The Importance of the Vervet (African Green Monkey) as a Biomedical Model

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The vervet monkey (*Chlorocebus aethiops*, formerly *Cercopithecus aethiops*) has long been among the most important non-human primate (NHP) models for biomedical research, with a PubMed citation record (over the past 10 years) close to that of rhesus macaque and much greater than that of any other NHP[1]. The dramatic recent growth in the use of vervet as a model derives in part from its recognized utility as an old world monkey alternative to the rhesus macaque, of which there is now a critical shortage for biomedical research. In contrast, Caribbean-derived vervets are abundant, disease-free, small statured, and easy to handle. In addition, HIV research also has an increasing focus on vervets because in contrast to macaques, they are readily infected with simian immunodeficiency virus (SIV) but do not progress to disease.

The vervet is an ideal system for genetic discovery, which will likely increase its use dramatically over the next few years. Unique among NHP species, the vervet currently offers opportunities for genetic investigation of a wide range of phenotypes in both managed extended pedigrees and large, demographically well-characterized feral population samples. The availability of both types of samples enables a combination of linkage and association approaches. These are complementary strategies which in humans, have now proven successful in identifying loci of large and small effect, particularly for the types of quantitative traits (QT) which can be most effectively studied in NHPs. Making optimal use of this opportunity for identifying variants responsible for QTs in vervet will require a genome-wide set of densely mapped SNPs, as is now available in humans and will soon be available in rhesus and baboon consequent to the completion of their reference genome sequences. Availability of a vervet complete genome sequence will spur a wide range of investigations, including opportunities to develop coordinated projects with genetic and genomic studies of humans and the other sequenced NHPs. In the rest of this document we highlight the evidence justifying the immediate and high priority commencement of the vervet genome sequencing. In particular we describe: 1) The history and characteristics of the most widely investigated vervet populations; 2) Some of the most important uses of the vervet in biomedical research; 3) The readiness of the community of vervet researchers to make effective use of the reference sequence.

History and characteristics of vervet populations

Vervets inhabit most of Sub-Saharan Africa and, taking their several sub-species in aggregate, are the most populous African NHPs. Europeans, in the 17th and 18th Centuries, brought a small number of vervets to the Caribbean islands of St. Kitts, Nevis, and Barbados. There they established feral populations that have expanded enormously, to a current level in the tens of thousands. These populations are demographically comparable to genetically isolated human

[1] A search for *Chlorocebus* or *Cercopithecus aethiops* brings up >10,000 research publications over this time, compared to ~5,000 for cynomolgus monkeys (*Macaca fascicularis*) and 2,500 for all species of baboon (*Papio* spp).

populations that have emerged from recent bottlenecks, and are anticipated to provide similar advantages for genetic association studies, e.g. extensive linkage disequilibrium (LD) surrounding trait loci. Initial investigations of St. Kitts vervets, using densely spaced SNPs on a single chromosome, confirm this expectation (N. Freimer, unpublished data).

Most of the vervets utilized in North America for biomedical investigations descend from these Caribbean populations. Vervets primarily from St. Kitts and Nevis have served as the founders of large research colonies in the US, for example at the New Iberia Research Center in Louisiana and at the Vervet Research Colony (VRC), established at UCLA in the late 1970s. The VRC is a multigenerational pedigree of genetically verified structure, consisting of more than 500 living individuals, covering up to eight generations, and descended from 57 vervets captured from St. Kitts in the late 1970s and early 1980s. The VRC has been the focus for the development of genetic and genomic resources for the vervet, as described later in this document, and for numerous ongoing linkage studies of QTs. The VRC, recently relocated from UCLA to the Wake Forest University Primate Center, is operated jointly by the two universities. Through an infrastructure award from NCRR, monkeys from the VRC have been made available to the scientific community for studies of diabetes, cardiovascular disease, AIDS, substance abuse, attention deficit disorder, alcoholism, reproduction, tissue regeneration, and gene expression, along with a wide range of phenotype data and biological materials. Management procedures have been designed to further increase the value of the VRC for genetics research by insuring that environmental exposures (diet, housing, data collection procedures) are equivalent for all individuals thus enhancing the ability to detect genetic influences on spontaneous variation in physiology, behavior and disease susceptibility.

Research colonies have been established on both St. Kitts and Barbados, and have been utilized for a wide range of investigations (e.g. Redmond 2007; Lemere 2004; Gerald 2002). The vervets available through these colonies provide the opportunity for association studies (both fine-mapping and genome-wide) of a wide range of phenotypes.

