The Value of Extended Pedigrees for Next-Generation Analysis of Complex Disease in the Rhesus Macaque

Amanda Vinson, Kamm Prongay, and Betsy Ferguson

Abstract

Complex diseases (e.g., cardiovascular disease and type 2 diabetes, among many others) pose the biggest threat to human health worldwide and are among the most challenging to investigate. Susceptibility to complex disease may be caused by multiple genetic variants (GVs) and their interaction, by environmental factors, and by interaction between GVs and environment, and large study cohorts with substantial analytical power are typically required to elucidate these individual contributions. Here, we discuss the advantages of both power and feasibility afforded by the use of extended pedigrees of rhesus macaques (*Macaca mulatta*) for genetic studies of complex human disease based on next-generation sequence data. We present these advantages in the context of previous research conducted in rhesus macaques for several representative complex diseases. We also describe a single, multigeneration pedigree of Indian-origin rhesus macaques and a sample biobank we have developed for genetic analysis of complex disease, including power of this pedigree to detect causal GVs using either genetic linkage or association methods in a variance decomposition approach. Finally, we summarize findings of significant heritability for a number of quantitative traits that demonstrate that genetic contributions to risk factors for complex disease can be detected and measured in this pedigree. We conclude that the development and application of an extended pedigree to analysis of complex disease traits in the rhesus macaque have shown promising early success and that genome-wide genetic and higher order -omics studies in this pedigree are likely to yield useful insights into the architecture of complex human disease.

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Introduction

Preventing or curing complex diseases such as cardiovascular disease (CVD), diabetes, addiction, macular degeneration, and arthritis, among many others, poses one of the biggest challenges to human health worldwide. Susceptibility to these diseases is determined by genes, by environmental influences, and by interaction both among genes and between genes and environment. Given this complexity, genetic analysis of complex disease aims to characterize the contribution of additive genetic effects relative to observed variation in the trait studied (i.e., heritability), to identify the comprehensive set of genetic variants (GVs) and their interactions that confer directional effects on the trait, and to describe how GVs and environment interact to influence trait expression. However, achieving any of these goals is no trivial matter because of the substantial analytical power required to detect the many GVs of varying effect size, and the corresponding large number of interactions expected to determine disease susceptibility. The need for sufficient power to support these analyses typically requires very large study cohorts of either unrelated (population-based) or related (family-based) individuals, either of which can be challenging to develop in many human populations. In contrast, large managed colonies of rhesus macaques (*Macaca mulatta*) with extensive pedigree information and close genetic similarity to humans are readily available at many primate research centers. Despite this obvious opportunity and the widespread use of the macaque as a model for human disease pathology, the application of pedigreed rhesus macaques to genetic discovery in complex human disease is currently nonexistent. In this article, we discuss advantages of feasibility and power gained when using extended pedigrees of rhesus macaques for genetic discovery based on next-generation sequence (NGS) data. We also present highlights of research conducted in rhesus macaques for selected complex human diseases to demonstrate how genome-wide genetic discovery in the rhesus macaque model would add to our knowledge of these diseases. Finally, we describe the development of an extended pedigree and sample biobank of Indian-origin

macaques at the Oregon National Primate Research Center (ONPRC) intended for genetic research in complex disease, and the results of initial studies of heritability in this pedigree for several quantitative risk factors for human disease.

Advantages of the Rhesus Macaque Model for Genetic Discovery in Complex Human Disease

Genetic Similarity to Humans and the Feasibility of Developing Extended Rhesus Macaque Pedigrees

Large, managed colonies of rhesus macaques such as those found at many primate research centers have inherent advantages that make developing extended pedigrees in macaques a viable alternative to population-based human study cohorts. These advantages start with a very close genetic and physiologic similarity to humans. The most recent common ancestor between macaque and human occurred approximately 25 million years ago. The rhesus macaque genome exhibits synteny for 89% of human genes and shares 93% mean sequence identity with the human genome (Rhesus Macaque Genome Sequencing and Analysis Consortium et al. 2007). This close degree of genetic similarity to humans, combined with the widespread use of rhesus macaques in physiologic studies of complex disease, makes this species an obvious choice for studying genetic effects on disease that will translate easily to the human condition, particularly for early stages of disease that are difficult or impossible to study in humans.

Rhesus macaques are also polygamous breeders and naturally form multi-male, multi-female groups in which males compete for access to females, a social grouping pattern reproduced in the group-housed macaques at the ONPRC. Male macaques can reproduce as early as 2 years of age, and large paternal half-sibship cohorts are common. Compared with the standard human generation time of 20 years, the breeding strategies and generation times found in rhesus macaques can produce very broad, multi-generational pedigrees over a much shorter time span. Further, because animals may live to their mid-20s in captivity, animals from several successive generations may be available for sampling and phenotyping at any single point in time. Combined with the multiple years of pedigree information available for many primate research center colonies, these advantages of breeding patterns and generation time make it eminently feasible to characterize very large and complex pedigrees with substantial analytical power for genetic studies. Moreover, because animals receive comprehensive veterinary care on an ongoing basis throughout the year, blood samples and whole-body phenotypes of interest may be collected on hundreds of animals per year at nominal cost by working closely with veterinary and animal care personnel during routine exams. In contrast, the human generation time and mobility common to many Westernized countries frequently limit human studies to population-based study designs with unrelated individuals. Such studies may require sample sizes that are orders of magnitude larger in order to reproduce the analytical power found in a smaller number of close relatives.

Power for Analysis of Rare Variants In Extended Rhesus Macaque Pedigrees

In recent years, the focus of most gene mapping studies in humans was on the contribution of common genetic variants to common complex disease, an approach implemented by the International HapMap Project (International HapMap Consortium 2003) and aided by the design of commercial technologies for surveying genetic variation in populations of unrelated individuals. However, the very small proportion of disease or trait heritability ultimately explained by this approach has led to much recent discussion of the basis for this "missing heritability." Although many hypotheses have been proposed to explain missing heritability, including epigenetic processes (Furrow et al. 2011), methodological deficiencies (Ehret et al. 2012; Zuk et al. 2012), and gene–environment or other interaction (Kaprio 2012), the hypothesis repeated most frequently is the previously neglected contribution of rare or low-frequency variation to heritability of complex disease (Manolio et al. 2009). Concurrent with the realization that rare variants may account for a substantial portion of missing heritability in complex disease, the cost of sequencing a whole genome has plummeted, and the ability to afford sequencing of whole or partial genomes for large numbers of individuals is now within reach. With the rapid approach of the $1000 genome, the field of complex disease genetics is shifting from genome-wide association studies (GWASs) based on common variants assessed using microarray technology to analysis of NGS data (exome and whole-genome), which reveals the entire complement of genetic variation, both common and rare, and allows the testing of contributions from rare variants to complex disease.

