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Genome typing of nonhuman primate models: implications for biomedical research

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The success of personalized medicine rests on understanding the genetic variation between individuals. Thus, as medical practice evolves and variation among individuals becomes a fundamental aspect of clinical medicine, a thorough consideration of the genetic and genomic information concerning the animals used as models in biomedical research also becomes critical. In particular, nonhuman primates (NHPs) offer great promise as models for many aspects of human health and disease. These are outbred species exhibiting substantial levels of genetic variation; however, understanding of the contribution of this variation to phenotypes is lagging behind in NHP species. Thus, there is a pivotal need to address this gap and define strategies for characterizing both genomic content and variability within primate models of human disease. Here, we discuss the current state of genomics of NHP models and offer guidelines for future work to ensure continued improvement and utility of this line of biomedical research.

The need to advance understanding of nonhuman primate genomes

With the accelerating development of whole-genome sequencing techniques and related methods, such as high-throughput genotyping and whole-exome (re)sequencing (WES; see Glossary), personalized medicine is no longer just a visionary goal in human medicine. Currently, knowledge of the patient's genotype can aid in the selection of targeted drugs or special therapeutic treatments; cancer research and treatment have shown early successes in this regard [1,2]. Given this recent progress, with more anticipated soon, there is a critical need to interpret and apply the results of animal-derived disease modeling and drug safety or efficacy testing within the framework of individual human genetic and genomic variation. To achieve this, ideally, the same extent of information regarding genetic background should be available for both the patient population and the preclinical animal model.

Information on genetic variation among individuals is particularly important for NHPs, which are widely used as model organisms in biomedical research. For most of these primate models, there is clear evidence of biologically meaningful genetic differences among species and, as with

Glossary

3Rs (reduction, refinement, and replacement): an ethical framework for the use and care of animals in scientific experiments. This includes the replacement of animals wherever possible, the refinement of husbandry and procedures during experiments, and the reduction in terms of improving approaches and increasing efficiency of experiments [45,46].
Clustered regularly interspaced short palindromic repeats/CRISPR-associated 9 system (CRISPR/Cas9 system): a system used by prokaryotes for immunization processes by integrating exogenous DNA to keep track of viral attacks and to defend their organism from invaders with similar DNA. New technologies now allow for the transfer of this system to eukaryotes. Customized Cas9 nucleases are guided by small RNAs to the target site in the genome to manipulate specific genes [41,44,47].
Genome-wide association studies (GWAS): identification of SNPs in the genome and analysis of their distribution in different (phenotypic) populations. Based on these data associations between specific SNPs and disorders, physiological traits or gene functions can be detected [48].
Personalized medicine: based on genomic information from individuals and patients, associations between genes and diseases are detected and used for personalized marker-assisted diagnosis, prevention, and treatment [49,50].
Transcription activator-like effector nucleases (TALENs): genome-editing tool using nucleases that are fused to a DNA-binding module, which can be customized for sequence-specific targets [51].
Whole-exome (re-)sequencing (WES): technology of next-generation sequencing to determine all variants of known coding regions (exons) in the genome [52]. Available genome sequences provide reference genomes for comparative sequencing or resequencing technologies, which are used to identify mutations and polymorphisms between organisms.

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humans, substantial levels of genetic variability within species frequently linked to geographical origin [3–6]. Although there is evidence that the genomic makeup of NHPs can have considerable influence on the outcome of biomedical experiments [7–12], the number of detailed genetic studies accounting for these differences is still low compared with the total number of biomedical studies conducted with NHPs.

Accordingly, for the broad scientific and industrial community using NHP models, some fundamental guidelines are needed on how to apply successfully modern genomic tools to NHP research. We argue that there is an urgent need to increase the number of sequenced NHP genomes, as well as of studies investigating the influence of genetic variation on experimental outcomes. Furthermore, research would benefit from improved knowledge of biomarkers for disease, genetic disorders, and toxicology, as well as of the respective differences between human and NHP genomes. Here, we highlight some of the most important aspects that should be considered to direct future developments and strategies for primate genetics in modern biomedical research.

