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# Next-generation genome editing

**_Nature Biotechnology_ supports recent calls for public engagement concerning the risks and benefits of genome editing in the human germline, particularly given our poor knowledge of what we should change in the human genome.**





The CRISPR-Cas9 genome editing system is so inexpensive, so easy to use, so reproducible in different labs, and in such widespread use, it was always going to be used by someone, somewhere, sometime to alter human reproductive cells. Last month, researchers from Sun Yat-sen University in China described just that. In a paper in *Protein & Cell* (18 April 2015; doi:10.1007/s13238-015-0153-5), they report the use of CRISPR-Cas9 to cleave the beta globin gene in human tripronuclear zygotes. This now places genome editing technology front and center stage as the go-to tool for human germline modification. Because we know so little about how particular variants introduced into particular genetic backgrounds influence phenotype, it also means a thorough debate about the risks and benefits of human germline modification has never been more important.

Ever since the advent of recombinant DNA technology, the ability to make heritable human genetic modifications has been a source of controversy. Germline modification was among the chief concerns discussed by scientists at Asilomar 40 years ago. To this day, the NIH's Recombinant DNA Advisory Committee (RAC), which was created in response to Asilomar, has said it will "not entertain proposals for germline alterations." In March, two independent groups of researchers called on the community to engage with the public before genome editing research on the human germline proceeds any further (*Science* **348**, 36–38, 2015; *Nature* **519**, 410–411, 2015).

Modification of human germ cells raises concerns about lack of consent from offspring who are subjected to genome alteration, compounds unintended harms resulting from genome alterations to future generations and impacts society by altering genetic diversity of populations without prior knowledge of the consequences.

Much of the debate in germline gene therapy has focused on whether intervention in the human germline should be permissible at all. It has been widely argued that a bright line should be drawn between somatic cell gene modification and germline changes, and between disease correction and physical enhancement. As a result, germline modification by gene therapy was an activity to be avoided rather than pursued, particularly with the risk of insertional mutagenesis after genomic integration of foreign DNA; in 2001, for example, the RAC stopped a phase 1 trial of an intravenous hemophilia B gene therapy because AAV vector sequence was detected in the semen of a trial participant.

As a consequence, germline gene therapy has been something of a non-starter. In contrast, the efficiency, expediency and economy of CRISPRs and other tools, such as zinc finger nucleases and TALENs, have prompted researchers to reevaluate the possibility of altering genes in human reproductive cells.

That is not to say that genome editing of germ cells is without its own technical challenges. For example, the modification efficiency of CRISPR-Cas9 is dependent not only on the desired target sequence and cell type but also on whether the change is a deletion induced by nonhomologous end-joining of double-strand breaks or base corrections by homology-directed repair (HDR) using an oligonucleotide. The former is much more efficient than the latter at present, although there may be ways of suppressing host ligase activity to promote HDR. Thus, knockout of a dominant mutation is at present easier than correction of a loss-of-function mutant.

Another major issue is reducing the number of off-target changes made during genome editing, which again is sequence dependent. For CRISPR-Cas9, use of a nickase is one way to improve specificity. That said, any off-target changes induced at the epigenetic level—alterations potentially affecting modified offspring later in life—also would require close monitoring.

Even if off-target changes can be suppressed to acceptable levels, screens to identify germ cells containing a desired allele remain a work in progress. Advances in single-cell deep sequencing will help, particularly if modifications are carried out and screened in spermatogonial stem cells or oocyte progenitors, rather than single-cell biopsies from human embryos.

Finally, genetic mosaicism, resulting either from germ cell division before completion of Cas9 nuclease action or from residual endonuclease activity after the one-cell stage, may also compromise whether desired genetic changes are present in the correct adult tissues or at the correct levels in the body to produce a desired phenotype. Microinjecting Cas9 as a degradable protein rather than as RNA has been suggested as one way to help reduce mosaicism.

These technological challenges are likely to be surmountable. But we remain a long way from knowing which nucleotides to change.

The fact is we have identified relatively few naturally occurring alleles in the human population with sufficient penetrance that we would want to target (e.g., for monogenetic diseases such as familial adenomatous polyposis or Huntington's). In most other cases, we have little evidence of how different genetic backgrounds would affect the phenotype associated with a variant. Essentially, to countenance germline genome editing today would be to carry out a series of blind human experiments. Without a much better understanding of variants in a particular genetic background, of epistasis and of genetic networks, predicting the phenotypic consequences of changing even one allele will be difficult, let alone changing more.

That does not mean that now is not the right time to have a conversation about the applications of germline genome editing or that there may not be cases where it could be responsibly pursued. At the moment, only 25 countries ban human germline modification; the rest of the world has much more permissive regulations. Given the difficulty of policing people who are intent on using CRISPR-Cas9 to modify human germ cells, encouraging wide debate with the public, policy makers and ethicists is the best way to ensure the greatest consensus can be reached on how best to use this powerful technology for the well-being and health of all.