Pedigree-based approaches to mapping causal rare variants for complex disease from NGS data have several significant advantages over population-based approaches (Wilson and Ziegler 2011). For variants of approximately 1–5% frequency, population-based cohorts have been shown to have lower power to detect causal variants than same-sized family-based studies. This is due to the dilution of the effect size at the level of the population caused by the low frequency of variants and their effects (even when severe) on relatively small numbers of individuals. Thus, to the extent that rare variants contribute to heritability in complex disease, studies based on thousands or many thousands of unrelated individuals may be required to identify them. In contrast, rare variants are shared by many relatives in pedigrees, thus increasing their frequency in the study. This enrichment for rare variants in pedigrees results in increased locus-specific heritability (a function of both allele frequency and effect size) and improved power to identify causal variants. Moreover, pedigree-based studies are not only able to enrich for

rare variants but also may enrich for phenotypes of interest by focusing on lineages with many affected individuals or with substantial trait variation (Wilson and Ziegler 2011).

Family-based studies in rhesus macaques have the potential for even greater power to detect causal rare variants than such studies in humans because of the relative ease of developing very large and complex pedigrees that are not possible in human populations. The increased mobility and generation time of human subjects frequently limits family-based genetic studies in human populations to collections of nuclear families or sibling pairs or groups, a study design with significantly reduced power compared with the use of extended pedigrees (Williams and Blangero 1999). Moreover, rhesus macaque colonies may exhibit a level of coancestry similar to that found in human genetic isolates as a result of limited gene flow, and animals typically experience the same managed environment (e.g., diet, housing), both factors that enhance power to detect genetic signal over noise. Given the advantages outlined above, pedigree-based approaches appear a far more desirable choice than population-based approaches for the study of complex disease genetics in the rhesus macaque model.

Value of Rhesus Macaque Pedigrees to Preclinical and Translational Studies

Large rhesus macaque pedigrees may also provide unique advantages in preclinical and translational studies, such as the ability to estimate the relative contributions to disease risk or response to therapeutics from inherited risk variants, specified environments, and family history. The advantages to conducting such studies in pedigreed rhesus macaques include the usual ones of power, feasibility, and genetic similarity, and add knowledge of inheritance patterns, access to detailed clinical family history, and the ability to rigorously control environmental factors. The potential utility of decomposing response or disease risk into underlying factors is suggested by a recent study by Do and colleagues (2012) that showed that family history may be a better predictor of disease liability than single nucleotide polymorphism (SNP)–based models for high frequency diseases with large heritability (e.g., coronary artery disease, Alzheimer's disease), whereas for diseases of low frequency (e.g., celiac disease, Parkinson disease), SNP-based models perform as well or better than family history. Although differences in DNA sequence and allele frequencies between rhesus macaques and humans may preclude the application of genetic risk models developed in monkeys to all clinical disease in humans, such approaches may have particular utility for diseases influenced by genetic variants that are highly conserved between macaques and humans. Another potential translational application of large rhesus macaque pedigrees is the development of immortalized cell lines from animals of known relationship. Such a resource would allow the estimation of heritability and the application of gene mapping and systems genetics approaches to molecular and cellular

response to experimental challenge and drug therapies measured in vitro.

General Challenges to Detecting Causal Rare Variants from NGS Data

Given the interest in the contribution of rare variants to complex disease, the enrichment for rare variants in pedigrees, and the ability to comprehensively survey all genetic variation in the genome at ever-decreasing cost, it is not surprising that there has been renewed interest in family-based designs in genome-wide studies. Both linkage analysis and association methods, as well as approaches that incorporate both types of information, may be applied in pedigrees with genome-wide sequence data to narrow the region of interest and to identify potentially causal variants. However, there are considerably more variants discovered in a NGS study, and because of this, there will be much greater correlation among these variants than seen previously from studies based on more sparse marker data, both within and across linkage disequilibrium blocks (Thomas et al. 2011; Tintle et al. 2011). This increased correlation may be caused by linkage or gametic phase disequilibrium, quality of the sequence alignment, or simple random chance. Failure to address this issue appears to inflate type I error and apparent power for both family- and population-based methods when there is a true but unknown genetic contribution to the phenotype.

To analyze rare sequence variants, it is necessary to aggregate them into new derived variables, and the success of various approaches to solve this problem can rely on assumptions about the direction of effects and may be constrained by variable numbers of sequence variants within a gene or other defined region of analysis (Ye and Engelman 2011). However, if there is prior information available on the variants themselves or on their effects on the trait (e.g., nonsynonymous or synonymous substitution effects, candidate genes, biological pathways, or gene networks) that reflects the true underlying etiology of a trait, the use of this prior information may be used to aggregate variants informatively and improve power to detect causal variants (Chen et al. 2011). The informative aggregation of variants may also alleviate the multiple testing burden in sequence-based studies, a problem that is greatly magnified by the sheer number of variants discovered in these studies relative to the number of markers used in a standard GWAS. A lack of prior information on which to aggregate sequence variants, particularly in intergenic regions, may prove a considerable challenge for studies relying on whole-genome sequence data. These and related problems are currently an active area of research.

The Rhesus Macaque Model for Common Complex Human Disease

The rhesus macaque is a well-established model for pathologic processes in many complex human diseases, and a

number of studies have employed a candidate gene approach to explore genetic contributions to these pathologies. However, we currently lack appropriate study populations to enable a move beyond the candidate gene approach toward genome-wide genetic discovery in this species. To underscore the need for pedigreed study populations that will support genome-wide studies in the rhesus macaque model, we briefly summarize key findings from studies of pathology and genetic contributions to pathology in both humans and macaques for a representative set of diseases with significant impact on public health. For these and other human diseases in which significant heritability is undisputed but for which many contributing genetic factors remain unknown, an extended pedigree of phenotyped rhesus macaques can provide a powerful resource for discovering new genetic variants that influence disease risk, and for translating these findings to the human condition.

