Towards Predictive Oral and Maxillofacial Medicine: Perspective on Zavras et al

Paul C Edwards
2012
Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, 114(5), 549–551.

The President's Council of Advisors on Science and Technology recently characterized personalized medicine as "the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease or their response to a specific treatment. Preventive or therapeutic interventions can then be concentrated on those who will benefit, sparing expense and side effects for those who will not[1]."

Alternatively termed precision medicine, molecular medicine, genomic medicine, and prospective medicine, this approach to patient care formalizes and fine tunes the long-standing practice in the health care field of tailoring a patient's management, be it in adjusting the frequency of preventive screening appointments or in the definitive treatment of their disease, to his or her unique set of characteristics by incorporating insight obtained from the large number of recent advances in genomics, proteomics and molecular biology. By stratifying a patient's disease susceptibility or targeting an individual to a specific treatment protocol to increase the likelihood of a positive response to therapy, the ultimate objective of personalized medicine is improved patient care. The prospect of stratifying patients by both disease susceptibility and expected response to therapeutic intervention would also be of benefit in identifying therapies that might be of potential benefit within subsets of the population having specific disease susceptibility markers that might otherwise be statistically masked by larger subsets of the population dominated by non-responders.

The concept and practice of personalized medicine is no stranger to the practicing oral and maxillofacial pathologist, who incorporates these principals into clinical practice on a daily basis, whether it be in assessing a patient for a possible deficiency of red blood cell glucose-6-phosphate dehydrogenase prior to instituting therapy with Dapsone for management of the patient with pemphigoid unresponsive to first line therapy, performing fluorescent in situ hybridization to identify a possible ETV6-NTRK3 gene fusion in order to differentiate acinic cell carcinoma from mammary analogue secretory carcinoma, or identifying the presence of high risk human

Accepted Author's Manuscript. Final version published as:

Edwards, P. C. (2012). Toward predictive oral and maxillofacial medicine: perspective on Zavras et al. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, 114(5), 549–551. http://dx.doi.org/10.1016/j.oooo.2012.08.440

papilloma virus (HPV) in oropharyngeal carcinoma by means of in situ hybridization and/or immunohistochemistry for the surrogate marker p16 in order to predict prognosis and, possibly, guide therapy.

While proteomics will inevitably play an increasingly important role in the future of personalized medicine, currently much of the focus in this field has been in the area of short nucleotide polymorphisms (SNPs). SNPs are single nucleotide variations within the genome that are estimated to represent close to 90% of all human genetic variation. To date, millions of SNPs have been identified and listed in the SNP database[2] administered by the National Center for Biotechnology Information in collaboration with the National Human Genome Research Institute. Although the majority of these SNPs are likely to be of no clinical significance, a yet to be determined percentage are believed to affect an individuals response to agents such as drugs and microbial challenges as well as their predisposition to developing specific health conditions.

Within the past 10 years, there has been a near exponential growth in the use of genome-wide association (GWA) studies in order to try to identify single nucleotide gene sequence variations that may predispose individuals to the development of complex, non-single gene inherited diseases typified by most types of malignant neooplasms. By this approach, large numbers of individuals affected by a disease (the case group) and unaffected healthy individuals (the control group) are compared for the presence of statistically significant differences in the allele frequency of the examined SNPs. A commonly used alternative to GWA studies is the hypothesis-driven candidate gene study, involving the targeted assessment of one or more areas of a specific gene.

In this issue of OOOOE, Zavras and colleagues present preliminary data demonstrating a possible association between short nucleotide polymorphisms in ERCC5 (“excision repair cross-complementing associated rodent repair deficiency, complementation group 5”), an enzyme involved in DNA damage repair, and variances in the risk of developing squamous cell carcinoma in their patient population. Specifically, they demonstrated that homozygosity for a specific single nucleotide polymorphism (the C allele at rs751402) in the proximal promoter region of ERCC5 is potentially associated with

lower rates of oral squamous cell carcinoma, whereas rs751402 T alleles appeared to be associated with an increased risk.

