Gene-edited chickens in the fight against bird flu

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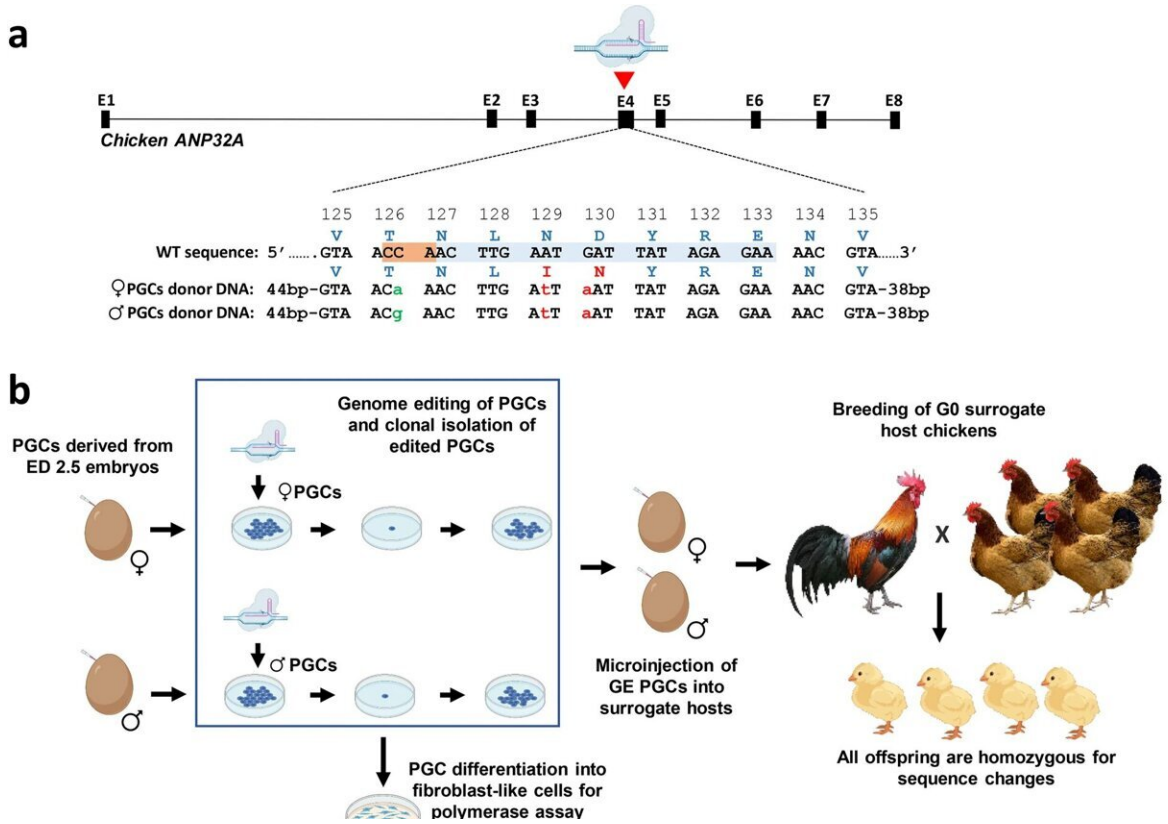

Breeding strategy for homozygous ANP32A$^{N129I\text{-}D130N}$ chicken. **a** ANP32A editing strategy: two nucleotide changes (red letters) introduce asparagine (N) position 129 (N129I) and aspartic acid (D) position 130 (D130N) missense mutations. The third nucleotide change (green letters) is a synonymous mutation in the gRNA PAM and serves as a marker control for allelic contribution from the male and female surrogate hosts. **b** Male and female PGC cultures were derived from the blood of individual chick embryos. The PGCs were edited, and clonal lines of GE PGCs were propagated and analyzed. GE PGCs were

differentiated into fibroblast-like cells for IAV polymerase assays. To generate GE chicks, GE PGCs were mixed with B/B dimerisation compound (to induce cell death of host embryo germ cells) and injected into iCaspase9 host embryos, which were incubated to hatch. After hatching, the surrogate hosts were raised to sexual maturity and directly mated. All offspring from eggs laid by the surrogate hosts were biallelic for the edit and contained the parent-specific PAM nucleotide change. **c** the activity of reconstituted IAV polymerase was assessed in fibroblast-like cells derived from ANP32A^{knockout} (Knockout), ANP32A$^{N129I-D130N}$ (N129I-D130N) or wild-type (WT) PGCs. Cells were transfected with avian IAV polymerase (PB2/627E - black bars) or human-adapted isoforms (PB2/627K - gray bars), Firefly minigenome reporter and Renilla reporter control plasmids and then incubated at 37 °C for 48 h. Wild-type chicken ANP32A (chA) cDNA was co-expressed with minigenome plasmids to rescue polymerase activity in ANP32A$^{N129I-D130N}$ cells. Data shown are Firefly activity normalized to Renilla plotted as mean ± SEM derived from (n = 3) three independent experiments each consisting of three technical replicates. Error bars represent standard error of mean (SEM). One-way ANOVA and Dunnett's multiple comparison test were used to compare polymerase activity in the GE cells with polymerase activity in WT cells. Unpaired two-tailed t-test was used to compare ANP32A$^{N129I-D130N}$ and ANP32A$^{N129I-D130N}$ +chA data. Statistical annotations are defined as *$P \leq 0.05$, ****$P \leq 0.0001$. **d** Image: wild-type (WT) hen (left) and homozygous ANP32A$^{N129I-D130N}$GE hen (right, blue ring on right shank). Credit: *Nature Communications* (2023). DOI: 10.1038/s41467-023-41476-3