Atherosclerosis

The rhesus macaque has a lengthy history as a nonhuman primate model for human atherosclerosis, particularly in response to experimental diets rich in fat and cholesterol. Depending on the type and amount of dietary fat and cholesterol and the length of time fed, rhesus macaques develop either mild hypercholesterolemia similar to that commonly seen in the general human population or the more extreme hypercholesterolemia seen in familial human disease (Bhattacharyya and Eggen 1987; Bond et al. 1980; Newman et al. 1974; Pronczuk et al. 1991; Rudel 1997). Coincident with this increase in plasma cholesterol and in direct proportion to the length of time on the experimental diet, rhesus macaques develop increasingly severe atherosclerosis that is strikingly similar to that observed in humans. Observed stages of lesion development include the retention of lipid-rich macrophages (foam cells) and other immune cells in the subendothelium, the appearance of fatty streaks in the aorta and coronary arteries, the progression from fatty streak to uncomplicated fibrous atheroma with proliferation of smooth muscle cells, and eventually the progression to more complicated lesions characterized by intimal necrosis, calcification, and hemorrhage (Armstrong 1976; Bond et al. 1980; Davis and Wissler 1984; Gresham 1976). Of particular note, rhesus macaques fed experimental diets have also been observed to progress to clinically relevant stages of disease (Williams et al. 1991), including ischemia with significant stenosis of the coronary arteries and sudden death resulting from occlusive thrombosis and myocardial infarction (Bond et al. 1980).

Despite these observations and the indisputable contribution of genetics to CVD in humans (Roberts and Stewart 2012; Schunkert et al. 2011), virtually nothing is known about the genetic basis of susceptibility to atherosclerosis or associated risk factors in rhesus macaques. This is unfortunate, given that the rhesus macaque model offers researchers the ability to investigate rigorously aspects of atherogenesis that may be difficult or impossible to study in humans, such as genetic susceptibility to early disease processes and gene-by-environment interaction. The likelihood that such studies would be fruitful may be inferred from the virtually complete identity between macaques and humans for a gene known to influence proatherogenic lipid levels in humans, *LDLR*, which codes for the low-density lipoprotein (LDL) receptor. The rhesus macaque *LDLR* exhibits 95–100% identity with the human *LDLR* and its corresponding protein at multiple levels of organization, including gene length, open reading frame length, protein length, and mRNA sequence, and 85–100% identity across functional domains in the protein (Kassim et al. 2011; Südhof et al. 1985). This degree of genetic similarity in *LDLR* corresponds well with clinical symptoms shared between macaques and humans with familial hypercholesterolemia (Kusumi et al. 1993), associated with mutations in *LDLR* in both species (Hummel et al. 1990). Given the increases in circulating lipids, correlated increases in atherosclerosis, and clinical symptoms of CVD in rhesus macaques fed experimental diets, and the similarities observed in macaques and humans for familial disease, it seems likely that genome-wide studies conducted in rhesus macaques will identify both known and novel genetic variants that increase susceptibility to atherosclerosis in both species.

Obesity and Type 2 Diabetes Mellitus

Both spontaneous and induced obesity are well-known in rhesus macaques, and, similar to humans, macaques with increasing adiposity progress from dysregulation of glucose metabolism to insulin resistance and ultimately to clinical type 2 diabetes mellitus (T2DM). In studies of spontaneous (noninduced) obesity in individually housed, male rhesus macaques of middle age or older, only one-third could be described as nonobese, whereas two-thirds were either moderately or very obese, despite maintenance on a low-fat, low-cholesterol chow diet (Kemnitz and Francken 1986; Kemnitz et al. 1989). Obese macaques showed a pattern of abdominal excess fat deposition very similar to that seen in obese humans, with abdominal circumference accounting for the largest differences in body dimensions between obese and nonobese animals. Changes in body size are correlated with age and sex, even in free-ranging macaques; body weight and central fat deposition are greatest in females aged 10 to 14 years and in males aged 15 to 19 years (Schwartz and Kemnitz 1992). These findings suggest that rhesus macaques have a natural propensity toward weight gain and obesity in middle age and beyond, similar to observations in humans.

Obesity in rhesus macaques is associated with metabolic dysfunction. Obese macaques have elevated fasting serum insulin, higher fasting plasma glucose levels, slower glucose disappearance rates, and higher triglycerides than nonobese monkeys (Kemnitz and Francken 1986). In particular, central

obesity appears to be the most strongly predictive of metabolic dysfunction; abdominal circumference, which together with body mass index (BMI) explains 96% of variance in body fat, is strongly correlated with fasting plasma insulin levels and with insulin resistance. Central obesity in rhesus macaques also appears to be an excellent predictor of T2DM, which develops following increases in body weight and fat, changes in plasma insulin, and acute response to glucose challenge, and may ultimately include pancreatic islet amyloid deposition and decreases in the number of islet β cells (reviewed in Bauer et al. 2011; Bodkin and Hansen 1988; de Koning et al. 1993; Kahn et al. 2001).

The role of genetic influences on obesity in humans is well-established, as indicated by very large recent GWAS of BMI and waist-to-hip ratio, a measure of central obesity in humans (Heid et al. 2010; Speliotes et al. 2010). One two-stage GWAS conducted in a total 249,796 individuals found new loci and replicated previous results for 32 variants associated with BMI that accounted for 6–11% of genetic variation in this trait. Because the distribution of fat has a different genetic etiology than that influencing BMI, a separate GWAS of waist-to-hip ratio was conducted in 190,803 individuals. This study revealed 14 loci associated with waist-to-hip ratio that explained approximately 1% of total variance in this trait, with several of these loci exhibiting evidence for sexual dimorphism. In rhesus macaques, despite evidence supporting a relationship between central obesity and metabolic dysregulation that predisposes animals to the development of T2DM, no studies have yet investigated a genetic basis for traits related to obesity.

In addition to spontaneous obesity featuring impaired glucose tolerance, insulin resistance, and progression to T2DM, the rhesus macaque also develops additional symptoms of human metabolic syndrome when challenged with experimental diets associated with insulin resistance in humans. Bremer and colleagues (2011) recently tested the hypothesis that rhesus macaques consuming beverages sweetened with fructose (containing approximately 50% sucrose and high-fructose corn syrup) would develop insulin resistance and metabolic syndrome, with some progressing to overt T2DM. After being fed fructose as a supplement to a standard commercial monkey chow diet daily for 1 year, all macaques developed components of the metabolic syndrome, primarily as increased adiposity, insulin resistance, inflammation, and dyslipidemia, and 4 of the 29 macaques developed overt T2DM.

