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Multicenter Initiative Seeking Critical Genes in Respiratory Papillomatosis

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Abstract

Objectives: To determine the host genes that govern susceptibility to recurrent respiratory papillomatosis (RRP). RRP is caused by human papillomavirus (HPV) 6 and 11. Millions of babies are exposed during the birthing process, but relatively few develop the disease and the aggressiveness of the course is highly variable. Genetically encoded host susceptibility is postulated. Determining the host genes that govern susceptibility will enhance our understanding not only of RRP but also of host-viral interaction in general.

Study Design: A genome-wide association study on familial triads consisting of an RRP-affected child and his or her parents. Using the HapMap data from the human genome project, we will identify those alleles that are over-transmitted by the parents to their affected offspring as compared to those alleles that are under-transmitted.

Methods: Approximately 400 patients and their parents will be recruited through a collaboration between the Center for Genomic Sciences and the RRP Task Force. DNA will be extracted from blood specimens and viral typing will be performed on biopsy specimens. Patients will be genotyped using single nucleotide polymorphism (SNP) markers and compared to their respective parents' genotype using the transmission disequilibrium test. Both a genome scan and a candidate gene approach will be utilized.

Results: Institutional Review Board authorization has been obtained at three hospitals and the process is underway at 18 more. Patient and parent recruitment has begun. Specimens have been forwarded to Pittsburgh, Pennsylvania, where the DNA has been extracted and is being stored.

Conclusions: A novel approach combining a nationwide patient resource and the mapping power of the subcentimorgan human haplotype map has been developed to elucidate the biological mechanisms of RRP by determining the genetically encoded susceptibilities of host-virus interaction.

Keywords

Genome; HapMap; polymorphisms; larynx; funding; career

INTRODUCTION

Millions of Americans are infected with one or more types of human papillomavirus (HPV). The scores of different HPVs cause a wide range of diseases, from common skin warts to cervical cancer. In children unfortunate enough to acquire the condylomatous viral types via vertical transmission from their mothers, the infection produces the most common benign neoplasm of the larynx, recurrent respiratory papillomatosis (RRP). The most common presentation of RRP is chronic dysphonia; if this disease is not controlled, it will lead to death through airway occlusion. The underlying pathophysiology of HPV infection on the vocal folds and other airway sites is incompletely understood, which has hindered the development of rational medical therapy. RRP can affect adults as well as children,¹ but it is children who are more commonly affected and it is in children that the disease tends to be more aggressive. To reduce variability, the study described herein focuses on juvenile-onset RRP disease.

Recurrent respiratory papillomatosis is caused by an infection with HPV 6 or 11. Millions of fetuses are exposed to these viruses during labor yet relatively few develop RRP. After being present for months or years, the disease usually tapers into remission leaving most patients with lifelong hoarseness. Between diagnosis and remission, some patients require only a few therapeutic procedures, whereas others require scores. The biological bases of these differences are not understood. The lack of a robust animal model for RRP has further hindered research efforts in this area.

Recent advances in understanding of infectious diseases in general and epidemiologic and laboratory HPV-based data suggest that host susceptibility is under genetic control. In this paper, we outline a novel approach to determine the genetic basis for host susceptibility to HPV using pediatric RRP as a model. The necessary ingredients for a successful gene discovery project are detailed: access to an informative patient population, laboratory systems that perform high-density genotypic analyses, the pending availability of a human haplotype map, and a source of funding. Determining the genetic basis of host susceptibility will provide insight into the fundamental pathophysiology of RRP and papillomavirus infections in general. This in turn will ultimately facilitate development of rational therapy.

MATERIALS AND METHODS

Ascertainment of Subject Population

Recurrent respiratory papillomatosis is not a common disease; the incidence is approximately 1 per 100,000 children per year.² Since these children generally endure the disease for an extended period of time, the prevalence is naturally higher than the incidence. It is estimated that between 700 and 3000 children will be managed for this disease in the United States during the course of a year.

Any robust study requires a large number of subjects to generate data from which conclusions can be drawn. Over a 3-year period, the busiest known center for pediatric RRP managed 38 patients.³ Among the pediatric institutions contributing to the largest collected series of RRP, the average number of patients treated in the 3-year period was 20. It is patently clear that large populations can be recruited only through multicenter collaborations. In the case of the RRP Task Force, this represents 20 centers.

The RRP Task Force

The RRP Task Force has been enrolling RRP patients from all over the United States since 1997. The RRP Task Force together with the Centers for Disease Control and Prevention maintains a database of all enrollees and each year up to 200 new patients were entered. The enrolled subjects have all been identified by Task Force otolaryngologists who constitute a subset of doctors from the American Society of Pediatric Otolaryngology and the American Bronchoesophagological Association who have a particular interest in the disease. For inclusion in this study, patients must have undergone an endoscopic examination with biopsy of the larynx and a histopathologic diagnosis made by a pathologist.

Center for Genomic Sciences, Allegheny-Singer Research Institute

The Center for Genomic Sciences (CGS), located in Pittsburgh, Pennsylvania, is a gene discovery laboratory with extensive experience localizing genes for a variety of human conditions, including pediatric gastroesophageal reflux,⁴ Crouzon syndrome,⁵ and hereditary pancreatitis.^{6,7} With the accumulated expertise and infrastructure that exist within CGS for conducting genotyping and statistical analyses, the authors have the means for the facile handling, tracking, preparation, and analyses of the large number of blood/DNA specimens that will be associated with this study.

