LGMD-1D DNAJB6 Foundation
and International Autosomal Dominant Muscular Dystrophy Registry

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Corona Virus and You

(A Pulmonary Doctor's Perspective)

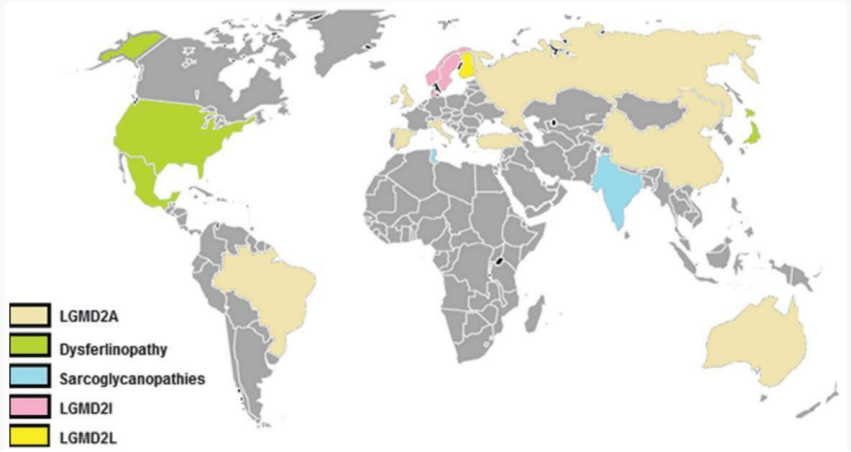
Every fall and winter we are assaulted by 200 respiratory viruses of which a handful are coronaviruses not including the one in question today. About 50 % of Americans get an influenza shot every year against the deadliest virus, influenza A and B. COVID 19, the coronavirus of current concern, and Influenza A and B can cause symptoms from the common cold to lethal pneumonia in all subgroups of people. Populations at risk are well known and should include those with muscular dystrophy who may have limited breathing muscle reserve. A mild disease not lethal to most could have mortal consequences in this population. Simple noninvasive breathing devices called BIPAP could be enough to serve as a bridge to health in hospital settings. BIPAP machines as well as conventional ventilators are likely to be in ample supply.

Aside from hygiene, isolation and personal protective equipment (see CDC guidelines), medications for treatment are still under investigation as of this writing. The news is very dramatic and fluid but stick with trusted sources of information such as the CDC.

Many companies are working on a vaccine which will be important for this fall if available by then. Luckily the COVID 19 is not prone to antigenic shift which means it does not put on a new disguise to avoid the vaccine induced immunity.

Progress on our international registry for autosomal dominant muscular dystrophies

We have made significant strides in our registry; now over 50 members around the world, from Russia to Australia and all points in between



There is a pressing need to organize the relatively few autosomal dominant muscular dystrophies since their genetic therapies will be similar.

LGMD-1D and ALL AUTOSOMAL DOMINANT MUSCULAR DYSTROPHIES REGISTRY: REGISTRATION FORM

A Review of Gene Therapy

Elizabeth G. Phimister, Ph.D., *Editor*

Prime Time for Genome Editing?

Fyodor D. Urnov, Ph.D.

In 1902, Garrod discovered that a difference in human metabolism (alkaptonuria) can be transmitted through the germline in accordance with Mendel's first law. A half century later, Ingram discovered the molecular basis for such transmission in human disease — specifically, sickle cell anemia: a focal change in the DNA that leads to a single amino acid chain in β-globin. And 60 years after that, a gene therapy was finally approved and prescribed for use in a genetic disease: a virus vector designed to ferry a normal copy of the adenosine deaminase gene to affected cells of patients with severe combined immune deficiency.