Genetic variation in NHP models and its relevance to biomedical research

NHPs, such as macaques (*Macaca* spp.), baboons (*Papio* spp.), African green monkeys (*Chlorocebus* spp.), and the common marmoset (*Callithrix jacchus*), are commonly used as models in biomedical and pharmacological experiments and during preclinical safety assessments. Based on phylogenetic studies using single or multilocus approaches, remarkable genetic variation has been found to occur not only between species, but also between populations of the same species from different geographic origins (Box 1). This is especially important because native geographic origins of test animals from commercial breeding centers are often unknown or, at best, uncertain. For example, cynomolgus macaques (*Macaca fascicularis*) exported from China, where they do not occur naturally (see Figure I in Box 1), originate from various countries in Asia [13,14]. In addition to the inherent inter- and intraspecific variation, in many of these NHP models, past interspecific hybridization has led to even more complex mosaic genomes, which comprise patches of different species ancestry (Box 1). Moreover, a comparative analysis of rhesus macaque and human genetic variation found that the rhesus macaque is three times as diverse as humans [15]. This high level of within-species diversity is a fundamental biological parameter that is an important consideration for any scientific study of rhesus macaques, which are commonly used as models for human disease. Evidence from mitochondrial DNA and other studies suggest that rhesus macaques are not unusual in having large amounts of genetic variation, and that high levels of variability are typical for NHPs [16].

Although relatively few studies have explicitly analyzed the genetic variation of experimental animals, there is increasing evidence that the genomic makeup of NHP models can considerably affect the outcome of biomedical experiments. This has been observed when animals from different populations (i.e., from different geographic regions) or unrecognized hybrids are used within one experimental approach. For example, in simian or human immunodeficiency virus (SIV/HIV) research, primate genetic variation can affect viral replication as well as the control of an infection [17–23]. Moreover, an acquired immune deficiency syndrome (AIDS)-like state similar to that seen in humans is primarily developed in rhesus macaques (*Macaca mulatta*) of Indian origin, whereas a more moderate disease progression is typical in rhesus macaques of Chinese origin [21,24]. Given these observations, it has become routine to genotype loci that are known to influence SIV/HIV disease progression, such as tripartite motif-containing protein 5 (*TRIM5*) or major histocompatibility complex (MHC) genes. Based on these data, research subjects and study groups are screened for genetic characteristics and chosen depending on those results before any SIV/HIV study [7,20,25].

Genetic divergence also affects physiological traits. Cynomolgus macaques of Mauritian origin are known to develop faster and to achieve sexual maturity earlier than cynomolgus macaques of Asian mainland origin [26]. In a vaccine study, cynomolgus macaques from Mauritius responded differently to *Shigella* immunization compared with cynomolgus macaques from non-Mauritian origins. Mauritian animals did not develop clinical shigellosis because of distinct gut microbiota [27]. These examples underline the advantages of knowing the geographic origin and genetic background of potential study subjects, before beginning any experiments. However, more importantly, even individual variation is known to influence disease susceptibilities and other disorders. In an experiment testing behavioral and neurobiological reactivity to a brief mild stress, rhesus macaques of a single family pedigree showed individual phenotypic variation that correlated with DNA sequence variation in the corticotrophin-releasing hormone receptor 1 gene (*CRHR1*), which is associated with the diathesis for anxiety and depression in humans [10]. A similar influence of genetic variation has been reported for other diseases, such as malaria susceptibility [8], and for toxicology studies [28]. For additional examples, readers are referred to a basic overview recently provided in a 2013 special issue of the *ILAR Journal* entitled 'Progress in Genetics and Genomics of Nonhuman Primates', dedicated to the topic of future trends in genomics of NHP models and its importance for biomedical research, including aspects of genetic variation in NHPs (for an outline, see [29]).

These examples highlight the importance of understanding genetic variation within NHPs and its relevance to toxicology, physiology, and human disease models. Recently, investigators have suggested that 'differences between species, ultimately arising at the genetic level, must be considered for informed research approaches' [30]. For many study designs, it is prudent to know whether inter- or intraspecific differences occur, the significance of these differences, and if they can be controlled for. From a practical standpoint, several questions arise: what is the best way to obtain relevant genetic data for potential research subjects, how many data and what type are required to design a given experiment effectively, and how should this information be incorporated into future study designs or selection of test animals? These

Box 1. Genetic variation and hybridization in nonhuman primate models

Several of the NHP models have wide geographic distributions, including macaques mainly on the Asian continent, and baboons and African green monkeys in Africa. Given the wide geographic range and reduced or absent gene flow between populations, different (sub)species of these genera exhibit notable genetic and sometimes even phenotypical and/or behavioral variation [4–6,53,54]. SNPs clearly differentiate rhesus macaques from India and China [3,34,55]. Similarly, a SNP analysis of cynomolgus macaques identified several polymorphisms unique to geographical populations of cynomolgus macaques from Indonesia and Indochina despite unrecognized phenotypic differences [14].