Patient Population and Control Subjects

People with RRP who were diagnosed before the age of 18 will constitute the study population. The attending otolaryngologist actively caring for the patient will invite the patient and his or her mother and father to participate as a study subject. After informed consent has been obtained, the following will be collected: a one-page clinical data collection form completed by the physician, a one-page (double-sided) socioeconomic and family history questionnaire completed by the mother, 9 mL of blood from each member of the triad, and several small papilloma biopsy samples. Specimens are collected while the patients are under general anesthesia. The materials are coded and sent by courier to the CGS. In addition, participating surgeons are asked to fill out a disease progression form annually to help distinguish subjects with aggressive disease from those with indolent disease.

The design of this study is fundamentally different from that of most genetic investigations. Each patient's genotype will be compared to the genotype of the parents. In situations in which a parent is unavailable, the unaffected siblings of the proband will be included in the study to try to reconstruct the missing parental genotype.

Regulatory Authorization

The study design calls for the collection of private health information, the collection of blood, the collection of pathologic specimens, and the genotyping of individual human beings. Since the federal guidelines for the protection and privacy of human subjects in research calls for local regulation within the context of local customs and laws, each hospital's institutional review board needs to independently review the protocol and procedures. Processing a score or more of such applications is a major undertaking.

Viral Typing

DNA extracted from papilloma specimens will be subjected to polymerase chain reaction with group-specific primers, and viral type will be assessed using specific probes in a liquid hybridization assay followed by gel-retardation. In this way, the study will be controlled for whether HPV 6 as opposed to HPV 11 was the causative virus. Since HPV typing is not performed by the majority of clinicians, this study will accumulate one of the largest samples linking HPV type to aggressiveness of disease. Some studies have suggested that HPV 11 tends to be associated with a more aggressive course,⁸⁻¹⁰ so the viral type could be an important variable to adjust for in the final genetic statistical analysis.

Genetic Techniques

The DNA of patients and of their parents will be analyzed by two differing but complementary techniques: genomic scans and a candidate gene approach. In the genomic scan approach, a series of markers, or "mileposts," is analyzed throughout the entire genome of each individual to determine which version of a particular gene is carried. The genomic scan approach has the central advantage of requiring no *a priori* knowledge of the gene or genes involved in RRP susceptibility. Although a variety of markers are available, in this study we will use single nucleotide polymorphisms (SNPs, pronounced "snips"). A SNP is a single base change that may or may not have a functional consequence. The important feature of an individual SNP is that the location in the genome is known. More than two and a half million SNPs have been identified, and fortunately they are evenly distributed over the entire genome. SNPs are used because of their high density throughout the genome and because their genotyping can be highly automated, rapid, and cost-effective.

In the candidate gene approach, we will analyze our patient specimens for mutations in genes previously identified to be important in the various aspects of viral pathogenesis (Table I). This approach has the advantage of focusing the search in limited areas, but the corollary is that gaps in current understanding could render the discovery project fruitless.

Currently, no information is known on the heritability of RRP, and it is not known how many genes are involved in the etiology of RRP susceptibility. It is therefore not possible to accurately calculate the number of triads needed for this RRP study. To ensure that at least some of the genes involved in RRP susceptibility can be mapped and identified, between 400 and 800 triads are being recruited.

RESULTS

At several meetings of the RRP Task Force over the past 2 years, the members have repeatedly expressed enthusiasm for this study and a willingness to collaborate with the CGS. The Institutional Review Board process has been completed at three institutions, is ongoing at six others, and is being initiated at 12 more institutions. Recruitment of subjects has already begun. The recruitment rate has been encouraging. Good-quality DNA has been extracted from blood specimens and papilloma specimens.

The proposed study formed the scientific basis of a National Institutes of Health (NIH) grant application by the principal investigator, Farrel Buchinsky, under the co-mentorship of Drs. Post and Ehrlich. The grant submission was favorably received and therefore federal funds have been made available to conduct the study.

DISCUSSION

Voice

Recurrent respiratory papillomatosis is a significant cause of dysphonia¹ in children and adults. Initial management consists of debulking the papillomas by various means but almost always requires repeated procedures.³ It is not rare to hear of a patient having undergone more than 100 procedures to control the problem. Voice disturbance is the most frequent symptom from the time of presentation, through the years of repeated surgical therapy, and frequently persists even after remission. Besides the resulting emotional distress, there are great financial costs associated with the treatment of this chronic disease. Bishai *et al.*¹¹ estimated that the direct lifetime medical cost of treating a single person with RRP is \$200,000.

Besides direct ablative therapy, numerous other therapies have been proposed and tried. None of them are satisfactory. Interferon- α is the most well known and has been the subject of two randomized trials.^{12,13} A plethora of adjuvant therapies are described in the literature.¹⁴ Controls, randomization, and adequate sample size are generally lacking.

Etiology

Polymerase chain reaction-based detection assays have demonstrated HPV 6 or 11 in pathological specimens in more than 90% of cases.^{15,16} Interestingly, the virus is also detected in normal tissue adjacent to the papilloma.¹⁷ It is thought that the virus in the normal tissue is latent and is activated by surgical trauma. Several studies have commented on a possible association between the type of virus (i.e., 6 or 11) and the clinical course or the likelihood of remission.^{8-9,18-20} There is some evidence that HPV 11 is associated with a more aggressive course, but the connection is tenuous. Several studies showed no association.^{18,19}

In cervical cancer, the HPV types have been widely accepted as influencing the clinical course (HPV 16 and 18 being considered “high-risk”). Furthermore, the physical state of the HPV DNA has also been shown to be important in the pathogenesis of cervical cancer. Instead of the HPV DNA existing in the episomal state, its insertion and integration into the

host chromosomal DNA is critical in the disease process.²¹ Similarly in RRP, integration of HPV 11 DNA has been demonstrated in wart specimens.²² Rady *et al.*²² studied multiple specimens from a patient with aggressive RRP who went on to develop malignant transformation. Episomal and integrated forms of HPV 11 sequences were observed in benign warts, but only integrated forms of the HPV 11 were observed in malignant papilloma tissue samples. Additional studies have looked at integration but only in patients who underwent malignant transformation. As part of this research initiative, we will assess the frequency and chromosomal location of HPV 6 and 11 DNA integration into the host DNA of benign papilloma specimens. Exact localization of any potential integration “hot spot” will be performed, since the involved genes will be considered candidate genes for RRP susceptibility.