Moving forward, the pace of progress in treating genetic disease at its root cause is certain to be more commensurate with the unmet medical need. Innovations in DNA sequencing and computation allow rapid and inexpensive whole-genome sequencing of DNA from an affected person; at present, approximately 5000 medical conditions have been traced to the kinds of small changes in a single gene of the type described by Ingram. In parallel, efforts in putting DNA repair to applied use have yielded a technology, genome editing, that relies on a targeted double-stranded break to drive genetic change. The efficiency and specificity of genome editing in primary human cells and organs has passed regulatory review by the Food and Drug Administration and the European Medicines Agency to support multiple ongoing clinical trials in indications as wide-ranging as the hemoglobinopathies (ClinicalTrials.gov numbers NCT03432364 and NCT03655678), hemophilia B (NCT02695160), and congenital blindness (NCT03872479).[1] The "editor" in these trials is a DNA-cutting enzyme, such as a zinc finger nuclease or a complex consisting of a short strand of RNA and the enzyme Cas9, discovered in the CRISPR (clustered regularly interspaced short palindromic repeats) bacterial adaptive immune system. However, a group of scientists — Anzalone, Liu, and colleagues[2] — have recently described a new, "turbocharged" version of this approach.

A BRIEF HISTORY OF GENOME EDITING

A decade ago, the first patients treated with a gene-editing approach received an infusion of their own gene-edited cells, engineered with a zinc-finger nuclease (disrupting the gene *CCR5*, with the goal of slowing progression of human immunodeficiency virus [HIV] infection).[3] Excitement over the potential for treating and potentially curing certain genetic diseases by editing the genome became widespread after the discovery that Cas9, an enzyme used by bacteria to cut the genomes of invading parasites, can be programmed to induce a double-stranded break in any DNA sequence through the straightforward act of designing a 20-nucleotide RNA strand complementary to 20 bases in the DNA target.[4] Strikingly, Cas9 proved to be a versatile and efficient genome editor in a wealth of contexts, including many clinically relevant primary human cell types.[1,5] Although it is truly inspiring that our ability to identify a disease-causing mutation is, in principle, now commensurate with our ability to repair that mutation in a targeted fashion, it is also noteworthy that not one of the three aforementioned clinical trials involve editors that are designed to directly repair the disease-causing mutation.

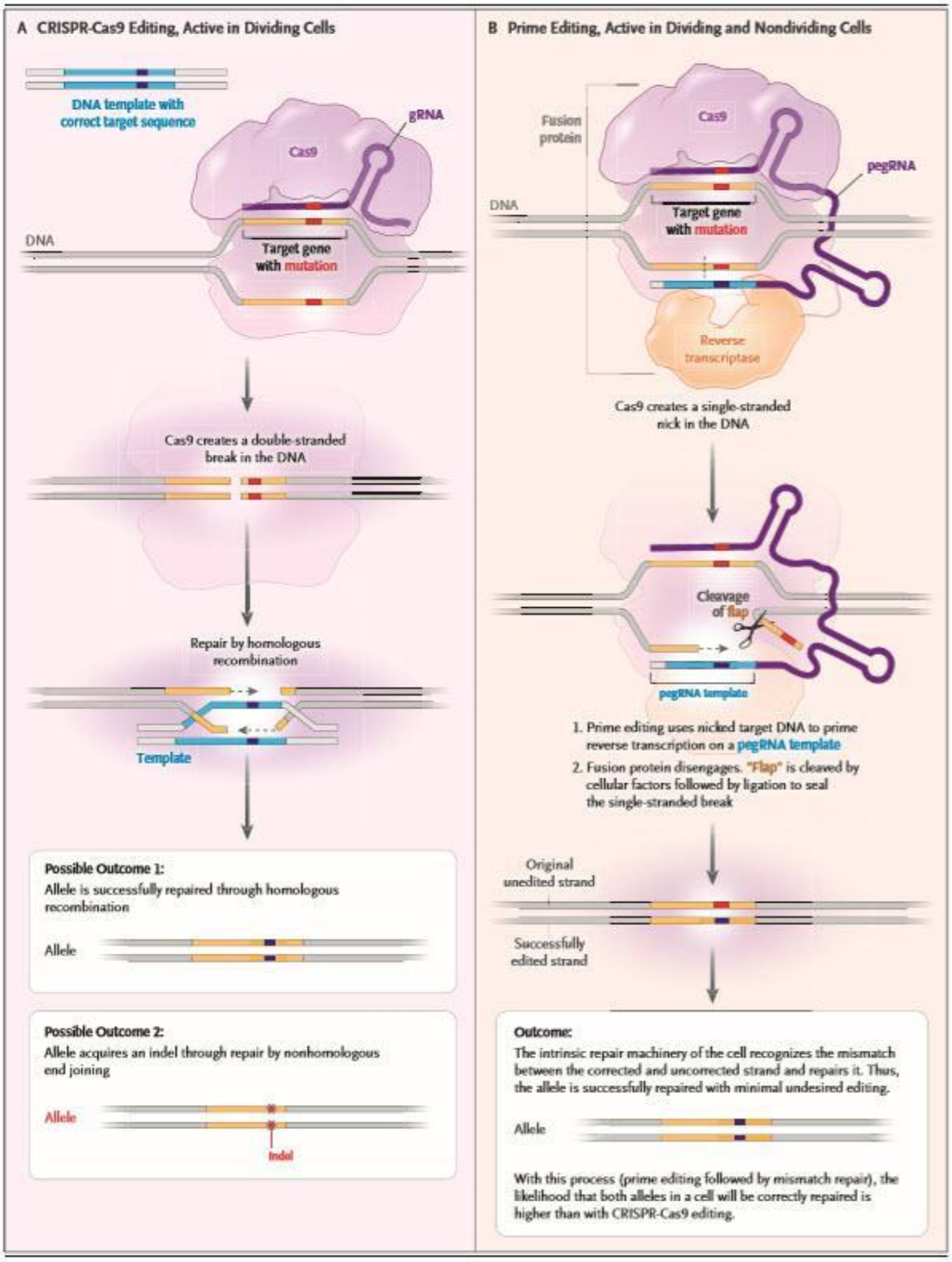
This is in part because a double-stranded break used in genome editing to activate a given region of DNA for targeted genetic change is repaired by one of two competing pathways: the direct ligation of the two ends in a process