Moreover, there is evidence for ancient and ongoing intrageneric hybridization between most species of these genera in areas where their ranges meet [6,12,56–59]. Continuing hybridization over generations may lead to the transfer of certain genes into the genome of another species, a process called 'introgression'. In some cases, this can even cause genome-wide admixture of involved species, leading to a mosaic genome [60–62].

For example, the genome of cynomolgus macaques from the Asian mainland, north of the Isthmus of Kra, is introgressed by rhesus macaques (Figure I). Studies using Y chromosomal and mitochondrial markers revealed that Asian mainland cynomolgus macaques carry Y chromosomal fragments of rhesus macaques from China and Myanmar, but not from India, whereas they retained their original cynomolgus macaque mitochondrial genome [5,58]. A recent genome analysis [12] showed that up to approximately 30% of the genome of Asian mainland cynomolgus macaques is of rhesus macaque origin. Given that most cynomolgus macaques used in biomedical research are derived from breeding centers in China and Vietnam, it is obvious that these animals are likely not pure cynomolgus macaques [63,64]. Likewise, although presumably not introgressed by rhesus macaques, cynomolgus macaques imported from Indonesia and the Philippines are genetically highly diverse [5,65], which is unsurprising considering the wide distribution of the species and the isolation of certain island populations (Figure I). In addition, Mauritian cynomolgus macaques, which most likely originated from a few Indonesian founder animals and, hence, are thought to be relatively homogeneous, carry a combination of insular mitochondrial DNA and continental Y chromosomal DNA [5]. Thus, cynomolgus macaques from different geographic regions exhibit extreme genetic variation.