Vertical Transmission

Human papillomavirus types 6 and 11 are notorious for causing a far more common disease: anogenital warts (also referred to as venereal warts or condylomata acuminata). The incidence is roughly 100 per 100,000 population per year.^{23,24}

Although millions of women have vaginal HPV, relatively few children develop RRP. Several studies have looked at the prevalence of maternal genital warts and HPV infection during pregnancy. The prevalence of detectable DNA (of any type of HPV) ranges from 9 to 55%.^{25–29} Exposure to HPV 6 and 11 is necessary, but it is by no means sufficient for the development of respiratory papillomatosis. The vertical transmission of RRP led to the recommendation of performing elective cesarean sections on mothers with condylomata acuminata; however, the transmission rates are so low (estimated to be less than 0.25%)³⁰ that the recommendation is not universally followed.

Risk Factors

It is not known why only the unfortunate few get this disease. Speculations abound: patient immunity; immature immunity; timing, length, and load of virus exposure. Shah *et al.*³¹ repeatedly demonstrated the risk triad for juvenile onset RRP: first-born child to a young mother by vaginal delivery (Table II). The risk triad is statistically significantly over-represented among children with RRP when compared to the general population. However, statistically significant Z tests for proportions do not explain all the variability and do not negate the role that genetics may play in the susceptibility.

For many years, physicians working with RRP have accepted the speculation that prolonged exposure during the second stage of labor in primigravid women explained the disproportionately high incidence of RRP in first-born children.³¹ Another explanation comes from the combination of (1) work on transmission of herpes simplex,³² in which the mother’s serologic status was an independent risk factor, and (2) cohort studies looking at seroconversion in response to incident infections of genital HPV infection. Carter *et al.*³³ showed a relatively long delay (6–12 mo) between infection and seroconversion. We can reasonably expect these risks to congregate on a young woman who may have recently initiated sexual activity. It is quite conceivable that she may become pregnant and acquire HPV in a similar time frame. Delivery of her baby could precede seroconversion and

therefore the fetus would be exposed to the virus without the potential protection provided by passive immunity. Unfortunately, these plausible explanations have not been proven, and the logistics that would be required to systematically study them are daunting.

It is not known why some children run an aggressive course (10 procedure in a lifetime or 4 procedures per year or distal spread) while others run a relatively benign course. Epidemiologic data indicate that younger age at diagnosis (<3 y) is associated with more aggressive disease.³ There have been conflicting or as yet unreproduced results relating aggressiveness to viral type,⁸ helper T-cell profiles,³⁴ and down-regulation of the transporter associated with antigen presentation (TAP-1).³⁵

Role of Genetics in Disease Susceptibility

It is clear that host genetics play a significant role in governing susceptibility to infection.³⁶ Malaria is probably the best known example.³⁷ No one doubts that malaria is caused by *Plasmodium* parasites; however, children who are heterozygous for the sickle cell mutation in their P-globin gene are less likely to succumb when infected. More recently, other pediatric infectious diseases have been shown to have a genetic component, including otitis media. In a major study using monozygotic and dizygotic twins, Casselbrant *et al.*³⁸ demonstrated that approximately 73% of otitis media susceptibility is genetically mediated. Another good example of genetic influences in a disease known to be caused by an infectious agent is acquired immunodeficiency syndrome (AIDS). Genetic differences in the gene that codes for a chemokine receptor on macrophages, monocytes, and T-cells (*CCR5*)³⁹ has been associated with decreased susceptibility to human immunodeficiency virus (HIV) infection and with prolongation of the time from infection to death. There are eight or more additional genes that govern susceptibility to HIV. While each gene may only account for a small part of the variability seen by clinicians and epidemiologists, collectively, host genetic factors begin to explain and predict more of the variability than ever before.⁴⁰

Recognizing that clinical factors, such as vaginal delivery or young age of mother, play a role in one's risk of developing RRP does not diminish the possible role of genetic factors. The well-recognized etiologic roles of day-care attendance and exposure to smoke in the incidence of chronic otitis media does not belittle the very significant role of hereditary factors in that disease,³⁸ nor does the phenomenon of promiscuity increasing the risk of contracting AIDS belittle the important mutation discoveries that were made in that disease.

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Evidence that Susceptibility to RRP is Genetically Determined

Genetic susceptibility to RRP is an area that has received little attention. Work published by Bonagura *et al.*⁴² showed that 16 patients with RRP were more likely than expected to have certain major histocompatibility complex (MHC) class II types: DQ3 and DR11 and one split of DR5. Of the 16 patients, 6 were children and 10 were adults. Since 15 of the 16 patients were non-Hispanic whites from all over the United States, the authors chose the composite white database from the 11th International Histocompatibility Workshop to serve as their controls for allele frequency. The same findings were not seen in a 62-member Finnish cohort⁴³ of adult-onset RRP, although there was a suggested association between the

detection of HPV in papilloma specimens and DQB1*0501. The reference population for this study was 93 Finnish cadaveric kidney donors. Most recently, Gelder *et al.*¹⁰ published MHC data on a cohort of 60 RRP patients in the United Kingdom and compared them to 554 cadaveric organ donors who are representative of the British population. Of the 60 cases, 36 had juvenile-onset and 24 had adult-onset disease. The authors found that human leukocyte antigen (HLA) DRB1*0301 was disproportionately present in their group of patients (55%) as compared with the control group (25%). This finding would be compatible with the notion that a particular form of a MHC protein is rendering the host susceptible to the disease by abolishing or suppressing an immunologic attack. Subsequent *in vitro* testing was not able to demonstrate that the presence of this allele resulted in diminished peripheral blood mono-nuclear cell proliferative responses to HPV-11-like particles.

