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The Ethics of Using Transgenic Non-Human Primates to Study What Makes Us Human

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Abstract

An ongoing flood of comparative genomic data is identifying human lineage specific (HLS) sequences of unknown function, and there is strong interest in investigating their functional effects. Transgenic apes, our closest evolutionary relative, have the highest potential to express HLS sequences as they are expressed in *Homo sapiens* and likewise experience harm from such transgenic research. These harms render the conduct of this research ethically unacceptable in apes, justifying regulatory barriers between these species and all other non-human primates for transgenic research.

INTRODUCTION

The publication of the draft sequence of the common chimpanzee genome in September 2005 was heralded as an advance that confirmed what Darwin[1] and Huxley[2] posited more than a century ago: humans share common ancestors with African great apes[3,4]. In addition to highlighting vast homologies between human and chimpanzee DNA, primate comparative genomics is also providing insights into the question of what makes members of *Homo sapiens* different from their nearest evolutionary relatives.. There is great interest in identifying the genetic factors that underlie the traits that are traditionally taken to be the hallmarks of our species: higher cognition[5], complex language and vocalizations systems[6], skin phenotypes[7], immune and reproduction system function[8,9], sensory perception[10,11], degree of self and social awareness[12], and other idiosyncrasies of our physiology, anatomy and behavior [13]. While computational predictions of function can be made from large data-sets, determining which sequences are functionally related to complex lineage-specific phenotypes can likely be accomplished by experimental investigation.

One potentially appealing experimental strategy is to investigate functional effects of HLS sequences in transgenic non-human primates (NHPs). Ideally, the species chosen for this research should be those who share the greatest genetic similarity with *Homo sapiens*, to increase the likelihood that they will express the HLS sequences in ways that give a clear indication of their function in humans. While the long generation times of apes makes them less than ideal for genetic studies, their highly similar genetic background with humans makes them a particularly attractive host species for transgenic studies of the function of HLS sequences. However, we argue that the scientific insights that can potentially be gained by this approach are outweighed by ethical concerns regarding the generation of such “humanized” apes. The same evolutionary proximity that makes this research strategy attractive renders it ethically unacceptable in apes, including greater apes (chimpanzees, orangutans, bonobos, gorillas) and lesser apes (gibbons), because apes have the greatest potential to produce human-like phenotypes and, as such, carry a unique potential for harm.

HUMAN LINEAGE-SPECIFIC SEQUENCES

In addition to the human genome sequence[14], we now have the complete draft genome sequences of chimpanzee[4] and rhesus macaque[15]. Draft assemblies are currently available for the gorilla, orangutan, marmoset, and lemur (http://www.ensembl.org/index.html), and a draft Neanderthal genome sequence has recently been published[16]. In addition, draft sequences of bonobo and baboon are underway (http://www.genome.gov/10002154). These data-sets are providing a rich and unprecedented resource for mining lineage-specific genomic changes among humans and NHPs. Evolutionary genomics has already uncovered a wide range of HLS sequences. Genes affected include those that show striking HLS increases or decreases in copy number[17,18,19], genes that have changed dramatically at the sequence level specifically in humans[20], and genes that show altered brain expression between human and chimpanzee[21,22].

These genes span a range of functional characteristics, from those with no known function to those implicated in various anatomical and physiological processes[5], including several linked to higher cognitive functions[20,22,23]. We can expect that, as more and more primate genomes are completed, the list of HLS genomic changes will significantly expand and further stimulate interest in understanding the functional consequences of such changes. This in turn can be expected to generate an increased interest in using transgenic animals as a means of studying HLS gene function.

TRANSGENIC RESEARCH USING NHPs

For uniquely human sequences or genes, transgenic research using NHPs could likely be accomplished with existing techniques, either through homologous exchange of HLS sequence with homologous sequence of the transgenic host species or addition of HLS sequences to the host genome[24,25,26,27]. However, new advances will be required to study functional consequences of differences in expression, since precisely altering expression levels in transgenic research by increasing copy number or modifying the regulatory region of a gene remains technically challenging. Nevertheless, improving our abilities to control the number and genomic location of copies of integrated transgenes and levels of temporal and spatial expression is a high priority goal within human gene transfer research, due to the critical implications for the safety and efficacy of human gene therapy and other treatments.