Genetic studies of T2DM in rhesus macaques are only just beginning. Notably, a recent exploratory GWAS conducted in 14 macaques with prediabetes or T2DM and eight healthy control macaques using a human SNP microarray (Hansen et al. 2011) revealed a candidate causal genetic variant associated with T2DM. Although a lack of power prevented results from achieving a standard threshold of significance, Hansen and colleagues (2011) combined results from a lowered significance threshold with analysis of gene expression in liver, heart, and skeletal muscle from 51 macaques to identify *CBLN2*, a member of a gene family coding for a precursor protein CER, which is expressed in rat pancreas and controls levels of plasma insulin. The association of this gene with T2DM in rhesus macaques received independent support from its association with insulin resistance in 1449 T2DM patients and 1482 matched control subjects from Finland and Sweden. These results underscore the urgent need for more powerful mechanisms of genetic discovery in the rhesus macaque, and provide encouraging evidence that further genetic study of T2DM in this species is likely to be productive.

Macular Degeneration

Given the rapid expansion in the number of adults aged 65 years and older in the United States expected in the next 20 years, common late-onset diseases such as age-related macular degeneration (AMD) will likely be the subject of increasing investigation. Studies of AMD in humans have demonstrated high heritability for both early and late forms of disease and have repeatedly implicated SNPs in both a gene coding for complement factor H (*CFH*) and in a transcribed region of unknown function (age-related maculopathy susceptibility 2 [*ARMS2*]) (Holliday et al. 2013). Rhesus macaques are an excellent physiologic model for AMD based on the close resemblance between the macaque and human eye (Francis et al. 2008; Hope et al. 1992). These similarities include a macula with a foveal pit containing structural characteristics important for spatial acuity, a feature shared only among the Old World monkeys, apes, and humans. Macaques spontaneously develop both drusen (yellow-white protein aggregates in the retinal pigment epithelium) that accumulate with age and pigmentary changes in the macula that mimic hallmarks of early to intermediate human macular disease.

In humans, AMD is associated with two SNPs found in *ARMS2* and in the adjacent promoter region of *HTRA1*, which codes for a serine protease and is known to be in complete linkage disequilibrium with *LOC3877145/ARMS2* in white, Japanese, and Chinese populations (Dewan et al. 2006; Yang et al. 2006). A recent sequencing study of these two locations in rhesus macaques revealed that, as in humans, these gene regions are adjacent, and additionally described two SNP variants associated with drusen in macaques, one variant in each region. Unlike in humans, these two variants were in high but not complete linkage disequilibrium. In macaques, the *HTRA1* variant has functional consequences for gene expression, but the role of the *ARMS2* variant is still not fully understood. The replicated association of the *ARMS2* and *HTRA1* genetic variants with drusen in macaques suggests that this species is likely to be an excellent genetic model for AMD; however, the ability to expand to genome-wide studies of AMD in macaques is currently constrained by the lack of

resources to enable such studies, including appropriate study populations.

Alcohol Abuse and Dependence

Rhesus macaques share many features of alcohol abuse disorders with humans, including behavioral and physiologic responses to alcohol and genetic influences on these responses (Barr 2013; Grant and Bennett 2003; Schwandt et al. 2010). For example, chronic alcohol consumption has been shown to alter hypothalamic-pituitary-adrenal (HPA) axis activity, the neuroendocrine stress response pathway (Boschloo et al. 2011). Candidate gene studies testing this hypothesis in rhesus macaques have demonstrated that *NPY* (Lindell et al. 2010), *DRD1* (Newman et al. 2009), and *CRH* (Barr et al. 2008), genes coding for proteins involved in neuroendocrine regulation of stress, are associated with HPA axis dysfunction and with alcohol consumption in this species. The interaction between HPA axis dysfunction and alcohol consumption in rhesus macaques suggests that, as with humans, there may be common genetic links between neuroendocrine stress response and alcohol use.

Numerous studies have also found strong similarities between macaques and humans linking alcohol-related behaviors to the function of neurotransmitters associated with mood, emotion, and the experience of reward. Dysfunction in the pathway regulating serotonin has been linked with a low response to alcohol, impaired impulse control, and early-onset alcoholism in humans (Hinckers et al. 2006). In support of a similar effect in macaques, one recent study identified an additive effect on HPA axis dysfunction in macaques from variants in two genes that influence serotonin signaling (*TPH2*, involved in the biosynthesis of serotonin, and *SLC6A4*, which codes for the serotonin transport protein), in addition to those found in the *CRH* gene (Ferguson et al. 2012). Similar to humans, rhesus macaques have a variable number repeat polymorphism in the transcriptional control region of the serotonin transporter gene (*SLC6A4*), commonly known as 5HTT-LPR, that influences its expression and predicts intoxication in rhesus macaques reared without mothers, suggesting a genotype-by-environment interaction effect on intoxication (Barr et al. 2003). The endogenous opioid system in humans is implicated in the experience of reward and has also been linked to vulnerability to addiction (Oswald and Wand 2004). Similar to humans, macaques carry a nonsynonymous mutation in *OPRM1*, the mu-opioid receptor gene, that has been associated with increased alcohol-induced stimulation, increased alcohol consumption, and increased frequency of intoxication, particularly in males (Barr et al. 2007), also consistent with findings in humans. These results suggest a substantial genetic component to alcohol-related physiologic response and behavior and highlight the need for genome-wide genetic analysis of traits related to alcohol abuse and dependence.

The Potential of the Rhesus Macaque as a Model for Other Complex Human Disease

Acute and Chronic Colitis in Rhesus Macaques

In addition to serving as models for human disease, captive-bred rhesus macaques also spontaneously develop disease that appears similar to human disease but for which the macaque-specific etiology and overlap with human disease are not well understood. Colitis in rhesus macaques is characterized by dehydration, lethargy, loose stool and/or dysentery, electrolyte imbalance, and metabolic acidosis. Both acute and chronic colitis represent a significant cause of morbidity and mortality in managed colonies of rhesus macaques. In 2010, among the 3181 outdoor-housed rhesus macaques at the ONPRC, 250 animals accounted for more than 700 clinical cases of diarrhea, an incidence rate of 7.8%. Of these, 103 animals were euthanized because of severe, acute colitis or chronic colitis and wasting, resulting in a mortality rate of 3.2% (Prongay et al. 2013). Similar rates have been reported elsewhere (Hird et al. 1984; Schneider et al. 1960).