MHC studies have also been conducted among cohorts of women to investigate associations with cervical cancer and its precursor, genital HPV infection. It is interesting to note that there was a decreased risk of cervical squamous cell carcinoma⁴⁴ and of transient or persistent genital HPV infections⁴⁵ associated with DRB1*0301 whereas Gelder¹⁰ demonstrated this allele to be disproportionately present in the RRP patients.

Numerous studies have investigated the interaction of HPV-encoded E6 protein and the tumor suppressor gene product p53. E6 associates with p53 and induces its degradation. Storey *et al.*⁴⁶ demonstrated that a polymorphism in the p53 gene gives rise to p53 proteins with different rates of E6-associated degradation. Among people with invasive cervical carcinoma, there was an association between the p53 polymorphism and HPV 16 seropositivity.⁴⁷ Several case-control studies in a variety of ethnic groups have not reproduced Storey *et al.*'s striking observation.^{48–50}

Human papillomavirus is specific to humans, but other species also are infected by papillomaviruses. For instance, canine oral papillomavirus predictably gives rise to oral papillomas in thoroughbred beagles.⁵¹ However, the probability of developing oral papilloma when outbred dogs are exposed to the virus is considerably lower (Benson AB, personal communication). Unlike in humans, beagles almost always demonstrate regression of the papilloma after a few weeks. In one *in vivo* experiment that suggested a host genetic predisposition to disease, one beagle was noted to have nonregressing disease that was refractory to therapy.⁵² The virus in that dog was geno-typed and found to be no different from the prototype. Viral isolates from the nonregressing papilloma were used in other dogs in which the disease progressed and then resolved in the conventional way. It appeared that in this case, differences in the host and not in the virus were driving the recalcitrant phenotype.

In a rabbit model of papillomavirus infection, there was an association between rabbit leukocyte antigen type and the ability to clear the infection.⁵³ Two alleles in linkage disequilibrium, DR α -B and DQ α -E, were strongly linked to wart regression. Cottontail rabbit (Shope) papillomavirus was used to inoculate rabbits and the course of the resultant skin warts were noted. Most rabbits demonstrated regression of their warts but some showed persistence. One allele at the DR α locus was associated with 100% regression in 26 rabbits but of the 51 rabbits that did not carry the allele, only 55% regressed.

Challenges in Finding Genetic Factors in RRP

Several issues make RRP a difficult disease to explore for genetic factors. Large, multigenerational families with RRP have not been described and would be very difficult to ascertain. The genetics of RRP susceptibility is unknown. This is to say that the pattern of inheritance is unknown and probably unascertainable (other than to say it is complex). Large numbers of children affected with RRP have not been available to a single research group. Ideal control populations do not exist; they would consist of children who are known to have been exposed to HPV 6 or 11 but did not develop the disease.

Fortunately, advances in (1) the construction of high-density maps (SNPs) and the success of the Human Genome Project; (2) high throughput genotyping procedures (such as genome scans on a chip); (3) analytical methodologies for establishing linkage from incomplete sets of genotypic data; (4) the development of high throughput sequencing; and (5) the work of the RRP Task Force have provided possible solutions to the above problems.

Classically, genetic etiologies for diseases were suspected when rare disorders were prevalent in specific families. In such cases, pedigree analysis (segregation analysis) was used to classify a trait's inheritance pattern as autosomal dominant, autosomal recessive, X-linked, or mitochondrial. For example, cystic fibrosis segregates as an autosomal recessive disorder, whereas Crouzon syndrome shows an autosomal dominant mode of inheritance. Determining the genetic etiology of monogenic disorders requires the cooperation of one large family or several smaller families, each of which has multiple individuals affected with the disease in question. Gene mapping becomes more complex when multiple genes underlie a particular trait and the trait does not show a simple mendelian inheritance pattern. In these situations, it is rare to find more than two affected individuals in a single family. It is still possible, however, to map susceptibility loci for these polygenic traits. Examples of such complex traits include diseases such as type 2 diabetes mellitus, inflammatory bowel disease, and coronary artery disease. RRP represents an even more challenging trait for linkage because there is almost never more than one affected person per kindred.

These considerations dictate that a case-control design would be required to perform an association study. In this way, a researcher would hope to find an allele that is disproportionately frequent in the cases as compared to the controls. In essence, this is what the three HLA studies in RRP have done,^{10,42,43} having limited themselves to region 21 on the short arm of chromosome 6 where the MHC loci are to be found. This is a reasonable region in which to focus a search, since many investigators believe that RRP persists because the host fails to mount an effective T-cell-mediated attack on infected cells. These three studies have not had consistent findings. It may be that allele frequency is subject to population stratification; affected and unaffected groups come from different populations and it just so happens that the populations differ in the frequency of the marker allele. An inclusive explanation of the differences in the studies would be that different genes confer susceptibility in different populations.