ERCC5 is one of the main constituents of a group of proteins involved in the nucleotide excision repair (NER) pathway, by which smoking-related DNA adducts, carcinogens covalent bound to DNA, are repaired. As unrepaired DNA damage resulting from exposure to environmental carcinogens is believed to be an early event in the process of carcinogenesis, there is strong biologic plausibility for the hypothesis that an individual's variation in the efficacy of DNA repair activity could have an association with differences in the risk of carcinogenesis. There is additional corollary data that would appear to strengthen such an association. For one, it is known that mutations in this gene cause one subtype of xeroderma pigmentosum, a genodermatosis characterized by increased susceptibility for skin cancer development following exposure to ultraviolet light. Looking at oral squamous cell carcinoma in particular, there is preliminary evidence that polymorphisms in cell cycle control genes may be associated with increased oral cancer susceptibility (e.g. CCND1 gene encoding for protein Cyclin D1[3]). More than 70 non-synonymous SNPs (i.e. single nucleotide polymorphisms leading to a change in protein sequence) have been identified in the coding region for ERCC5. As noted by Zhu et al[4], several studies have indicated that polymorphisms in ERCC5 may be associated with the risk of developing breast, lung, bladder, and esophageal cancer. However, many of the published hypothesis-driven candidate gene studies have provided conflicting results. A recent meta-analysis[5] of 44 published case-control studies, comprising 23,490 cases and 27,168 controls looking at the association between another widely studied ERCC5 SNP (rs17655) and overall cancer risk, concluded that it was unlikely that this specific polymorphism contributes to increased susceptibility to cancer risk.

The results of this preliminary hospital-based case control hypothesis-driven candidate gene study are of obvious interest to the readers of OOOO. Nevertheless, without diminishing the findings from this study, it is important to point out that thousands of potential SNP-disease predisposition associations have been identified, with many more characterized on a daily basis. The larger impact of this study lies in the fact, by building upon a larger body of existing data, it

highlights the progress that has been made, and the challenges ahead, in moving towards the goal of predicting individual disease risk.

The authors acknowledge a number of limitations in their study. Women were under-represented relative to the general population in the control group (25%) as well as in the case group (3.7% female). Information on smoking, alcohol, and areca nut use was collected in a binary "current user" or "nonuser" form, without stratifying individuals based on duration or lifetime quantity of consumption. There were also marked differences in identifiable risk factors between the case group and the control group, although the authors did adjust for these disparities as part of their statistical analysis. While there are accepted differences in the statistical analysis and approaches to matching controls in genetic association studies compared to conventional case control studies, especially where it comes to environmental confounding[6], selection of controls for smaller candidate gene driven studies should ideally follow classical epidemiological control recruitment guidelines, such that the controls have the same opportunity to develop the disease as the cases[7].

The findings from this study should also not be generalized to other populations, because, as necessitated by genetic association case control studies such as the current one, the subjects were specifically selected to represent a population of genetically similar individuals (in this case, from a localized geographic region of Taiwan).

Most significantly, for a case control study, the overall magnitude of the effect was relatively small, with an odds ratio of 2.1 for the TT homozygotes. When assessing risk susceptibility in conditions with a strong environmental component, the strength of the association is of particularly significance.

While further validation by larger population-based case-control studies is needed, these results, if supported by future studies, raise the intriguing prospect of being able to stratify a patient's risk of oral cancer susceptibility, thereby potentially guiding prevention strategies.

This component of personalized medicine, better termed predictive risk testing, predictive medicine, or, when focusing on the field of oral pathology, “predictive oral and maxillofacial medicine”.

A number of issues must be addressed before this approach can be introduced into clinical practice with any degree of confidence. For example, it is clear that lifestyle and environment (e.g. smoking history) have a significant influence on the development of many conditions that affect the oral and maxillofacial region, regardless of specific genetic makeup. In many cases, these environmental factors may have a more significant effect than specific putative disease associated-SNPs. Therefore, in order to have a meaningful clinical impact, this type of approach should identify individuals with a substantially elevated risk of developing the condition in question. Ultimately, in order to increase predictive power, panels of highly individualized and statistically validated predisposition SNPs will need to be developed (e.g. an oral cancer risk factor SNP panel).

In order to justify undergoing testing, and the associated potential psychological considerations associated with both true-positive and false positive results, there must also be an appropriate intervention that would ultimately reduce or counterbalance a patient’s inherent increased risk.

Looking at the potential introduction of a single SNP-based predictive test (e.g. rs751402 for the assessment of oral cancer risk), the obvious issue would be to determine to whom this test would be targeted: the patient at higher risk for oral cancer due to a long history of cigarette and alcohol consumption, the patient with a clinically diagnosed leukoplakia, or the individual with mild dysplasia? In this scenario, it could be argued that the clinician’s efforts and the additional health care costs involved in performing this test could be put to better use encouraging changes in high risk behavioral patterns. On the other side of the equation, one can’t help but wonder what effect a test result suggesting a decreased risk of developing the condition in question e.g. oral cancer, might have on the patient’s health behavior? Would this potentially encourage the individual to continue with their smoking habit, under the false reassurance that they are “low risk”?