Macaque colitis may have heterogeneous underlying etiologies, although evidence suggests a significant role for both genetic and environmental factors, the hallmark of complex disease. To date, most research has focused on environmental causes of colitis. Of note, some of the most successful models of environmental colitis are developed around pathogens that can be isolated from both sick and healthy animals. Of these, the rhesus model of acute bacillary dysentery, using oral administration of *Shigella flexneri*, is perhaps the best characterized (Kinsey et al. 1976; Mulder 1971; Pucak et al. 1977; Takeuchi et al. 1968) and provided early insight into the microscopic features and cellular pathophysiology of this syndrome. Bacterial models of chronic colitis, using *Camphylobacter* species (Tribe and Fleming 1983) and *Escherichia coli* (Kang et al. 2001), two bacteria commonly isolated from healthy animals, have also been developed and may prove to be useful models for human inflammatory bowel disease (Sestak et al. 2003). In addition, the association between viral infection and diarrheal disease remains a dynamic area of research. Rhesus macaques experimentally infected with simian immunodeficiency virus may develop chronic colitis, similar to human immunodeficiency virus patients. Disease severity and lesion location varies among individuals, and these differences have recently been correlated with interleukin 6 and SOC-3 gene expression (Mohan et al. 2007). This variation in symptoms is also seen among animals infected with adenovirus. Early studies implicated adenovirus in chronic colitis (Sestak et al. 2003; Stuker et al. 1979), but recent reports suggest the virus is also prevalent in asymptomatic carriers (Roy et al. 2012). Among animals infected with the same bacterial or viral pathogens, substantial variability in symptoms suggests the possibility of genetic variation among individuals in susceptibility or response to infection.

In addition to genetic susceptibility to environmental pathogens, genetic variation in response to other environmental

stress may also contribute to colitis. Early weaning and social stress are both strongly correlated with disease. For example, nursery-reared animals have diarrhea rates 7.5 times higher than infants reared with mothers (Elmore et al. 1992), and the rate of diarrhea among nursery-reared animals is 3 to 4 times higher than that for animals reared in any other housing type (Hird et al. 1984). Indoor, group-housed animals and animals in single or paired housing have higher diarrhea rates than outdoor, group-housed animals (Hird et al. 1984; Sestak et al. 2003; Wolfensohn 1998). Historically, these differences have been attributed purely to a lack of maternal or other environmental influence, but associations are increasingly made between genetic variation and environmental factors likely to cause social stress. For example, polymorphisms in the gene coding for the serotonin transporter (*SLC6A4*) have been implicated in differences in behavioral response to stress between macaque infants with secure attachments to their mother and infants lacking parental attachment (Suomi 2011).

Dietary sensitivities also offer a new and potentially powerful model of genetic susceptibility to colitis. A subset of rhesus macaques with noninfectious chronic colitis appear to have inherited gluten sensitivity, similar to human celiac disease, although genetic analysis is still ongoing (Sestak et al. 2011). This finding suggests that the rhesus macaque may be a good model for genetic susceptibility to human celiac disease, which has been associated with variants in the *TAGAP* gene (Eyre et al. 2010).

Degenerative and Inflammatory Arthritis in Rhesus Macaques

Rhesus macaques spontaneously develop both degenerative and inflammatory arthritis, and similarities in structural, mechanical loading, and connective tissue properties in the joint make this an attractive model for human disease (Châteauvert et al. 1989; Châteauvert et al. 1990). Naturally occurring osteoarthritis and spondyloarthropathy has been extensively studied in a troop affiliated with the Cayo Santiago colony at the Caribbean Primate Research Center in Cayo Santiago, Puerto Rico. Spondyloarthropathy was identified in 20% of the animals aged 8 years or older and, similar to humans, females had higher disease incidence (Pritzker et al. 1989; Rothschild et al. 1997). A metabolically associated degenerative osteoarthritis similar to human calcium pyrophosphate dihydrate crystal deposition disease was also identified in this population (Pritzker et al. 1989; Rothschild et al. 1999). Additionally, estrogen-depleted bone loss has been confirmed in studies of aging adult female macaques at the Wisconsin National Primate Research Center (Colman et al. 1999). More recently, age-associated disc space narrowing and osteophytosis was confirmed in a cohort of animals followed for 11 years at the same center (Duncan et al. 2011).

The heterogeneous and progressive nature of these various arthropathies suggests significant similarities to human disease, and their pathology has been explored experimentally in rhesus macaques. For example, type II collagen-induced arthritis (CIA), a model for rheumatoid arthritis, is induced in 70% of animals inoculated with type II collagen (Bakker et al. 1990). Genetic susceptibility to CIA has been implicated in rhesus macaques by association of CIA with the major histocompatibility complex (MHC) class I region (Bakker et al. 1992), and further studies demonstrated that the MHC class 1 allele Mamu-B26 was protective for CIA (Vierboom et al. 2005; Vierboom et al. 2007). A similar pattern of immune-mediated resistance to CIA was noted in 2000 at the Wisconsin National Primate Research Center after an outbreak of *Shigella* bacteria. Similarly, a cohort of macaques was reported to develop reactive arthritis, an inflammatory spondyloarthropathy, in response to infectious enterocolitis (Rothschild 2005), and a protective effect of the MHC A locus allele, Mamu-A*12, was associated with resistance to this arthropathy (Urvater et al. 2000). Further research in rhesus macaques aimed at clarifying the heterogeneity among arthropathies, characterizing genetic susceptibility and environmental influences on disease, and exploring overlap with human disease is urgently needed.

The ONPRC Research Pedigree and Sample Biobank

Development of an Extended Pedigree at the ONPRC for Genetic Analysis of Complex Traits in the Indian-Origin Rhesus Macaque