Surmounting the Conceptual Challenge

For the study of RRP, it is not possible to use linkage analysis, since families with many affected individuals are not available. Therefore, gene mapping must be carried out using an association study. For association studies, it is possible to either use case-control or the triad design. For this study, it was decided to use the triads, since this design will overcome the problem of detecting association due to population substructure instead of linkage disequilibrium. Since Americans are an outbred population of many different ethnic groups, population substructure is a particular problem of association studies. This is an inherent problem for case-control designs, and recently developed methods only partially resolve the issue.⁵⁴

In each parent's gonads, the process of meiosis results in random segregation of alleles during gametogenesis. The two parentally transmitted alleles (case alleles) are compared to the two alleles (control alleles) that were not transmitted to the affected offspring. This technique of using nontransmitted parental alleles as controls was first described by Falk and Rubinstein in 1987.⁵⁵ The analysis of this type of data was further developed by Spielman *et al.*,⁵⁶ who developed the transmission disequilibrium test (TDT) (Fig. 1). Since the parents' nontransmitted alleles are used as the controls, population substructure will not increase the false-positive rate. The TDT has hitherto only been used to look for a specific allele at a specific locus. The TDT is usually only used to study candidate genes or, after a gene has been mapped, to aid in fine mapping. With the development of high-density SNP-based approaches, however, it has become feasible to perform TDT on a genome-wide basis.

The human genome project has been completed; there is now a reference map for 98% of the 3 billion base pairs that make up the code for human life. Attention is now turning to polymorphisms, since this is where we expect to find the links between genetic differences among populations or individuals and the diseases they acquire and the variations in their response to therapy. To this end, more than 2.5 millions SNPs have been mapped. It is estimated that the human genome contains some 5.3 million common SNPs. Therefore, most genes will contain or be closely adjacent to multiple SNPs that can be used for linkage and association studies. Fortunately, it does not appear as if it will be necessary to ascertain the status of millions of SNPs to genotype an individual. Recent observations by Patil *et al.*⁵⁷ show that it is not necessary to characterize every single known SNP for a particular individual. Simulations by Zhang *et al.*⁵⁸ testify to the validity of only using a select few SNPs to identify a particular haplotype at a particular locus. These highly informative SNPs have been called "tagSNPs" and an effort is underway to map the entire genome with respect to these tagSNPs. In October 2002, the National Human Genome Research Institute (a branch of the NIH) announced the creation of the International HapMap Project that will create a linkage disequilibrium map of the human genome and provide the information to the research community.⁵⁹

Developing Clinician-Scientists and Funding

A successful research program requires money, resources, protected time, and a trained investigator. Since this project straddles the ground between basic science research and a frustrating clinical entity that renders individuals permanently dysphonic, a grant application

was submitted to the National Institute on Deafness and other Communication Disorders (NIDCD) in February 2002. An External Advisory Committee was convened to oversee the project as well as the career development of the principal investigator. Drs. Bettie Steinberg and Stephen O'Brien, who are arguably the world's leaders in the fields of RRP research and the discovery of viral susceptibility genes, respectively, were willing to serve on the committee. The grant was subjected to the NIH's usual rigorous scientific peer review process and was awarded funding following one full grant application cycle.

For many reasons, there is a dearth of individuals developing careers at the interface between clinical practice and scientific investigation.

Many years ago, the model of a physician-scientist flourished, particularly in academic medical centers. The notion of spending half of one's time treating patients and the other half conducting research and teaching was viable. Frequently, the individual who chose this route would earn less than a peer who chose strict private practice but would find adequate compensation in the multifaceted gratification and in the high job security. Two major phenomena have maimed this model in the United States. Marketplace changes powered by the reimbursors of medical care have decreased the financial resources available to physicians and medical school departments through their clinical activities. Equally significantly, reimbursements to the large medical centers in which these physician-scientists have traditionally worked have been curtailed. Most physicians and medical centers have responded by cutting expenses (such as research and labor costs, such as nursing) and attempting to raise clinical volume, mostly through captured market share.

The other major change was the enforcement of billing regulations. Residents performed vast amounts of clinical work and were supervised to the level that each individual resident's skills demanded. Seeking to reduce health care costs, the government spearheaded billing fraud enforcement, and there have been several high-profile cases. Gone are the days of a physician working in a laboratory while one or two of his residents see patients, keeping the clinical revenues flowing. These changes raise barriers to a clinician-scientist career.

The NIH's mentored research career development award programs (K08 for scientific research and K23 for patient-oriented research in the clinical arena) seek to change this dynamic (<http://grants.nih.gov/training/kawardhp.htm>). The vast majority of K award funds are used to buy back the clinician's time from his or her department. The program also provides support for research costs (for example, supplies). Generally speaking, the dollar amount for supplies is not sufficient to completely carry out the proposed project; therefore, these types of efforts are generally placed in the context of a larger laboratory effort. Most importantly, the young physician conducts the research under the mentorship of experienced scientists, in a well established laboratory, with the consent and support of the department and institution. Funds are also applied to the rounding off of a physician's education to better equip him or her to become an independent scientist. The NIH has provided funding programs; the next step is for senior faculty and chairs to directly or indirectly support the process by apportioning the time and the effort to provide the critical mentoring.

CONCLUSION

The case for a genetic role in the development of RRP has been made and the current obstacles to determining that role have been defined. A combination of a novel technical approach, the cooperation of a group of concerned physicians who collectively have access to the largest population of patients with RRP ever assembled, the expertise of a gene discovery center, and the support of the National Institute on Deafness and Other Communication Disorders provides the components necessary to elucidate these genetic factors.