A common application of transgenic animal technology has been the generation of mouse models to study a number of human disorders, including neurodegenerative disorders28, autoimmune diseases29,30, and cancer[31]. Nevertheless, even though there are National Institutes of Health-supported efforts aimed at “humanizing” mice by development of transgenic strains that contain human-specific alleles (RFA-MH-08-050) and a recent study has examined the function of human alleles of the FOXP2 gene in transgenic mice[32], there are limits to what scientists can learn about the function of human genetic sequences in mice because of the significantly different genetic backgrounds of the two species. There are also considerable anatomical and physiological differences between these species that may interfere with such an approach. In particular, there are large differences in relative size and composition between rodents and humans in areas of the brain associated with higher cognition. Thus, the possibility of anything near human cognition resulting from HLS sequences transferred to a mouse using conventional transgenic approaches is remote.

Recently there has been a shift towards using NHP models[33]. The creation of chimeras through the interspecies transfer of stem cells is one means of exploring the function of the HLS sequences. The ethical concerns raised by the interspecies transplantation of human brain, retinal stem cells, and other cells to nonhuman embryos or fetuses are addressed in the literature34,35,36,37. The production of chimeras entails the transfer of an entire genome to

the host species, while the creation of transgenics, which is the focus of this article, indicates the transfer a genomic sequences(s) to the host. The first transgenic NHP was produced in 2001, and in 2008 a rhesus macaque that expressed hallmark features of Huntington's Disease became the first transgenic monkey model of human disease, raising the hope of ultimately developing new therapies38. Another experimental approach is the recently reported generation of transgenic NHPs to study mitochondrial gene replacement in primate offspring26. The goal of developing new therapeutic approaches using these methods is an admirable one, yet it raises important ethical questions and points to the need to distinguish between the use of apes and all other NHPs ("monkeys" hereafter).

The basic rationale for pursuing transgenic research on HLS sequences is that it could provide important information regarding the basic scientific question of what makes members of *Homo sapiens* different from their nearest evolutionary cousins. The research continuum from bench to bedside suggests that results of transgenic research may also produce clinical benefits, especially given that HLS gene variants have been found to be disproportionately enriched in genomic locations implicated in human diseases[19,21]. Another key factor is evidence that evolutionary mechanisms that have produced rapid genomic changes in the human lineage often produce human disease as a byproduct[25]. It is possible that HLS variants that affect human phenotype could provide important insights into disorders and clinically relevant phenotypes that are difficult to study by other means, including cognitive disease[25], neurodegenerative disorders[38], social behavior disorders such as autism[39], dementia[40], speech articulation defects[41,21], and gene delivery systems[42] for therapeutic use. Since this research is still in the early stages and the benefits still theoretical, it is appropriate to examine proactively the ethical concerns related to NHP transgenic research.

ANIMAL WELFARE REGULATIONS

The prospect of conducting transgenic NHP research differs according to country; no international animal welfare regulations exist at present but the OIE World Assembly of National Delegates adopted a 5th Strategic Plan for pursuing OIE global mission in animal health and welfare in 2010 (http://www.oie.int/eng/session2010/press_p_releases.htm). Currently, transgenic research involving all NHPs could be approved in countries, including the United States and China, that have no specific bans[43,44,45,46,47]. The use of great apes for most research is effectively banned in the United Kingdom, New Zealand, the Netherlands, Austria, and Sweden[48,49,50]. The ban in Austria and Sweden also includes the lesser apes[51]. And the European Union is now considering updates to their animal welfare laws that would mirror requirements in the United Kingdom[52,53]. Although monkeys are used for research in the United Kingdom, regulations require additional oversight, including a special license for the individual researcher, project, and institution[47,54].