Another important resource for vervet association studies is provided by scientifically managed populations of African vervets, for example several hundred pedigreed and non-pedigreed vervets available through the Primate Unit of the South African Medical Research Council, (de Vries, 2007). These vervets offer unique opportunities for comparison of very distantly related vervet populations.

Uses of the vervet in biomedical research
We highlight here some of the most prominent current uses of the vervet.

- Vervet as an alternative to rhesus: recognizing the critical shortage of rhesus monkeys for biomedical research, the NCRR Strategic Plan called for the development of breeding colonies and extensive phenotyping and genetic resource development for species such as vervet. The vervet is similar to rhesus in behavior and physiology (e.g., Disotell 2000; Higley 1996; Raleigh 1992; Coe 1992; Zeigler 1990) and is about equally closely related to humans (Page 2001; Raaum 2005). At the same time it is more readily accessible (from Caribbean populations), is less expensive, and may be accompanied by fewer health and safety risks (Baulu 2002; Gordon 2005).

- Investigation of neurobehavioral phenotypes: Neurobehavioral phenotypes form a central focus of vervet investigation. Behavioral observations over several decades have identified heritable traits relating to aggression, maternal-infant interactions, anxiety, and novelty seeking and impulsivity (Fairbanks 1999-2004). More recent efforts have

established paradigms that demonstrate the enormous value of NHPs as models intermediate between humans and rodents for elucidating cognitive processes such as working memory and response inhibition that are relevant to human diseases such as schizophrenia and attention deficit disorder respectively (James 2007). Such a project occupies a central role in the new Consortium for Neuropsychiatric Phenomics, one of only nine interdisciplinary research consortia funded through the NIH Roadmap. Investigations of neurochemical markers in the VRC have led to the first published QTL linkage for a primate neurobehavioral trait, the concentration of the dopamine metabolite homovanillic acid (HVA) in cerebrospinal fluid (CSF), (Freimer 2007).

- Investigation of neurodegeneration: The vervet has provided a longstanding model for Parkinson's disease, including, most recently, translational studies using neural stem cell approaches (Redmond 2007). The recent discovery that Caribbean-derived vervets develop cerebral amyloid beta plaques with aging, and that these deposits are associated with gliosis and neuritic dystrophy, makes them an important new model for Alzheimer's disease research and treatment (Lemere 2004).

- Investigation of neuroanatomic variability. High-resolution structural magnetic resonance imaging (MRI) of the brain has been completed for nearly 400 vervets in the VRC. Variability in brain structure is extraordinarily heritable in this population compared to human samples (Roger Woods, unpublished data), and can be >95% for features such as (body size adjusted) total brain volume or cerebral volume. These findings indicate that there should be high power to genetically map such variation, but also likely reflect the environmental homogeneity characterizing this pedigree. The MRI files will be available to the scientific community through a web browser, providing an additional reference resource for investigations of vervets.

- Investigation of the immune system and infectious disease: The vervet is growing in importance as a model species for AIDS research. African populations of the vervet are natural carriers of SIV and unlike Asian macaques, do not show signs of illness when infected. Caribbean-derived vervets are free of SIV and thus represent an excellent model for experimental study of the immune response on initial infection. Several labs are using this model to understand how the immune system prevents disease progression, with potential implications for the care and prevention of HIV-related diseases in humans (Goldstein 2006; Pandrea 2006). This community of researchers will look particularly to sequence variations identified between African and Caribbean vervet populations for clues to the biology of individual response to HIV.

- Investigation of metabolic phenotypes: Traits relevant to diabetes and metabolic syndrome are an increasing focus of vervet research, for example, VRC-wide screening and heritability analysis for markers of metabolic syndrome (Kavanagh 2007). With the relocation of the VRC to Wake Forest, the diet of the entire colony will be switched to one resembling a typical American diet, greatly expanding the scope of studies in this area. The vervet monkeys have already been used by Wake Forest investigators to examine cholesterol gallstone disease, as they are one of the few old world monkeys to develop cholesterol stones. Vervets have also been used as models for mechanistic studies of diet-induced hyperlipoproteinemia and coronary artery disease with the associated dietary fat type and cholesterol sensitivities of these endpoints. The investigation of hepatic lipid metabolism using isolated liver perfusion has been

conducted in vervets with dietary fat and cholesterol-induced nonalcoholic fatty liver disease.