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BIBLIOGRAPHY

1. Derkay CS. Recurrent respiratory papillomatosis. *Laryngo-scope* 2001;111:57–69.
2. Armstrong LR, Preston EJ, Reichert M, et al. Incidence and prevalence of recurrent respiratory papillomatosis among children in Atlanta and Seattle. *Clin Infect Dis* 2000;31: 107–109. [PubMed: 10913405]
3. Armstrong LR, Derkay CS, Reeves WC. Initial results from the national registry for juvenile-onset recurrent respiratory papillomatosis. RRP Task Force. *Arch Otolaryngol Head Neck Surg* 1999;125:743–748. [PubMed: 10406310]
4. Hu FZ, Preston RA, Post JC, et al. Mapping of a gene for severe pediatric gastroesophageal reflux to chromosome 13q14. *JAMA* 2000;284:325–334. [PubMed: 10891964]
5. Preston RA, Post JC, Keats BJ, et al. A gene for Crouzon craniofacial dysostosis maps to the long arm of chromosome 10. *Nat Genet* 1994;7:149–153. [PubMed: 7920632]
6. Whitcomb DC, Gorry MC, Preston RA, et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996;14:141–145. [PubMed: 8841182]
7. Whitcomb DC, Preston RA, Aston CE, et al. A gene for hereditary pancreatitis maps to chromosome 7q35. *Gastroenterology* 1996; 110:1975–1980. [PubMed: 8964426]
8. Rimell FL, Shoemaker DL, Pou AM, et al. Pediatric respiratory papillomatosis: prognostic role of viral typing and cofactors. *Laryngoscope* 1997;107:915–918. [PubMed: 9217130]
9. Rabah R, Lancaster WD, Thomas R, Gregoire L. Human papillomavirus-11-associated recurrent respiratory papillomatosis is more aggressive than human papillomavirus-6-associated disease. *Pediatr Dev Pathol* 2001;4:68–72. [PubMed: 11200493]
10. Gelder CM, Williams OM, Hart KW, et al. HLA class II polymorphisms and susceptibility to recurrent respiratory papillomatosis. *J Virol* 2003;77:1927–1939. [PubMed: 12525627]
11. Bishai D, Kashima H, Shah K. The cost of juvenile-onset recurrent respiratory papillomatosis. *Arch Otolaryngol Head Neck Surg* 2000; 126:935–939. [PubMed: 10922223]
12. Healy GB, Gelber RD, Trowbridge AL, et al. Treatment of recurrent respiratory papillomatosis with human leukocyte interferon. Results of a multicenter randomized clinical trial. *N Engl J Med* 1988;319:401–407. [PubMed: 3398891]
13. Leventhal BG, Kashima HK, Week PW, et al. Randomized surgical adjuvant trial of interferon alfa-n1 in recurrent papillomatosis. *Arch Otolaryngol Head Neck Surg* 1988; 114:1163–1169. [PubMed: 3046638]
14. Pransky SM, Kang DR. Tumors of the larynx, trachea, and bronchi In Bluestone CD, Stool SE, Kenna MA, eds. *Pediatric Otolaryngology*. Philadelphia: W.B. Saunders, 1996: 1402–1414.

15. Glynn M, Sanford T, Hoover L, et al. Characterization of human papillomavirus in airway papillomas by histologic and biochemical analysis. *Ann Otol Rhinol Laryngol* 1999; 108:1073–1077. [PubMed: 10579235]
16. Rihkanen H, Aaltonen LM, Syrjanen SM. Human papilloma-virus in laryngeal papillomas and in adjacent normal epithelium. *Clin Otolaryngol Allied Sci* 1993;18:470–474. [PubMed: 8877222]
17. Maran A, Amelia CA, Di Lorenzo TP, et al. Human papillomavirus type 11 transcripts are present at low abundance in latently infected respiratory tissues. *Virology* 1995;212: 285–294. [PubMed: 7571398]
18. Penaloza-Plascencia M, Montoya-Fuentes H, Flores-Martinez SE, et al. Molecular identification of 7 human papillomavirus types in recurrent respiratory papillomatosis. *Arch Otolaryngol Head Neck Surg* 2000;126: 1119–1123. [PubMed: 10979126]
19. Gabbott M, Cossart YE, Kan A, et al. Human papillomavirus and host variables as predictors of clinical course in patients with juvenile-onset recurrent respiratory papillomatosis. *J Clin Microbiol* 1997;35:3098–3103. [PubMed: 9399501]
20. Pou AM, Rimell FL, Jordan JA, et al. Adult respiratory papillomatosis: human papillomavirus type and viral coinfections as predictors of prognosis. *Ann Otol Rhinol Laryngol* 1995;104:758–762. [PubMed: 7574251]
21. Badaracco G, Venuti A, Sedati A, Marcante ML. HPV16 and HPV18 in genital tumors: significantly different levels of viral integration and correlation to tumor invasiveness. *J Med Virol* 2002;67:574–582. [PubMed: 12116007]
22. Rady PL, Schnadig VJ, Weiss RL, et al. Malignant transformation of recurrent respiratory papillomatosis associated with integrated human papillomavirus type 11 DNA and mutation of p53. *Laryngoscope* 1998;108:735–740. [PubMed: 9591556]
23. Lukasiewicz E, Aractingi S, Flahault A. [Incidence and management of condylomata acuminata by French general physicians]. *Ann Dermatol Venereal* 2002;129:991–996.
24. Chuang TY, Perry HO, Kurland LT, Ilstrup DM. Condyloma acuminatum in Rochester, Minn., 1950–1978. I. Epidemiology and clinical features. *Arch Dermatol* 1984;120: 469–475. [PubMed: 6546656]
25. Sedlacek TV, Lindheim S, Eder C, et al. Mechanism for human papillomavirus transmission at birth. *Am J Obstet Gynecol* 1989;161:55–59. [PubMed: 2546429]
26. Eppel W, Worda C, Frigo P, et al. Human papillomavirus in the cervix and placenta. *Obstet Gynecol* 2000;96:337–341. [PubMed: 10960622]
27. Hording U, Iversen AK, Sebbelov A, et al. Prevalence of human papillomavirus types 11, 16 and 18 in cervical swabs. A study of 1362 pregnant women. *Eur J Obstet Gynecol Reprod Biol* 1990;35:191–198. [PubMed: 2159427]
28. Soares VR, Nieminen P, Aho M, et al. Human papillomavirus DNA in unselected pregnant and non-pregnant women. *Int JSTD AIDS* 1990;1:276–278.
29. Schneider A, Hotz M, Gissmann L. Increased prevalence of human papillomaviruses in the lower genital tract of pregnant women. *Int J Cancer* 1987;40:198–201. [PubMed: 3038757]
30. Shah K, Kashima H, Polk BF, et al. Rarity of cesarean delivery in cases of juvenile-onset respiratory papillomatosis. *Obstet Gynecol* 1986;68:795–799. [PubMed: 3785792]
31. Shah KV, Stern WF, Shah FK, et al. Risk factors for juvenile onset recurrent respiratory papillomatosis. *Pediatr Infect Dis J* 1998;17:372–376. [PubMed: 9613648]
32. Brown ZA, Selke S, Zeh J, et al. The acquisition of herpes simplex virus during pregnancy. *N Engl J Med* 1997;337: 509–515. [PubMed: 9262493]
33. Carter JJ, Koutsky LA, Hughes JP, et al. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. *J Infect Dis* 2000; 181:1911–1919. [PubMed: 10837170]
34. Bonagura VR, Hatam L, DeVoti J, et al. Recurrent respiratory papillomatosis: altered CD8(+) T-cell subsets and T(H)1/T(H)2 cytokine imbalance. *Clin Immunol* 1999;93: 302–311. [PubMed: 10600342]
35. Vambutas A, Bonagura VR, Steinberg BM. Altered expression of TAP-1 and major histocompatibility complex class I in laryngeal papillomatosis: correlation of TAP-1 with disease. *Clin Diagn Lab Immunol* 2000;7:79–85. [PubMed: 10618282]