Animal welfare regulations and guidelines vary around the world, but all share a commitment to what is known as the Three Rs: replacement, reduction, and refinement. The Three Rs state that animals should only be used if there is no alternative and, when animals are necessary, only the most humane methods should be used on the smallest number of animals required for scientific validity[43,4549,55,56,57]. However, the Three Rs are criticized by some for not providing a way to give special consideration to certain species, such as NHPs[46].

THE ETHICAL ISSUES

Predictive and Diagnostic Uncertainty in Transgenic NHP Research

The transfer of an HLS gene or genomic variant into an NHP could have a specific, discreet effect that has a more or less dramatic impact on a phenotype of relevance to human function. However, biomolecular systems are interdependent, and most genes appear to have multiple functions that can be seemingly unrelated. So, such a transfer could just as easily have a wide array of unexpected and/or deleterious effects. For example, even if gene targeting could be carried out specifically, the plieotropic nature of most genes will often make the phenotypic impact of a transgenic intervention extremely difficult to predict or interpret and may not emerge immediate but only as offspring are produced, even if studied first in other animals[21,58]. This uncertainty raises the risk of producing unanticipated harm to transgenic NHPs. Moreover, if HLS gene transfer actually accomplishes its intended aim and produces a measurable change in a “human-like” NHP phenotype, this would raise another set of ethical concerns. Even if a transgenic NHP remotely displayed phenotypic traits that made it only slightly more similar to a human…there would be further ethical questions concerning the animal’s vulnerability to harm.

Ethical Obligations towards NHPs in Transgenic Research

The debate about animal welfare in general, and the use of primates in particular, is often carried out in the literature in terms of “moral status”, a term meant to describe what entities are owed in their own right rather than as instrumental value to others[59,60]. Moral status is not an all or nothing assessment since ethically justified regulations allow research on humans and prohibit capricious animal use. Additionally, consideration of our ethical obligations to humans and animals is not dependent on inherent capacities alone, but also includes relational elements. For example, this consideration is applied to humans who lack many human capacities and to companion animals like cats, dogs, and horses that lack higher order capacities. Merely labelling an entity as having or lacking moral status cannot do the ethical work of sorting out our particular obligations. Rather, ethical obligations are to be determined by carefully assessing the answers to at least five questions: What are the goals of research? What is the probability of success? Which animals are to be used? What effect will there be on the animals used? Are there any alternatives? These questions represent a moderate approach to the ethics of animal research: they assume that some animal research is warranted and can be ethically justified, but also recognize that not every scientific finding automatically outweighs the interests and welfare of the animals that its demonstration might require[61].

There is a growing literature on ethical issues in the creation and research use of transgenic and chimeric animals in general, and primates in particular. Some analysts try to articulate the unique concerns in transgenic research in terms of the "unnaturalness" of "crossing species boundaries," citing unspecified downstream evolutionary and ecological risks[62,63]. Others turn to notions of "animal integrity" or "species-specific dignity" to try to focus their critiques on the impact of transgenic manipulations on the animals themselves[35,64,65,66]. This approach is headed in the right direction, but it usually remains at a relatively abstract level that is hard to apply to the actual conduct of animal research. It can be both strengthened and sharpened by considering the purely biological challenges that any transgenic apes that actually express humanized phenotypes would face. At the far end of the spectrum, of course, are cognitive changes that might give transgenic apes enough self-awareness to appreciate the ways their lives are circumscribed and to suffer, albeit immeasurably, in the full psychological sense of that term. But it is not necessary to go that far. Imagine the life of the transgenic chimpanzee that, while no more self-aware than other chimps, is hairless, walks erect, lacks long canine teeth, or vocalizes like a human. There

may be no question of a "normal" primate life in the wild for this chimp: it is likely to be condemned by its engineering to live in a human environment. Outside the laboratory, this chimp could well be considered a grotesque curiosity and unusually susceptible to exploitation and inappropriate treatment for commercial gain. Within the laboratory colony, moreover, the transgenic chimp is unlikely to be able to be socially successful with its own species either and potentially viewed as strange and an outcast by those without its differences. Ill-suited for both worlds, the humanized chimp is likely to sustain more injuries and encounter more privations than the unengineered inhabitants of either species.

