to further investigate genetic influence), it is now possible to search the genome in an unbiased manner for genes whose common variation contributes to the trait in the population. The usefulness and impact of genetic research will be much more powerful when we can say that some combination of polymorphic variants in some number of genes, along with certain environmental (including treatment) factors, is associated with a much greater degree of the variation in the pathology or other trait.

“Personalized Medicine”

Dental and medical care is generally based on an examination and assessment of the patient’s sta-

tus, diagnosis, and prescription of treatment. The treatment is typically based on a likely positive response in the majority of individuals with the diagnosis. This approach will work for most patients most of the time. However, what will be effective for most of the population may not be the optimum treatment for others. Recently, the analysis of an individual’s response to treatment, largely determined by intrinsic genetic factors and individual behavior, has become more comprehensive, resulting in what has been termed “personalized medicine.”

The major direction personalized medicine has taken to date is in the field of pharmacogenetics, literally the study of the effect of genetics on pharmacology. Pharmacological efficacy often depends on metabolism and deposition, activities that generally involve enzymes and other proteins that can vary among individuals secondary to the corresponding genes that coded for them. Examples include not only determining individuals in whom certain medications would be of the greatest benefit, but also those in whom relatively uncommon or severe unwanted (side) effects might be more likely.²

The combination (interaction) of genetic and environmental (behavioral) factors in individuals is also gaining attention as an important aspect of patient care. For example, the treatment of the individual whose genes (genome) predispose to familial hypercholesterolemia will require a different treatment regimen than the individual with the genome that is not associated with increased plasma cholesterol levels.³ In addition to the individual’s genomic predisposition, their behavior (lifestyle/diet and exercise, in this case) may affect the individual differently based on individual genomic differences. This has been referred to as metabonomics (or metabolomics), or even pharmacometabonomics.⁴

Based on the intrinsic lure of treatments with better outcomes and fewer side effects, the market for molecular diagnostic tests is predicted to grow at an extraordinary rate over the next 10 years, particularly in the area of pharmacogenetics. However, personalized medicine is a paradigm that exists more in conceptual terms than in reality. There are currently only a few marketed drug-test products, and a lack of impact in actual clinical practice as enthusiasts had hoped and predicted.⁵

Is this a function of time, or another example of hype that ultimately does not live up to the promise or expectation? Ultimately only time will tell, but some have predicted it is only a matter of time with advances in technology.⁶ For example, the polymerase chain reaction, the process by which large amounts of DNA are replicated and therefore feasible to study that has revolutionized biology, was invented in 1983. Today 1 million single nucleotide polymorphisms (SNPs) and copy number variation (CNV) markers can be genotyped in an individual using a single microarray chip.^{7,8} How did we get to this point?

The Human Genome Project

The hallmark event in genetics research to date, the sequencing of the human genome (The Human Genome Project) was really only a step toward developing a “map” or understanding of not only how we are so similar in our genome, but how variation occurs among us.^{9,10} Our genome varies from one individual to the next, most often in terms of single base changes of the DNA called single nucleotide polymorphisms (SNPs, pronounced “snips”). The main research use of a human SNP map will be to determine the contributions of genes to diseases (or non-disease phenotypes) that have a complex, multifactorial basis.¹¹

More than 1.4 million SNPs have been identified in the human genome. SNPs that are physically close to each other in a DNA sequence are generally more likely to stay in the same order (and are said to be linked) than SNPs further apart. The combination of the linked SNPs on the same DNA strand (chromosome) is a haplotype. Linkage disequilibrium is the consistency of association of alleles (in this case SNPs) at two linked loci.