36. Hill AV. The genomics and genetics of human infectious disease susceptibility. *Annu Rev Genomics Hum Genet* 2001;2:373–400. [PubMed: 11701655]
37. Jameson JL, Kopp P Principles of human genetics. In Braunwald E, ed. *Harrison's Online*. Vol. 2003: AccessMedicine.com, 2003.
38. Casselbrant ML, Mandel EM, Fall PA, et al. The heritability of otitis media: a twin and triplet study. *JAMA* 1999;282: 2125–2130. [PubMed: 10591333]
39. Dean M, Carrington M, Winkler C, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the *CCR5* structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort. *ALIVE Study Science* 1996;273:1856–1862. [PubMed: 8791590]
40. Carrington M, Nelson G, O'Brien SJ Considering genetic profiles in functional studies of immune responsiveness to HIV-1 *Immunol Lett* 2001;79 131–140 [PubMed: 11595300]
41. O'Brien SJ, Moore JP The effect of genetic variation in chemokines and their receptors on HIV transmission and progression to AIDS *Immunol Rev* 2000;177 99–111 [PubMed: 11138790]
42. Bonagura VR, Siegal FP, Abramson AL, et al. Enriched HLA-DQ3 phenotype and decreased class I major histocompatibility complex antigen expression in recurrent respiratory papillomatosis *Clin Diagn Lab Immunol* 1994;1 357–360 [PubMed: 7496977]
43. Aaltonen L, Partanen J, Auvinen E, et al. HLA-DQ alleles and human papillomavirus DNA in adult-onset laryngeal papillomatosis *J Infect Dis* 1999;179 682–685 [PubMed: 9952376]
44. Madeleine MM, Brumback B, Cushing-Haugen KL, et al. Human leukocyte antigen class II and cervical cancer risk a population-based study *J Infect Dis* 2002;186 1565–1574 [PubMed: 12447731]
45. Maciag PC, Schlecht NF, Souza PS, et al. Polymorphisms of the human leukocyte antigen *DRB1* and *DQB1* genes and the natural history of human papillomavirus infection *J Infect Dis* 2002;186 164–172 [PubMed: 12134251]
46. Storey A, Thomas M, Kalita A, et al. Role of a p53 polymorphism in the development of human papillomavirus-associated cancer *Nature* 1998;393 229–234 [PubMed: 9607760]
47. Madeleine MM, Shera K, Schwartz SM, et al. The p53 Arg72Pro polymorphism, human papillomavirus, and invasive squamous cell cervical cancer *Cancer Epidemiol Biomarkers Prev* 2000;9 225–227
48. Pegoraro R, Moodley J, Naiker S, et al. The p53 codon 72 polymorphism in black South African women and the risk of cervical cancer *BJOG* 2000;107 1164–1165 [PubMed: 11002963]
49. Minaguchi T, Kanamon Y, Matsushima M, et al. No evidence of correlation between polymorphism at codon 72 of p53 and risk of cervical cancer in Japanese patients with human papillomavirus 16/18 infection *Cancer Res* 1998;58 4585–4586 [PubMed: 9788606]
50. Rosenthal AN, Ryan A, Al-Jehani RM, et al. p53 Codon 72 polymorphism and risk of cervical cancer in UK *Lancet* 1998;352 871–872 [PubMed: 9742979]
51. Ghim S, Newsome J, Bell J, et al. Spontaneously regressing oral papillomas induce systemic antibodies that neutralize canine oral papillomavirus *Exp Mol Pathol* 2000;68 147–151 [PubMed: 10816383]
52. Nicholls PK, Klaunberg BA, Moore RA, et al. Naturally occurring, nonregressing canine oral papillomavirus infection host immunity, virus characterization, and experimental infection *Virology* 1999;265 365–374 [PubMed: 10600607]
53. Han R, Breitburd F, Marche PN, Orth G Linkage of regression and malignant conversion of rabbit viral papillomas to MHC class II genes *Nature* 1992;356 66–68 [PubMed: 1347151]
54. Pritchard JK, Stephens M, Rosenberg NA, Donnelly P Association mapping in structured populations *Am J Hum Genet* 2000; 67 170–181 [PubMed: 10827107]
55. Falk CT, Rubinstein P Haplotype relative risks an easy reliable way to construct a proper control sample for risk calculations *Ann Hum Genet* 1987; 51(Pt 3) 227–233 [PubMed: 3500674]
56. Spielman RS, McGinnis RE, Ewens WJ Transmission test for linkage disequilibrium the insulin gene region and insulin-dependent diabetes mellitus (IDDM) *Am J Hum Genet* 1993;52 506–516 [PubMed: 8447318]