The above noted concerns regarding the applicability of predictive risk testing based on analysis of a single SNP become less of a concern, of course, as the field transitions to the use of panels employing hundreds of thousands to millions of validated SNPs encompassing a myriad of conditions. A number of these panel-based predictive risk-testing products, currently analyzing 500,000 to a million-plus SNPs, are being marketed directly to consumers (e.g. deCODEme™,; 23andme™), for as low as $300 US per test. For approximately $10,000 US, with a physician's referral, an individual can acquire their full genome sequence (e.g. Individual Genome Sequencing™, Illumina Inc.). An iPad™ app is even available (MyGenome) that permits users to explore which of their genetic polymorphisms could potentially predispose to health impacts. For those who do not have access to their own genome sequence, the genome of the Illumina CEO is preloaded in the app!

From a regulatory point of view, despite an explosion in the number of potential markers, oversight into the validity of these tests has been varied. In an investigation by the United States Government Accountability Office (GAO)[8], differing disease risk predictions were received from four different direct to consumer (DTC) genetic testing companies using identical DNA samples. Those predictions often conflicted with the donor's previously diagnosed medical conditions and/or family history. In contradiction to the GAO's findings, a separate peer-reviewed study published in Clinical Chemistry[9] reported a greater than 99.6% concordance in SNP analyses obtained from four different DTC tests, but did note disparities in the supplied risk estimates, which the authors attributed to differences in the SNPs analyzed as well as the reference populations used in the different companies' calculations. The latter observation reflects the fact that much of the underlying risk data in both genome-wide association and hypothesis-driven candidate gene studies are derived from Caucasian populations, rendering the validity of extrapolating these associations to non-Caucasian populations unclear.

As noted by Hamburg and Collins[10], while a multitude of genetic variations have been identified that can both predispose to the development of disease and predict a patient's response to therapy, the challenge remains that of incorporating this knowledge into

routine patient care. Currently, gene-based predictive risk testing has important limitations, in part because single SNPs for complex conditions such as oral squamous cell carcinoma do not entirely predict outcomes, in part by failing to account for environmental influences. High-level analysis of very large population-based data will be required before this approach can be widely accepted for clinical use. However, there should be no doubt that, in a relatively short period of time, as our knowledge base in this area continues to expand at an exponential rate, predictive risk testing will have a major impact on the practice of oral and maxillofacial pathology.

REFERENCES

[1]The President's Council of Advisors on Science and Technology. Priorities for Personalized Medicine". September 2008. Available online at: http://www.whitehouse.gov/files/documents/ostp/PCAST/pcast_report_v2.pdf (accessed 07/01/2012)

[2]Available online at: http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp; (accessed 07/01/2012)

[3]Huang M, Spitz MR, Gu J, Lee JJ, Lin J, Lippman SM, Wu X. Cyclin D1 gene polymorphism as a risk factor for oral premalignant lesions Carcinogenesis 2006;27:2034-7

[4]Zhu ML, Shi TY, Hu HC, He J, Wang M, Jin L et al. Polymorphisms in the *ERCC5* gene and risk of esophageal squamous cell carcinoma (ESCC) in eastern Chinese populations. PLoS One. 2012;7:e41500.

[5]Zhu ML, Wang M, Cao ZG, He J, Shi TY, Xia KQ et al. Association between the ERCC5 Asp1104His polymorphism and cancer risk: a meta-analysis. PLoS One. 2012;7:e36293.

[6]Zondervan KT, Cardon LR. Designing candidate gene and genome-wide case–control association studies. Nat Protoc. 2007;2:2492-501.

[7]Amos CI, Wu X, Broderick P, Gorlov IP, Gu J, Eisen T, et al. Genome-wide association scan of tag SNPs identifies a susceptibility

locus for lung cancer at 15q25. Nat Genet 2008;40:616-22.

[8]Direct-to-consumer genetic tests-misleading test results are further complicated by deceptive marketing and other questionable practices GAO-10-847T, Jul 22, 2010. Available online at: http://www.gao.gov/new.items/d10847t.pdf (accessed 07/01/2012).

[9]Imai K, Kricka LJ, Fortina P. Concordance study of 3 direct-to-consumer genetic-testing services. Clin Chem 2011;57:518-21.

[10]Hamburg MA, Collins FS. The path to personalized medicine. N Engl J Med 2010;363: 301-4.