Following the sequencing of the human genome, generation of a haplotype “map” of the human genome was undertaken. It consists of a high density of SNPs defining the small number of ancestral haplotypes (blocks of tightly correlated genetic variants that are in linkage disequilibrium) in each region of the human genome. Using this genome-wide linkage disequilibrium map, microarray chips and analysis software allows comprehensive and efficient testing of the association of genes with pathology and other types of traits.^{12,13}

It quickly became evident that some haplotypes are relatively common among most if not all populations, while some are more specific. Of the 3 billion or so bases in the human genome, it has been estimated that 99.9% are exactly the same from person to person. Still about 3 million (or 0.1%) are different, and that’s what makes us unique. The current database of SNPs (<http://www.ncbi.nlm.nih.gov/SNP/>) is a catalog of human genetic variation that is useful for defining how genes contribute to phenotype, as well as a map of human similarity and therefore presumed development and migration of human racial and ethnic groups.^{14,15}

It turns out that the linkage disequilibrium is an extremely sensitive indicator of population history, because the multiallelic nature of haplotypes is highly informative, and the change of haplotypes follows a predictable clock set by genetic recombination rates. Not only is the information about human population history interesting in its own right, but it is also important in the design of studies so that the appearance of spurious differences between affected subjects and control subjects secondary to background differences in haplotypes of the two groups may be minimized.¹⁶

Genome-Wide Association Studies (GWAS)

Association analysis is a method to determine if a particular polymorphic variant (marker allele) is more frequent in a group of subjects with the pathology or other type of trait of interest compared with a control group. This initially was often done analyzing polymorphic variants in or close to a candidate gene, usually selected because of previous knowledge of the function of the gene and its possible effect on the development of trait of interest. This, of course, limits discovery of genes that may be important (you are not likely to find something if you do not know where to look for it), particularly ones that may play more of a cumulative or modifying role. The development of haplotype maps, microarray chips, and the software to begin to make some sense out of the tremendous amount of data that such a study produces has led to the rapid use of genome-wide association studies. This is different from using microarray chips to study the linkage of polymorphic variants in a

family with a Mendelian “monogenic” pathology or other type of trait.

Association studies can be family based to control for population stratification (polymorphic variant frequency differences between cases and controls due to systematic ancestry differences), but the large number of polymorphic variants that may now be studied makes the population based (case-control) association type of study acceptable if appropriate controls are used. However, rigorous standards of statistical significance will be needed to avoid an excessive number of false-positive results, since each SNP has a low prior probability of a cause-and-effect relationship. Additional confidence that associations are strong and relevant in a population will be gained by replicating studies using large samples.¹³

Genome-wide association studies are starting to be published for susceptibility toward a variety of conditions such as inflammatory bowel disease,¹⁷ type 1 diabetes,¹⁸ type 2 diabetes,¹⁹ late-onset Alzheimer’s disease,²⁰ bipolar disorder, coronary artery disease, Crohn’s disease, rheumatoid arthritis, type 1 and type 2 diabetes,²¹ esophageal cancer,²² nicotine dependence,²³ colorectal cancer,²⁴ Parkinson’s disease,²⁵ exfoliation glaucoma,²⁶ childhood asthma,²⁷ control of human immunodeficiency virus type 1,²⁸ atrial fibrillation,²⁹ periodic limb movements in sleep,³⁰ sporadic postmenopausal breast cancer,³¹ estrogen receptor-positive breast cancer,³² myocardial infarction,³³ prostate cancer,³⁴ amyotrophic lateral sclerosis,³⁵ outcome of antidepressant treatment,³⁶ and multiple sclerosis,³⁷ among others.

Practically all of these genome-wide association studies have more to do with etiology than response to treatment, although an increasing number of studies looking at the response of a variety of treatments—when there is a variable response in a population—is anticipated. Thus future studies may focus on etiological (diagnostic) factors, or response to treatment factors, or both.

Undertaking Genome-Wide Association Studies in Orthodontics

A review of recent genome-wide association study articles show in general that the numbers of families in the studies are in the hundreds,

whereas studies that analyze nonrelated individuals with affected subjects and control subjects number in the thousands. This underlies the need for a broad collaboration if sufficient numbers are to be obtained for these studies to be useful. The question of what to do with this in randomized controlled trials (RCTs) is interesting. Are subjects still randomly assigned to a group, and then the genotyping performed afterward? Or should the genotyping be done beforehand, especially if it is to assist in assigning a diagnosis (perhaps based on the present of a particular polymorphic variant in a gene that has a major effect), and then individuals in the same diagnostic category randomly assigned to a group? Perhaps it should be a combination, with subjects having diagnostic markers determined before assignment with subsequent random assignment to a group, and then followed by further analysis looking for modifying genetic factors that are associated with variation within and between the groups.