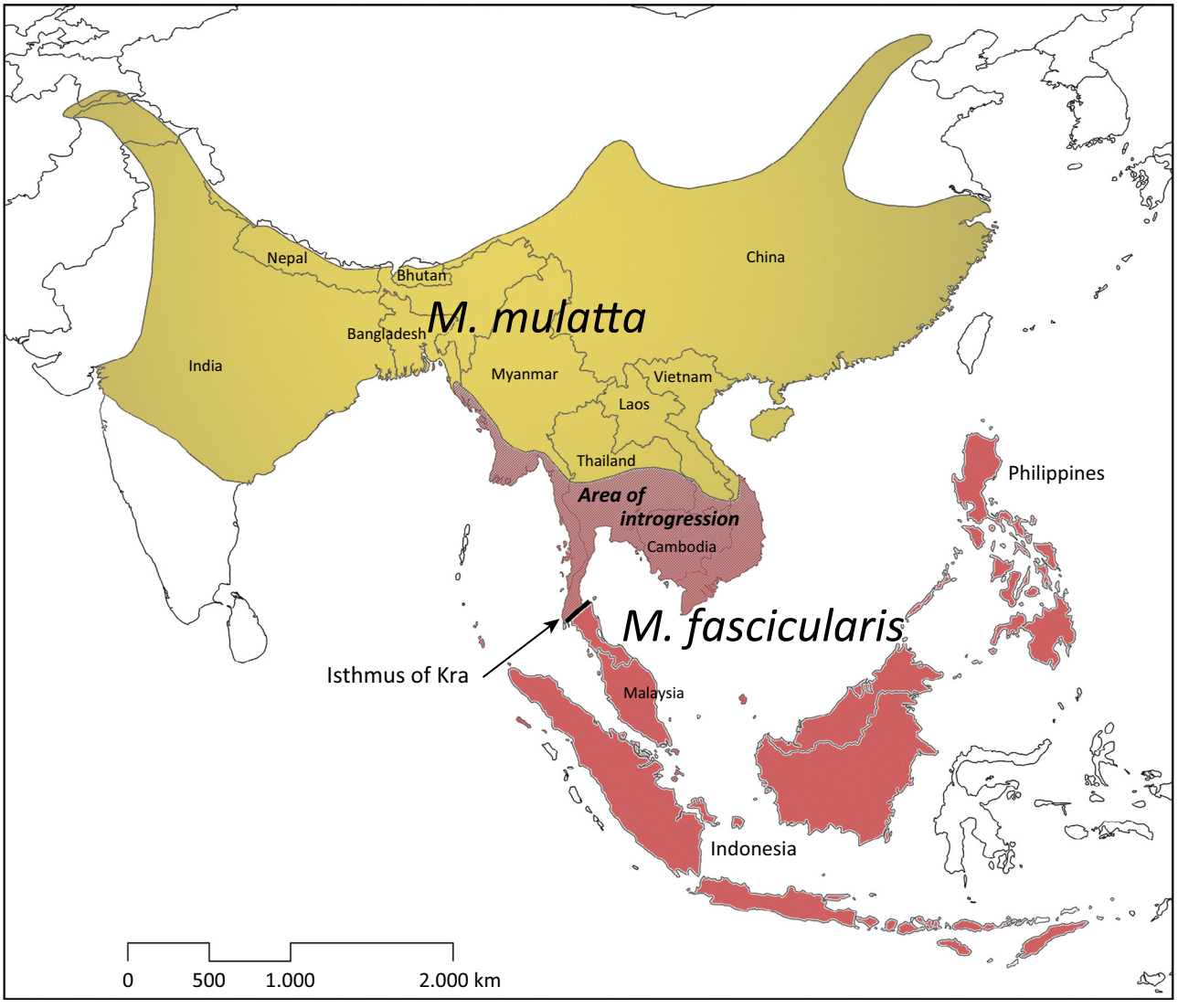

Figure I. Natural distribution of rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques. The hatched region indicates the putative area of introgression from rhesus into cynomolgus macaques. Adapted from [14,63].

considerations have important implications for breeding management, primate husbandry, and animal welfare. Below, we discuss potential solutions to these challenges and suggest guidelines for the field to adopt.

Applications of NHP genomics to biomedical research

In the era of affordable whole-genome sequencing, obtaining detailed information about the genetic makeup of NHPs is relatively straightforward. The genomes of several NHP species have now been sequenced in addition to those of the great apes, which were published within the past few years [9,16]. Whole-genome sequences (WGS) are now available for a Mauritian, Malaysian, and a reported Asian mainland cynomolgus macaque [12,28,31], for four Indian and two Chinese rhesus macaques [3,32–34], and for one Tibetan macaque (*Macaca thibetana*) [35]. WGS of other macaque species, as well as of baboons, African green monkeys, and marmosets, are in progress [16,36,37]. These data provide an important basis for understanding physiological characteristics and their underlying genetic causes (e.g., [38]). More importantly, however, these genome sequences facilitate high-resolution genotyping and resequencing of other individuals [28,39]. Thus, the fundamental tools are in place to expand understanding of individual NHP genetic variation. Comparisons of the few available WGS of rhesus and cynomolgus macaques from different populations are valuable examples of the power of genome sequencing [3]. However, due to the known inter- and intraspecies genetic variation in NHP models, in our opinion these examples provide only a glimpse of the potential of genome typing. To reduce the genetic 'noise' in biomedical studies, we need more complete and better-annotated reference genomes for animals from diverse geographic areas to be able to select individuals with appropriate genetic backgrounds for a single study. Given the striking differences in physiology, drug metabolism, and disease susceptibilities between cynomolgus and rhesus macaques, we also recommend the inclusion of several individuals from the Asian mainland introgression zone into WGS projects (see Figure I in Box 1). Although one approach is to determine whether significant genomic regions, relevant to the experiment, are affected by introgression, or whether they correspond to the phenotypical species, we also need information on the extent of introgression in the complete genomes of our study animals to estimate the influence of potential 'foreign' genetic material. Until we understand the implications of this admixture for various types of research projects, it is difficult to determine an 'acceptable' level of admixture, but such a threshold analysis would be useful.

To identify loci and alleles that are associated with specific physiological traits, differences in gene expression and/or disease risk, or which alter the rate of drug metabolism, we require more informed genome-wide association studies (GWAS). Although this is far from being a reality, we suggest establishing data banks of well-annotated WGS for significant populations of known geographic origin and for major breeding lineages of each NHP model species. With these reference data, it might be sufficient to characterize the geographic origin using only a few specific loci before experiments in future study designs. For such approaches, the analysis of single nucleotide polymorphisms (SNPs) might be one promising tool. In macaques, SNP analyses can already assist in determining geographic origins of animals imported from breeding centers and in the genetic-based selection of animals before experiments [14,35,40]. Additional typing of specific genomic regions, which are known to be associated with respective diseases or physiologies, will then further assist pre-selection of animals and help to minimize false results in experiments and to improve the reliability of biomedical studies.