Scientists have used gene editing techniques to identify and change parts of chicken DNA that could limit the spread of the bird flu virus in the animals.

Researchers were able to restrict—but not completely block—the virus from infecting chickens by altering a small section of their DNA.

The birds showed no signs that the change in their DNA had any impact

on their health or well-being.

The findings are an encouraging step forward, but experts highlight that further gene edits would be needed to produce a chicken population which cannot be infected by bird flu—one of the world's most costly animal diseases.

Scientists from University of Edinburgh, Imperial College London and the Pirbright Institute bred the chickens using gene editing techniques to alter the section of DNA responsible for producing the protein ANP32A. During an infection, flu viruses hijack this molecule to help replicate themselves.

When the ANP32A gene-edited chickens were exposed to a normal dose of the H9N2-UDL strain of avian influenza virus—commonly known as bird flu—9 out of 10 birds remained uninfected and there was no spread to other chickens.

Partial protection

The research team then exposed the gene-edited birds to an artificially high dose of avian influenza virus to further test their resilience.

When exposed to the high dose, half of the group—5 out of 10 birds—became infected. However, the gene edit did provide some protection, with the amount of virus in the infected gene-edited chickens much lower than the level typically seen during infection in non-gene-edited chickens.

The gene edit also helped to limit onward spread of the virus to just one of four non-gene-edited chickens placed in the same incubator. There was no transmission to gene-edited birds.

Viral evolution

Scientists found that in the ANP32A gene-edited birds, the virus had adapted to enlist the support of two related proteins—ANP32B and ANP32E—to replicate.

Following lab tests, scientists found that some of the mutations enabled the virus to utilize the human version of ANP32, but its replication remained low in cell cultures from the human airway.

Experts say that additional genetic changes would be needed for the virus to infect and spread effectively in humans.

However, the findings demonstrate that the single ANP32A gene edit is not robust enough for application in the production of chickens, according to the team.

Further edits

To prevent the emergence of escape viruses—viruses that adapt to evade the gene edit and cause infection—the research team next targeted additional sections of DNA responsible for producing all three proteins—ANP32A, ANP32B and ANP32E—inside lab-grown chicken cells.

In cell cultures in the lab, growth of the virus was successfully blocked in cells with the three gene edits.

The next step will be to try to develop chickens with edits to all three genes. No birds have been produced yet.

The study highlights the importance of responsible gene editing and the

need to be alert to the risks of driving viral evolution in unwanted directions if complete resistance is not achieved, experts say.

Bird flu is a major global threat, with a devastating impact in both farmed and wild bird populations. In the UK alone, the current outbreak of H5N1 bird flu has decimated seabird populations and cost the poultry industry more than £100 million in losses.

In rare instances, mutations in the bird flu virus allow it to infect people and cause serious illness. Efforts to control the spread of the disease are urgently needed.

"Bird flu is a great threat to bird populations. Vaccination against the virus poses a number of challenges, with significant practical and cost issues associated with vaccine deployment. Gene-editing offers a promising route towards permanent disease resistance, which could be passed down through generations, protecting poultry and reducing the risks to humans and wild birds. Our work shows that stopping the spread of avian influenza in chickens will need several simultaneous genetic changes," said Professor Mike McGrew, the study's principal investigator, from the University of Edinburgh's Roslin Institute.

"This work is an exciting collaboration that fuses our expertise in virology with the world-leading genetic capability at the Roslin Institute. Although we haven't yet got the perfect combination of gene edits to take this approach into the field, the results have told us a lot about how influenza virus functions inside the infected cell and how to slow its replication," said Professor Wendy Barclay, Imperial College London.

The work is published in the journal *Nature Communications*.

More information: Alewo Idoko-Akoh et al, Creating resistance to avian influenza infection through genome editing of the ANP32 gene

family, *Nature Communications* (2023). DOI: 10.1038/s41467-023-41476-3. www.nature.com/articles/s41467-023-41476-3

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