Large scale observational studies could be used to monitor treatment outcome. Individuals with a specific outcome could be analyzed for genetic polymorphic variant associations using chips with standardized variant panels (including ethnic-specific variants as indicated). Based on these retrospective studies these chips could be used for prospective observation to assess predictability.³⁸

While the genotyping has advanced to a startling stage, it is still only as good as the clinical diagnostic data with which it will be associated. Thus consistent and disciplined clinical evaluation will also be required. Because of the number of subjects that will likely be required to be studied, a combination of orthodontic residency clinics and private practices is envisioned. The advantage of the orthodontic residency clinic is that the residents can be focused on filling out data on patients as a part of their clinical and or research experience. One disadvantage is the turnover of residents both in terms of consistency of treatment and in collecting data. On the flip side, private practice is more consistent within relatively long time periods as to type of treatment and how the data will be collected, but the problem is finding the person power and time to do so in the busy office. In addition, all individuals who will discuss any aspect of the study with potential subjects to obtain informed

consent to be included in the study will likely require passing some type of human research protection examination.

Although for unit volume a greater amount of DNA can be obtained from blood samples, as orthodontists we are well positioned to obtain buccal samples using sterile brushes, or saliva samples in a tube with the required fixative, for reasonably expedient transport to a laboratory for extraction, quantification, and storage of the DNA for that subject. Alternatively, a large volume of blood is not necessary, and the amount used to determine a hematocrit from a quick ear lobe or finger tip lancet will generally give a good yield. Because of the greater and reportedly "cleaner" yield of DNA from blood samples, these have often been preferred in genome-wide association studies, although it has been reported that DNA from buccal samples (if collected and prepared "optimally") can be as good in these studies as DNA from a blood sample.³⁹ The handling of informed consent, clinical data collection, and samples for obtaining DNA can be a time-consuming activity that the busy office staff, and sometimes the doctor, although supportive, may not always be happy about.

Another consideration in addition to the number of subjects will be the cost of the chip procedure itself, and the data analysis. Although decreasing in price, the cost per person for the chips with the greatest number of SNPs is still in the several hundreds of dollars. The analysis may be staged to maximize efficiency and minimize sample sizes. Even so, the size of the resulting data sets, and the complexity of relating them to different clinic and or treatment data, will raise significant issues of analysis and interpretation usually requiring someone well versed in hereditary genomics.^{6,40}

Support of Genome-Wide Association Studies in Orthodontics

Genome-wide association studies are now a major emphasis for the National Institutes of Health (NIH), as evidence by their Web site,⁴¹ which in part states: "NIH, the Foundation for the National Institutes of Health, Pfizer Global Research & Development and others have formed a public-private partnership, the Genetic Association Information Network (GAIN), to fund genome-wide association studies. After peer-review of appli-

cations, GAIN announced its first round of studies in October 2006. The initial studies include bipolar disorder, major depression, kidney disease in type 1 diabetes, attention deficit hyperactivity disorder, schizophrenia and psoriasis." In a time of national budget constraints and the NIH budget not growing for the first time in several years, the importance of studies to analyze the diagnosis for and outcome of orthodontic treatment is sometimes not appreciated by nonorthodontists. It will be imperative for the profession to support this type of research for it to proceed, to show its validity, and to attract NIH funding.

Conclusion

Multiple factors and processes contribute to the response to orthodontic treatment. Some patients will exhibit unusual outcomes linked to polymorphic genes. Analysis of overall treatment response requires a systems analysis using informatics for integration of all relevant information. The influence of genetic factors on treatment outcome must be studied and understood in quantitative terms. Conclusions from retrospective studies must be evaluated by prospective testing to truly evaluate their value in practice. Genome-wide association studies are necessary to further the evidence base for the practice of orthodontics. Only then will we begin to truly understand how nature (genetic factors) and nurture (environment factors, including treatment) together affect our treatment of our patients.

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