known as nonhomologous end joining or the use of DNA of identical sequence (normally the sister chromatid) to patch the break through homology-directed repair. Repeated cycles of cutting and nonhomologous end joining will drive the gain or loss of a few base pairs, producing a knockout; alternatively, providing the cell with a repair template bearing the desired genetic change will lead to its homology-directed repair–driven transfer into the chromosome and thus to mutation repair. Although experiments in human somatic-cell genetics for research purposes routinely use both methods, in a clinically relevant setting the second method — homology-directed repair of a mutation with the use of an exogenous template — has several obstacles. Fundamental biologic features of homology-directed repair in primary human cells restrict the efficiency of repair to a small "window" of fewer than 20 bases surrounding the double-stranded break, and it is not always possible to engineer CRISPR-Cas9 to cut in such a window. Furthermore, the use of homology-directed repair to repair mutations requires an exogenous repair template, and this adds to the challenge of delivering the editing machinery to the target cell. Repairing a mutation on one allele is often accompanied by an unwanted knockout of the other allele as a result of end joining, which is the more prevalent of the two pathways of double-stranded break repair (Fig. 1A) — and in nondividing cells, such as neurons, homology-directed repair operates poorly or not at all.

Figure 1 (facing page). Two Genome-Editing Approaches.
Panel A shows gene editing with the use of clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 through DNA-template–based homology-directed repair of a Cas9-induced double-stranded break. Cas9 is guided to the mutated gene by a 20-nt segment of guide RNA (gRNA) that it carries. Once tethered through complementary binding of gRNA to target DNA, Cas9 cleaves it, forming a double-stranded break in each copy of the gene. In some cases, the double-stranded break is repaired by copying and pasting genetic information from a repair template (such as a DNA oligonucleotide or a virus) that has the normal variant of the gene (blue). The resulting allele is genetically corrected. In other cases, however, the double-stranded break is repaired by end joining, and this creates a small undesired insertion or deletion ("indel," indicated by red stars) in the gene. Panel B shows Anzalone and colleagues' technique of "prime editing,"[3] which uses nicked target DNA to prime reverse transcription on an RNA template. In prime editing, Cas9 is modified in three ways (relative to its form in CRISPR-Cas9 editing). First, its ability to cut both strands of DNA is altered so that it can cut only one strand and thus create a nick. Second, the RNA that it carries contains a "corrected" version of the part of the gene that is mutated, and therefore it is referred to as prime editing guide RNA (pegRNA). Third, the Cas9 enzyme is fused to a reverse-transcriptase enzyme: the resultant fusion enzyme can therefore nick the DNA (with the Cas9 portion) and transcribe RNA into DNA (with the reverse-transcriptase portion). This fusion enzyme binds to the mutated gene and creates a single-stranded nick. The DNA upstream (i.e., to the left) of the nick escapes from the Cas9 part of the enzyme and, in accordance with Watson–Crick base-pairing rules, binds to the extended portion of the pegRNA. Next, the reverse-transcriptase portion of the fusion enzyme synthesizes corrected DNA, using the pegRNA as a template. After reverse transcription of the edited DNA strand, the DNA repair machinery of the cell removes the redundant portion of original DNA, allowing the edited strand to pair with the complementary DNA strand in the chromosome. After the nick is sealed, the cell's DNA repair machinery corrects the complementary DNA strand, using the edited strand as a template.