Based on the advantages described in this paper, we aimed to develop a single, extended pedigree of Indian-origin rhesus macaques for the purpose of genome-wide genetic analysis of quantitative risk factors for complex human disease. This pedigree was characterized by developing custom Python scripts to select a set of animals from the approximately 4500-member colony of rhesus macaques housed at the ONPRC that optimized several criteria. Animals must (1) be of pure Indian ancestry, (2) have parentage assignment based on genotypes at 12 to 28 microsatellite loci, (3) be a member of a minimum three-generation vertical lineage, (4) be available for sampling, (5) have no ancestors in common outside the pedigree, and (6) form a single pedigree configuration. The resulting pedigree contains 1289 rhesus macaques, including 800 females and 489 males, and spans six generations (see Figure 1). This pedigree represents approximately 29% of the total population of rhesus macaques found at the ONPRC. Animals range in age from 1.6 to 28.5 years, corresponding to a developmental age range of 4.8 to 85.5 human years (the distribution of age by sex in this pedigree is summarized in Figure 2). Table 1 summarizes the most frequent relationship classes found within this pedigree; these relationships provide hypotheses of excess alleles shared
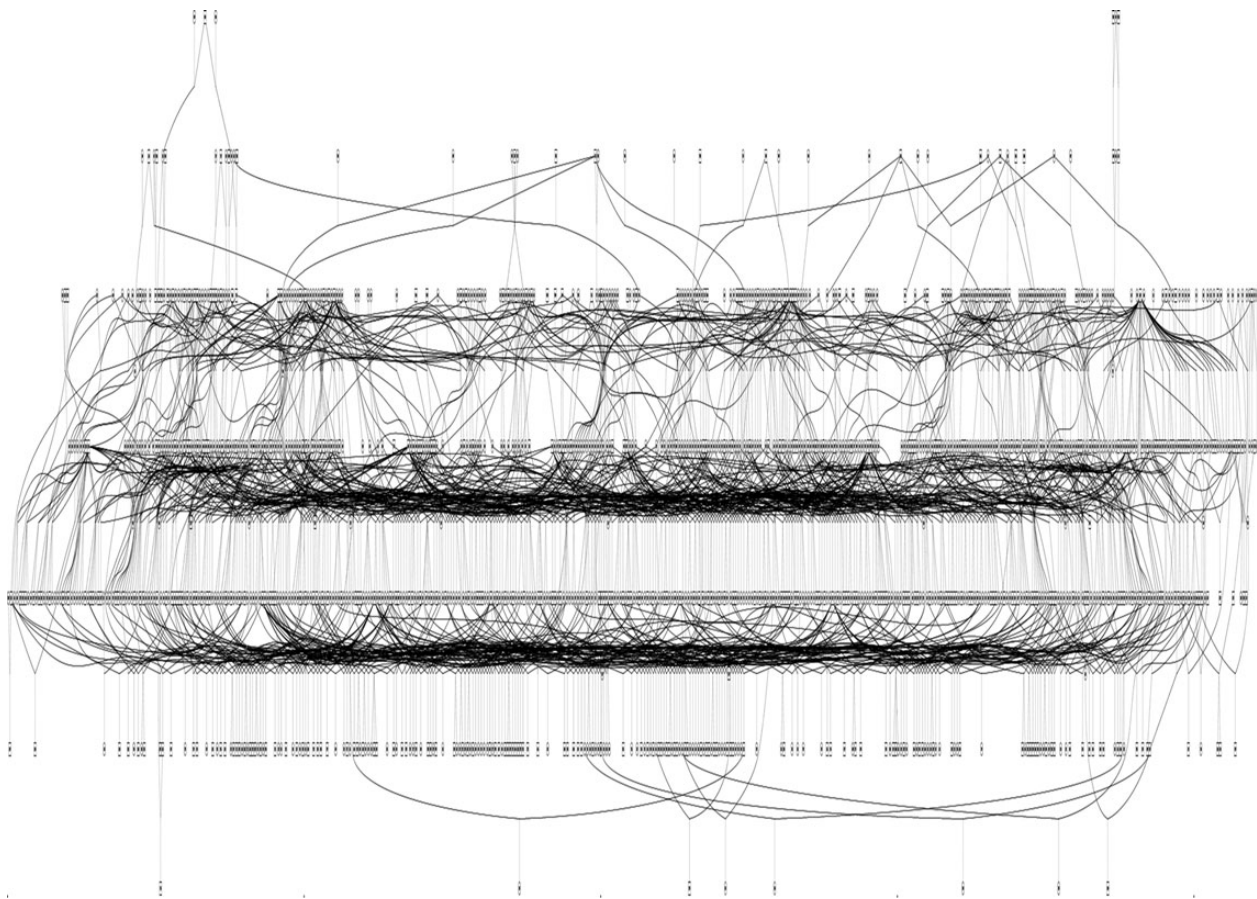
Figure 1 Diagram of the 1289-member research pedigree of Oregon National Primate Research Center rhesus macaques
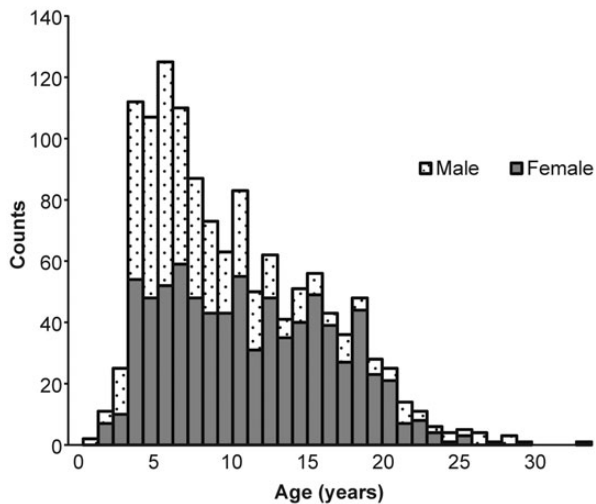

Figure 2 Distribution of age and sex among the 1289 rhesus macaques in the Oregon National Primate Research Center research pedigree.

identical by descent among macaques, and the large number and complexity of these relationships is the source of power in genetic analysis conducted in extended pedigrees.

Sample Biobank and Whole-Body Phenotypes

To enable extensive phenotyping of pedigree members, we collected whole blood samples on these macaques under an approved institutional animal care and use committee protocol. All samples were collected after an overnight fast and at a consistent time of day (approximately 10:00 AM) (see Table 2). Whole blood was processed for serum, plasma, leukocytes, and peripheral blood mononuclear cells and aliquoted separately in RNAlater (Ambion) to support transcriptome analysis and for storage in liquid nitrogen to enable viable cell assays. As of this writing, approximately 95% (n = 1229) of the macaques in this pedigree have at minimum a source of high-quality DNA that will enable analysis of NGS data, and approximately 66% (n = 852) have at least banked serum and plasma to support multiple biomarker assays. We have also collected multiple measures of morphometry and adiposity on approximately 730 of the same animals, including crown–heel, rump–heel, and crown–rump lengths, chest circumference, maximum girth, abdominal circumference, circumference of the upper arm and thigh, and measures of skin-fold thickness at the subscapular, upper arm/triceps, and thigh. Importantly, the vast majority of these samples and phenotypes were collected during routine processing of animals in group housing

Table 1 Summary of the most frequent relationship classes found within the single, 1289-member pedigree described