Furthermore, with the advances in personalized medicine, there is the crucial need to expand our knowledge of differences between human and NHP genomes and to determine how they can affect the usage of NHPs to model human disease and drug responses reliably. Comprehensive comparisons of WGS of humans and NHP models, in combination with results of GWAS, will enable us to detect coding variations and similarities and, thus, also increase our ability to detect true causal relations between genotype and disease, and their influence on biomedical studies.

Another promising tool in NHP research is the production of genetically modified primates for targeted approaches. Different methods are being developed, and the most promising are currently the nuclease-based clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (CRISPR/Cas9) system [41] and transcription activator-like effector nucleases (TALEN)-based gene modification [42]. Nucleases can be programmed to target specific sites in the genome, and to mutate or knock out genes, or alter their expression [43,44]. With these tools, NHPs can be efficiently used to model human diseases and to develop therapies for genetic disorders. However, as a basis for the usage of transgenic monkeys in biomedical research and personalized medicine, we need more information regarding gene functions and possible markers for toxicology and disease in both human and primate genomes.

Incorporating NHP genomics into breeding management and primate husbandry

As high-throughput sequencing techniques become significantly cheaper, new opportunities will arise because many institutions, pharmacological industries, and breeding facilities are essentially driven by cost efficiency. However, it is not yet clear whether sequencing and the associated data analysis burden will become affordable enough for the majority of these institutions and facilities to genotype multiple individuals of each primate species used in their facility. Therefore, although the value of whole-genome analyses in NHP models is without doubt, questions arise concerning how breeders, academia, and industry should prioritize these resources and how the financial and practical challenges of implementing the use of genetically well-characterized NHP models can be met.

Genomic information can be used to select certain genotypes for specific experimental approaches, which is expected to increase the accuracy and efficiency of scientific studies, as well as to decrease the number of test animals used. Although such approaches can contribute in substantial and practical ways to achieve reduction and refinement within the principles of the 3Rs (reduction, refinement,

and replacement), new problems might arise for breeding facilities and researchers. The development of a genotype-based selection method of test animals would lead to specific requests for particular subsets of animals or animals from specific geographic regions depending on the research question. For a market that is already increasingly led and, thus, also restricted, by national and international laws and directives on the usage of animals in experimental approaches, it is questionable whether such a selective approach is practical in all facilities. Moreover, the relatively long generation times of NHPs, difficulties in sperm collection and artificial insemination, as well as increasing efforts to improve primate husbandry and welfare, make implementation of selective breeding to enrich certain genotypes based on genomic data nontrivial. Finally, selective breeding for one set of genotypes today severely limits the future genetic potential of research and breeding populations, essentially locking in the current research interests and causing undesirable reduction in total genetic variability. Thus, the maintenance of genetic diversity within facilities has to be balanced with the need for selectively bred lines of NHPs. The dangers of overselection are real and must be avoided.

As a first step, in line with advances in NHP genomics, breeding centers and primate facilities should begin to pay more attention to separate and maintain populations from specific geographic regions, well-characterized breeding lineages, and groups that include introgressed or admixed individuals. As a long-term goal, it is possible that additional selectively bred lines could be established for major disease models to enable a genome-based selection of specific genotypes. However, this should only be done after careful consideration of the near- and long-term consequences, and when it will further refine NHP research and take advantage of other advances in personalized medicine.

Concluding remarks

More genomic information about within-species variation as well as between-species differences is required. One promising approach is to sequence whole genomes from sets of representative lineages within species from known geographic origins. As the cost of sequencing continues to decline, it will become feasible to analyze WGS on a population genetic scale [3,12,34]. In addition, we also recommend increasing effort be made in GWAS and other types of studies that explicitly test for potential influences of genetic variation in NHP models on biomedical experiments. Comparisons of these data with results from annotated human genomes will substantially increase knowledge on gene functions and enable us to reduce genetic background noise in both human and NHP studies. Moreover, such results will provide essential information for the application of transgenic primates in biomedical research and advance their usage in personalized medicine. With respect to primate husbandry and selective breeding, an overall approach that maximizes the genetic value of animals today while maintaining maximum flexibility and genetic potential for the future must be primary goals.

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