A TEMPLATE FOR REPAIR

Anzalone et al. appear to have untangled this Gordian knot of problems. The foundational discoveries that enabled genome editing resulted from curiosity-driven inquiry into fundamental biologic processes, and the study by Anzalone et al. is no exception. Certain types of mobile genetic elements, called L1 retrotransposons, "move" (although "copy and paste" is perhaps a more accurate metaphor) to new chromosomal locations by cutting one of two DNA strands at their destination, whereupon the L1 RNA molecule serves as a template for reverse transcription (that is, the synthesis of DNA using an RNA template), followed by its insertion into the nicked target. Anzalone decided to marry this mechanism with a fundamental property of Cas9, the enzyme central to genome editing: the ability to carry an RNA molecule. What if that RNA molecule included an extra stretch of sequence, complementary to a DNA region of interest, except for the desired genetic change? One could then use Cas9 to place that RNA directly next to that region, whereupon Cas9 would nick the target DNA. How would the RNA-encoded corrective sequence be incorporated into the


A CRISPR-Cas9 Editing, Active in Dividing Cells
DNA template with correct target sequence
gRNA
Cas9
DNA
Target gene with mutation
Cas9 creates a double-stranded break in the DNA
Repair by homologous recombination
Template
Possible Outcome 1:
Allele is successfully repaired through homologous recombination
Allele
Possible Outcome 2:
Allele acquires an indel through repair by nonhomologous end joining
Allele
Indel
B Prime Editing, Active in Dividing and Nondividing Cells
Fusion protein
Cas9
pegRNA
DNA
Target gene with mutation
Reverse transcriptase
Cas9 creates a single-stranded nick in the DNA
Cleavage of flap
pegRNA template
1. Prime editing uses nicked target DNA to prime reverse transcription on a pegRNA template
2. Fusion protein disengages. "Flap" is cleaved by cellular factors followed by ligation to seal the single-stranded break
Original unedited strand
Successfully edited strand
Outcome:
The intrinsic repair machinery of the cell recognizes the mismatch between the corrected and uncorrected strand and repairs it. Thus, the allele is successfully repaired with minimal undesired editing.
Allele
With this process (prime editing followed by mismatch repair), the likelihood that both alleles in a cell will be correctly repaired is higher than with CRISPR-Cas9 editing.

DNA? Cas9 has been used to ferry protein subunits (such as those that activate or repress gene expression) to target DNA. Why not, reasoned Anzalone, fuse a reverse transcriptase to Cas9? This remarkable protein chimera could find and nick a DNA target with its Cas9 module and then use the extended RNA brought in by Cas9 as a template to synthesize a DNA strand that would then — one would hope — be transferred to the chromosome (Fig. 1B). This frankly quixotic undertaking has succeeded in the petri dish, despite the challenges.

Anzalone et al. showed that the new engineered enzyme–RNA complex, which they have dubbed "prime editor," can correct point mutations, insertions, deletions, and combinations of these mutations in different types of human cell and that editing was equally efficient for changes 5 bp or 50 bp distant to the nick introduced by Cas9. They observed only a low level of undesired editing in the form of small insertions and deletions at the target (a known benefit of using the nick-inducing form of Cas9). Of greatest relevance to clinical translation, this process occurs, albeit with modest efficiency, in nondividing primary mammalian cells (mouse neurons).