Relationship	No.	Relationship	No.
Unrelated	890,106	Half-2nd cousins	1284
Parent–offspring	1966	Half-siblings and half-1st cousins	371
Siblings	165	Half-siblings and half-avuncular	73
Grandparent–grandchild	2150	Double half-avuncular	193
Avuncular	222	Double half-1st cousins	358
Half-siblings	6954	Half-2nd cousins, once removed	128
Great-grandparent–grandchild	1027	Half-1st cousins, twice removed	209
Grand-avuncular	56	Half-great-grand avuncular	98
Half-avuncular	13,179	Half-1st cousins and half-avuncular	142
1st cousins	156	Half-avuncular and half-grand avuncular	30
Great-great-grandparent–grandchild	84	Half-avuncular and half-1st cousins, once removed	330
Half-grand avuncular	3079	Half-1st cousins and half-2nd cousins	139
1st cousins, once removed	128	Parent–offspring and half-avuncular	16
Half-1st cousins	10,413	Half-1st cousins, once removed and half-2nd cousins, once removed	14
Half-1st cousins, once removed	6740	Half-sibs and half-2nd cousins	24
2nd cousins	27		

Table 2 Summary of sample inventory collected on the 1289-member pedigree

Sample type	Pedigreed macaques with samples/measures
Serum	854
Plasma	852
Leukocytes (for high-quality genomic DNA)	852
PBMCs for gene expression	708
PBMCs for viable cell assays	713
Measures of body morphometry	732
Measures of adiposity	725

See text for description of measures of body morphometry and adiposity. PBMC, peripheral blood mononuclear cell.

and did not require the assignment of animals to research. This approach minimizes costs and increases feasibility of large-scale phenotyping efforts and does not interfere with animal assignment to other research projects.

Power of the Pedigree to Detect Heritability in a Quantitative Trait

Our intended focus is primarily on the study of quantitative endophenotypes related to disease risk because they are closer to genetic regulation than clinical diagnoses and thus likely to be more informative for gene mapping. Given this focus, we assessed the power of this pedigree to detect significant heritability in quantitative traits across a wide range of true heritabilities (0.15–0.80) under the assumption that all individuals are phenotyped, using a maximum likelihood–based variance decomposition approach implemented in SOLAR software (Sequential Oligogenic Linkage Analysis Routines, v.4.2.0, Texas Biomedical Research Institute, San Antonio, TX). These results indicate that the power to detect an additive genetic contribution to quantitative trait variation using this pedigree is excellent, with approximately 85% power to detect trait heritabilities as low as 0.08 and 100% power to detect trait heritabilities as low as 0.16 (see Figure 3).

Power of the Pedigree to Detect Linkage to a Locus Influencing a Quantitative Trait

Because linkage analysis can reduce the genomic search space, lower the multiple testing burden, and provide prior information that may increase power to fine-map causal genetic variants, we examined the power of the pedigree to detect linkage to a locus influencing a quantitative trait (i.e., a quantitative trait locus [QTL]) using the same variance decomposition approach. Power to detect a locus with a log-of-odds (LOD) score of 3 was examined for QTL heritabilities up to 0.75, assuming that all individuals are phenotyped,
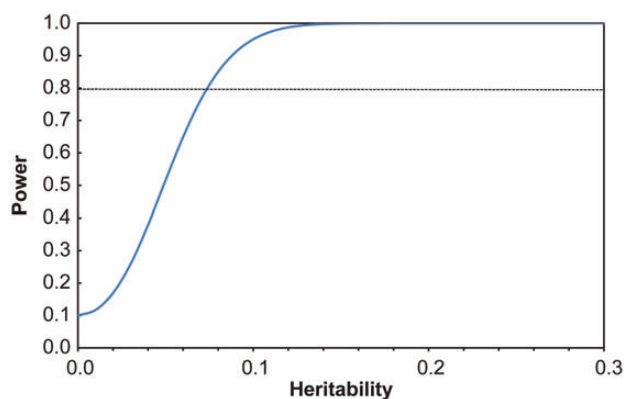
Figure 3 Power of the pedigree to detect heritability for a quantitative trait using a maximum likelihood–based variance decomposition approach. Analysis conducted in SOLAR (v.4.2 Texas Biomedical Research Institute, San Antonio, TX).
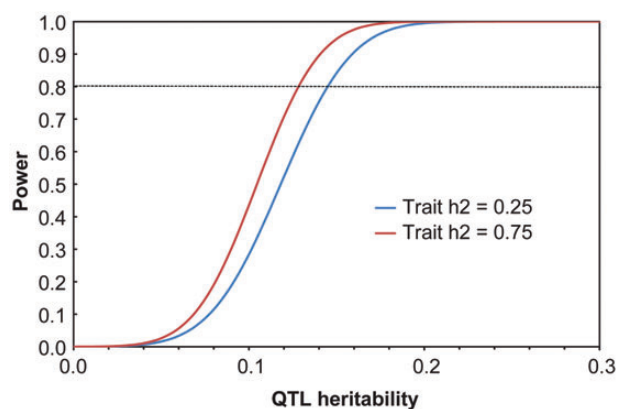

Figure 4 Power of the pedigree to detect linkage to a locus influencing a quantitative trait with a log-of-odds score of 3 across a range of quantitative trait locus heritabilities using a maximum likelihood–based variance decomposition approach. Power for two traits with total heritabilities of 0.25 (blue) and 0.75 (red) is considered. Analyses conducted in SOLAR (v.4.2, Texas Biomedical Research Institute, San Antonio, TX).

in two situations in which total heritability of the trait is 0.25 and 0.75 and residual heritability contributes to the power to detect the QTL (see Figure 4). The power of this pedigree to detect a QTL with an LOD score of 3 (i.e., indicating genome-wide significant evidence) is very good across this range of trait and QTL heritabilities. For example, considering a trait with a total heritability of 0.25, this pedigree provides greater than 80% power to detect a QTL heritability of 0.15; as total trait heritability increases to 0.75, we have greater than 80% power to detect a QTL heritability of 0.13. These QTL heritabilities are smaller or equivalent in magnitude to those reported in several previous QTL and eQTL mapping studies conducted in a large pedigree of baboons for LDL cholesterol levels, lipoprotein-associated phospholipase A_2 levels, and transcript levels implicated in immune

response (Vinson, Mahaney, Cox et al. 2008; Vinson, Mahaney, Diego et al. 2008; Vinson et al. 2011).