Apes share many capacities with humans such as the capacity for attachment and empathy, communication, internalization of social rules, giving, trading, revenge, social maintenance, and attending to group boundaries. Such capacities could exacerbate the harms to these animals. If "humanizing" expressions of HLS sequences only served to inhibit or destroy the characteristics that these apes already share with us, we have harmed them, even from a human "speciesist" point of view. Since it is hard to imagine a case of transgenic HLS sequence research in apes in which these social harms and the physical welfare concerns they generate would not arise, it is reasonable to conclude that this research will almost never be ethically acceptable. For policy purposes, this conclusion supports higher regulatory barriers, in terms of safeguards and review, against any transgenic HLS sequence research with apes.

Of course, monkeys also display many of these same social and behavioral characteristics. To that extent, should they enjoy the protections as apes when it comes to transgenic research with HLS sequences? Clearly there is an evolutionary spectrum between the great apes, monkeys, and lemurs, and any line-drawing that occurs across it will be to some extent arbitrary. The evolutionary and genomic distance between monkeys and humans is great enough to diminish the prospect that a simple HLS sequence insertion in a monkey genome will produce human-like phenotypes, or that those phenotypes will be as socially challenging for monkeys as they would be for apes. Because of the greater evolutionary distance between humans and monkeys compared to humans and apes, more genetic changes would be needed in monkeys to elicit a human-like phenotype than would be needed if apes were used as the host species. Consequently, there is a greater chance that using apes as HLS hosts would produce a transgenic with a human-like phenotype than if a monkey were the host species. Accordingly, because apes would more easily be humanized, and the traits would likely be more human-like than if a monkey host were the host species. For this reason, such humanized apes would be at greater risk for exploitation. From the ethical point of view, the more social and cognitive capacities primates possess, the weightier our obligations to them become. However, if a line is needed for policy and regulatory purposes, the line between the ape and monkeys is relatively clean. This reduced risk of harm of a human-like phenotype, suggests that transgenic HLS sequence research with monkeys may not always be ethically objectionable and could be regulated on a case-by-case basis by institutional animal care and use committees, including public representation in the form of a community member.

CONCLUSIONS

A few conclusions can be drawn from this discussion. First, because of the close evolutionary genomic proximity, transgenic apes have the greatest potential to express HLS sequences as they are expressed in *Homo sapiens* and consequently experience harm from transgenic research. This is in part due to the fact that humanized transgenic apes would likely exhibit traits that are the most human-like, putting them at greater risk of exploitation. Second, the challenge of assessing our ethical obligations to “humanized” apes is profound. The ethical concerns raised by the generation and use of apes as transgenic hosts to study

HLS sequence function render this research ethically unacceptable, justifying regulatory barriers between these species and monkeys. Concurrently, we recommend that the possible use of monkeys in transgenic HLS research be examined on a case-by-case basis by animal welfare review boards, with special attention paid to the unique issues in careful application of the Three Rs in primate research. Some information regarding HLS gene function can be potentially be obtained using HLS transgenic mice. Of course, another alternative would be to use clues given to us by comparative identification of HLS sequences in further studies of members of our own species. While the NHP genome is similar to ours, there are also significant differences. Thus, there are limits to what we can learn about the function of HLS variants until we study them in the human genome where they may exist as naturally-occurring variations. By this more direct means we can potentially identify deficiencies or mutations in HLS sequences, and phenotypes that seem to accompany these changes. Human HLS research is also laden with serious ethical questions about what it might mean for a human to lack the hallmarks of “humanness”, and how that research might be used and potential harms and abuses avoided. Those ethical questions deserve their own further analysis.

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