This substantial upgrade to the toolbox of genome editing opens the possibility of efficiently repairing mutations in key clinically relevant human cells that divide rarely or not at all. It is therefore of particular relevance to efforts to develop genome editing as an experimental therapeutic method for disorders of the nervous system and skeletal muscle. Of no lesser effect is the potential use of "prime" editing to address a fundamental challenge that experimental medicine faces in the age of personalized therapies.[6] Consider two people with the same disease who bear distinct etiologic mutations. It would be straightforward to design a CRISPR-Cas–based conventional gene-repair system for each one, but under current U.S. and European regulatory standards, this approach would require two applications — one for each CRIPR-Cas investigational new drug — doubling the effort and cost. Prime editing offers the prospect of using a single Cas9, armed with an extended RNA encoding an entire wild-type exon of a gene, to treat a cohort of persons, each of whom bears a distinct mutation in that exon. Thus, although prime editing is not going to replace double-stranded break–driven editing (recent clinical efforts in HIV, cancer, and the hemoglobinopathies do not require the prime approach), it could nonetheless be a helpful tool to correct genetic disease.

Genome editing, like other molecular interventions, needs to overcome barriers to delivery as well as immunogenicity, and it is currently at the beginning stages of the same cycle of early-stage clinical trial–based optimization that was essential to the success of, for instance, an approved viral gene therapeutic for β-thalassemia.[1] Prime editing is an impressive act of creativity by biochemists and bioengineers. I predict that the wave of such cross-disciplinary effort to accelerate the advance of editing to the clinic will, in the long term, overcome the technical barriers that lie in front of it.

Disclosure forms provided by the author are available at NEJM.org.

From the Innovative Genomics Institute and the University of California, Berkeley.

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LGMD1D Sponsored Blog:

All Things Muscular Dystrophy

Please visit and contribute if so inclined. We collect articles from lifestyle to research and post to inform our community. We have included a form for those who have an autosomal dominant muscular dystrophy who would like to register with our international registry (LGMD1 or other).

MDA and Invitae free genetic testing for MD!

Please follow the link to the Invitae guidelines and order panels. If your family has a specific gene to be tested then select that one gene and include that in your application (see page 10). If you don't have a specific gene but looking for a diagnosis then a whole comprehensive panel may be for you. Please call Invitae at 800-436-3037 to discuss the proper test before you start an application. I will help your application on page 10.

Please follow this link

MDA and Invitae free genetic testing for MD!

As a California licensed physician and an ordering physician with an account at Invitae, I have ordered a variety of genetic tests for individuals seeking genetic screening or confirmation. Although not a trained geneticist I have a functional knowledge of what genetic tests need to be done in the realm of muscular dystrophy. Genetic test ordering by paper or online can be unfamiliar and a tedious undertaking to most primary care providers and general neurologists.

DISCLAIMER: As a result I would be willing to act as an ordering physician facilitator for individuals needing and qualifying for free testing at Invitae. I would not be entering into a physician patient relationship with you but mainly a test ordering conduit. Discussing test results and verifying test results would be done with your primary care provider, primary neurologist, or calling Invitae genetic counselling and quality control staff at 800-436-3037.

If you consent to this process you would follow the link below which asks for important data for the Invitae ordering form without which I could not submit for your testing kit. Your data is protected by Google Suite and I follow HIPAA guidelines for personal medical data. Once the order is submitted and approved then a sputum kit with instructions is mailed to you. You will submit your specimen and the results may take 2-4 weeks to reach you.

Please let relatives and offspring who are interested know that this is available and a rare opportunity.

GENETIC TEST APPLICATION FOR INVITAE

Giving Tuesday Success!

Thanks to your generosity on Giving Tuesday in December, and matching funds from FaceBook, we received over 4,000 dollars. We have raised 50,000 dollars since inception in January, 2018. The next Giving Tuesday will be 12/1/20 so please mark your calendar. Due to the COVID 19 restriction on travel our natural history study is moving slowly but we stand ready to assist in offsetting travel expenses as per our mission statement.

Ways to Give All Year!

1. **OUR WEBSITE** **(a secure site with all the listings below)**
2. **AMAZON SMILE** **(list the LGMD-1D DNAJB6 Foundation for donation with each purchase at no cost to you.)**
3. **PAYPAL** **(Our foundation secure site)**
4. **CREDIT CARD** **(GuideStar charity secure portal)**