Readiness of the vervet research community to make use of the vervet genome sequence

The availability of phenotyped and pedigreed colonies is being matched by the development of genetic and physical mapping tools to interrogate the vervet monkey genome. A recently published 360 marker microsatellite based map (226 markers uniquely ordered at 1000:1 odds, with a 9.8 cM average resolution; Jasinska 2007, data available on the Web at www.vervetgenome.org) is being augmented with procedures for targeted SNP generation. These procedures make use of the available reference sequences from human and rhesus as well as the vervet physical mapping efforts (described below) from the McGill University and Genome Quebec Innovation Centre. Such SNPs have been used for fine-mapping of a QTL for dopamine metabolism to a region of ~5Mb on the vervet ortholog of human chromosome 10p (Freimer 2007). The next major step forward will be to generate a genome wide SNP discovery initiative (50-100 kb resolution).

The vervet monkey genome ultrastructure differs from that of other Catarrhines by its chromosome number (2n = 60), principally due to chromosome breakages. However, extreme karyotype variability characterizes the entire Chlorocebus genus as well as other genera within the Cercopithecini, making this a very interesting model for studying genome evolution. The availability of an end sequenced BAC library (library funded by the NIH and generated by Pieter de Jong, paired-end sequencing funded by Genome Canada and Genome Quebec) is being used to map structural rearrangements to a much finer level than the microsatellites or FISH results. The BAC end sequences can be used to define regions of syntenic colinearity as well as identify and characterize genomic structural rearrangements (the paired-end sequencing data and rearrangement maps are publicly available at http://www.genomequebec.mcgill.ca/compgen/submit_db/vervet_web. Currently at >6X template coverage, the paired-end sequence map already identifies ~1240 contigs of syntenic colinearity (to human) in aggregate covering >2.6 Gb of the sequenced human genome.

The vervet monkey is well-situated for leverage by comparative genomics with existing genome assemblies for human, chimpanzee, and rhesus, as well as other anticipated and ongoing non-human primate genomes. Sequence homology at the nucleotide level is sufficiently strong that 79% of all BACs can have ***both*** of their end-sequences uniquely placed on the existing primate genomes, providing the framework for synteny colinearity assessments at a BAC size level of resolution (150 kb). The ability to define syntenic segments between vervet and human immediately allows researchers to move from QTLs to gene content analysis. In cases where there are rearrangements we can modify our mapping and analysis accordingly, including being able to date the rearrangements as pre-or post- the divergence from rhesus. Whereas we use the human reference for the bulk of our genome colinearity and gene content analysis, we can also then project that information onto the rhesus to obtain a nucleotide level read out (from rhesus) that is even closer to the vervet genomic sequence. In fact, collaborators already use the rhesus local sequence as the template for primer design, thereby bypassing a resequencing step in the vervet.

Proposed next steps

The vervet monkey is an important biomedical research model poised to take advantage of a complete genome sequencing initiative. Characterized pedigreed populations (as well as large samples of unrelated phenotyped vervets) are supporting the genetic mapping of disease and behavioral QTs, and key genomic resources (including genetic, physical maps, gene expression studies, and cytogenomics protocols) are all well advanced and accessible on a shared bioinformatics platform. Given the status of vervet genetics and genomics research, we are immediately interested in moving to comparative genomics in order to identify and study the genetic bases and functional consequences of genetic variation within the vervet populations.

The needs for the next phase of the project revolve around the identification of high-density and genome-wide common SNPs to support QTL mapping in the phenotyped colonies (and in independent vervets under investigation around the world). We propose to select 6 individuals from the pedigreed colonies (representing a spectrum of genetic and phenotypic diversity) for complete genome sequencing and subsequent analyses of genomic variation and structural changes. *The generation of a genome-wide set of vervet SNPs, when combined with the existing genomic resources and the availability of extensive phenotyped samples, will permit rapid initiation of well-powered association studies. Such studies, together with efforts now underway in other NHP species, will provide previously unimaginable opportunities to elucidate the etiology of diseases and disease related traits.*

Finally, we also note that the vervet genome is extremely well-suited for massively parallel DNA sequencing approaches. As a member of the old world monkeys, conventional sequencing reads and Roche/454 type reads are highly alignable to existing reference genomes (human, chimpanzee, macaque). Anticipating the trade-off between massive output versus loss of specificity on the short read systems (Illumina, ABI Solid) we still expect to have an alignability success rate similar to human resequencing (Trials with Illumina and Solid are underway with John McPherson in Toronto to evaluate the two systems). The high level of alignability combined with our genome-wide recognition of sites of colinearity and rearrangements will further aid in separating polymorphisms from paralogs and CNVs, allowing us to create added value genetic mapping resources.

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