Power of the Pedigree to Detect Association between a Quantitative Trait and a Common Causal Variant

We also investigated the power of a measured genotype analysis conducted in our extended pedigree of rhesus macaques to detect association between a quantitative trait and a common, causal genetic variant or a genotyped marker in complete linkage disequilibrium with a causal variant. Simulations were conducted to derive expected χ^2 statistics based on a trait with a mean and variance similar to that for high-density lipoprotein (HDL) cholesterol levels in the baboon (*Papio hamadrayas, ssp.*), a total trait heritability of 0.20, and a SNP with a minor allele frequency of 0.20. The mean effect of the SNP was varied to account for 0.5–5% of the total trait variance at 0.5% intervals, and χ^2 was evaluated at each interval. Results from this analysis indicate that the pedigree has 80% power to detect associations accounting for 1.5–2.0% of the total variance in a similar trait (M. Mahaney, Texas Biomedical Research Institute, personal communication, 2013). These results indicate that this pedigree also has substantial power to detect effects of common variants on quantitative traits.

Heritability of Quantitative Risk Factors for Human Disease in the ONPRC Rhesus Macaque Pedigree

To confirm the power of the pedigree to detect genetic influences on quantitative traits, we investigated heritability for multiple quantitative risk factors of human disease, including endophenotypes and whole-organism level traits. Because lipid levels are well-established risk factors for CVD in humans and because they are significantly heritable in both humans and in baboons (Vinson, Mahaney, Cox et al. 2008), we assessed the heritability of fasting plasma lipids in a sample of 193 pedigreed rhesus macaques enriched for paternal half-siblings. We found heritability of similar or greater magnitude than that described in large human populations for all lipids in a standard lipid panel, including levels of total cholesterol, LDL cholesterol, HDL cholesterol, very-low density lipoprotein cholesterol, and triglycerides (Vinson, Mitchell, Toffey, Silver et al. 2013). Moreover, based on the standard use of these measures in studies of human obesity and risk for CVD both in the general population and in T2DM, we assessed heritability for abdominal circumference (normalized by crown–rump length), BMI (based on animal weight at sampling and crown–rump length), and animal weight in kilograms from measures in approximately 475 pedigreed macaques; these traits were also characterized by significant, although more moderate, heritability (Vinson, Mitchell, Toffey, Raboin 2013). Finally, we also assessed heritability for complete blood cell counts and related

parameters collected during the course of clinical treatment (i.e., not for research purposes) on 995 pedigreed macaques; white blood cell, red blood cell, and platelet counts, as well as measures of mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, hematocrit, and hemoglobin, were all significantly heritable, with heritabilities ranging from modest to substantial (Vinson, Mitchell, Toffey 2012). These results confirm that additive genetic contributions to important risk factors of human disease can be detected and measured in this pedigree. Based on these initial results, analyses of multiple additional endophenotypes implicated in risk for human disease are underway, including insulin, cortisol, vascular cell adhesion molecule 1 (VCAM-1), interferon γ, tumor necrosis factor α, interleukin 2, interleukin 6, interleukin 12p70, ghrelin, osteocalcin, and lipid subparticle size concentrations.

The Future: Next-Generation Sequencing in Pedigreed Rhesus Macaques

Recent advances in next-generation sequencing provide new avenues for genome-wide genotyping in the rhesus macaque, sidestepping the alternative requirement to generate macaque SNP genotyping arrays. As sequencing costs continue to decline rapidly, whole-genome sequencing is becoming an increasingly viable option for large study cohorts, including extended pedigrees, particularly when combined with imputation of genetic variants in unsequenced individuals. The yield of genetic variant data from whole-genome sequencing is expansive; for example, we have recently identified approximately 3.1 million high-quality SNPs per genome in six unrelated Indian rhesus macaques from 30–45X genome coverage (R Ramakrishnan, unpublished data). This amount of data may be collected in a small number of individuals selected from an extended pedigree and potentially combined with low-coverage sequence data to impute genotypes throughout the remainder of the pedigree (Pasaniuc et al. 2012; Uricchio et al. 2012).

As an alternative to whole genome sequencing, selective DNA enrichment techniques can reduce the amount of sequencing required and thus decrease both genotyping costs and data management requirements. Although such approaches will not necessarily identify all causative variants, the application of studies based on selectively reduced sequence data for analysis of complex disease has already been envisioned (Do et al. 2012; Kiezun et al. 2012). A variety of selection techniques is available. A combination of transcriptome sequencing (RNA-seq) and chromatin immunoprecipitation enriched sequencing (ChIP-seq) was recently used to selectively sequence gene-linked genomic regions in rhesus macaques. This approach yielded at least one SNP in each of the 16,797 annotated rhesus macaque genes and a total of 462,802 SNPs in all 14 individuals investigated (Yuan et al. 2012). Alternatively, we and others have found that human-based exon capture designs can recover 80–95% of the macaque-equivalent exons (Jin et al. 2012; Vallender et al.

2011). This "exome-seq" approach can be used for identifying SNPs and insertions/deletions and for defining haplotypes in 95% of coding genes. These genotype datasets can then be leveraged for subsequent genetic studies.

Conclusion

In this article, we have discussed advantages of both feasibility and analytical power when using extended pedigrees of rhesus macaques for genetic analysis of complex disease traits. These advantages include (1) close genetic and physiological similarity to humans; (2) the availability of very large populations with pedigree information at many primate research centers; (3) the ability to conduct large-scale sampling at nominal cost in managed colonies; (4) mating patterns and short generation times in rhesus macaques that can produce large cohorts of informative relative types, with animals in overlapping generations available for simultaneous sampling; (5) the environmental homogeneity and semi-isolation of managed rhesus macaque colonies that increases power to detect genetic signal over noise; and (6) the enrichment of rare variants in pedigrees, which increases power to detect influential rare variants in pedigrees compared with an equivalent number of unrelated animals. We have also presented short summaries of several representative human diseases investigated using the macaque model that highlight our current limited knowledge of genetic effects on these diseases. Throughout this paper, we have used these discussion points to make the case for the development and use of extended pedigrees for studying genotype–phenotype relationships in the rhesus macaque. Finally, we describe the results of our own efforts to develop a single, extended pedigree and corresponding biobank of Indian-origin rhesus macaques at the ONPRC, including a discussion of the pedigree design and power, and initial results of significant heritability for quantitative risk factors of CVD and metabolic disease. We conclude that the development and application of extended pedigrees to analysis of complex disease traits in the rhesus macaque show great promise for genome-wide genetic and higher order -omics studies in this valuable research model.

Acknowledgments

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