57. Patil N, Beroz AJ, Hinds DA, et al. Blocks of limited haplo-type diversity revealed by high-resolution scanning of human chromosome 21 *Science* 2001, 294 1719–1723 [PubMed: 11721056]
58. Zhang K, Calabrese P, Nordborg M, Sun F Haplotype block structure and its applications to association studies power and study designs *Am J Hum Genet* 2002,71 1386–1394 [PubMed: 12439824]
59. International Consortium Launches Genetic Variation Mapping Project National Human Genome Research Institute, 2003 2002
60. Johnston D, Hall H, DiLorenzo TP, Steinberg BM Elevation of the epidermal growth factor receptor and dependent signaling in human papillomavirus-infected laryngeal papillomas *Cancer Res* 1999,59 968–974 [PubMed: 10029092]
61. Andresson T, Sparkowski J, Goldstein DJ, Schlegel R Vacuolar H(+) ATPase mutants transform cells and define a binding site for the papillomavirus E5 oncoprotein *J Biol Chem* 1995, 270 6830–6837 [PubMed: 7896830]
62. Hemmi H, Kaisho T, Takeuchi O, et al. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway *Nat Immunol* 2002,3 196–200 [PubMed: 11812998]

Fig. 1.

Transmission disequilibrium test If a specific locus neither confers susceptibility to nor protects from recurrent respiratory papillomatosis (RRP), then individuals with the disease whose parents have the genotype depicted would be equally divided among the four genotypes, namely AC, BC, AD, and BD. On the other hand, if the “shaded” allele (**B**) conferred susceptibility, then the BC and BD individuals would outnumber the AC and AD individuals. By knowing the parents’ genotype, we know what each affected patient’s genotype could have been. We hereby have the basis to compare observed genotype with

expected genotype. In this way, we do not have to confront issues such as inappropriate control subjects, and we do not have to be overly concerned with stratification of allele frequencies in various gene pools

TABLE I.

Candidate Host Genes (and Their Loci) that May Confer Susceptibility to Recurrent Respiratory Papillomavirus.*

Gene Family	Example	Location	Rationale
Major histocompatibility complex	HLA-DRB1: major histocompatibility complex, class II, DR β -1	6p21.3	Membranous proteins that display proteolyzed remnants of virally encoded proteins either on the surface of the originally infected host cell or on the surface of specialized antigen-presenting cells ¹⁰
Processors of virally encoded proteins	TAP1: transporter 1, ATP-binding cassette, subfamily B	6p21.3	Transports peptides resulting from proteolysis of viral proteins. ³⁵ It facilitates movement from the cytoplasm of the host cell into the endoplasmic reticulum. From there, the viral peptide can bind to an MHC protein.
Growth factor cascade	EGFR, epidermal growth factor receptor	7p12	Epithelial cells from laryngeal papillomas have high steady-state levels of EGFR on their membrane. EGFR pathway is important in manifesting the changes in differentiation seen in the papillomatous phenotype. ⁶⁰
Interferons	IFN α 1 interferon, alpha 1	9p22	Induced by viral infection and interfere with viral replication. Interferon- α is the most well established adjuvant therapy. ¹²¹³
Interleukins and their receptors	IL2R α : interleukin 2 receptor, alpha	10p15-p14	Interleukins and their receptors constitute the cytokine signaling system between leukocytes and therefore coordinate the immune response
ATPase vacuolar acidification proteins	ATP6V0C ATPase, H ⁺ transporting, lysosomal 16 kDa, V0 subunit c	16p13.3	Acidify vacuoles in the cell surface receptor recycling pathway. Early phase protein 5 (E5) of HPV associates with protein ⁶¹ and interferes with acidification, thereby diminishing EGFR degradation, which in turn results in higher steady-state levels of EGFR on the keratinocytes' surface.
Toll-like receptors	TLR7 toll-like receptor 7	Xp22.3	Recognize pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents (including viruses), and mediate the development of effective innate and adaptive immune responses. Recently marketed cream for condylomata acuminata, imiquimod, works by binding to TLR7. ⁶²

* These genes comprise the major histocompatibility complex, cytokines, interleukins, growth factors, and genes whose products are known to interact with human papillomavirus-encoded proteins

TABLE II.

Observed and Expected Numbers of Cases of Juvenile-Onset Recurrent Respiratory Papillomavirus (RRP) with a Particular Feature.^{31 *}

Factor	Sample Size, <i>n</i>	Observed, <i>n</i> (%)	Expected, <i>n</i> (%)
Cesarean section birth	138	6(4)	27 (20)
First born	128	85 (66)	51 (40)
Mother <20 y old	100	33 (33)	14(14)

* Information was obtained from a questionnaire given to patients' families by their attending otolaryngologist. Data were collected and compared with the prevalence of the specific factor among the entire population of the United States. Population data was obtained from the National Center for Health Statistics

Sample Size = number of patients with RRP for whom information was available; *Observed* = frequency of specific factor among studied sample of RRP patients: absolute number (proportion among those patients for whom the information is known); *Expected* = frequency of specific factor if patients with RRP were no different from the population of the United States.

The differences between the sample of RRP patients and the general population were all highly statistically significant, meaning that the probability of having data this extreme or more extreme if in reality there is no difference between RRP patients and the general population is less than 0.01.