Lay person summary

Imagine a creature that grows to be as tall as a two-story building, with a heart weighing up to 25 pounds, and that can sprint faster that 30mph (girafeconservation.org). Although they sound other-worldly, you have probably actually seen one. Given their incredible stature, it is no wonder that giraffes are incredibly popular zoo animals as well as inspiration for business logos and toys. But we stand to learn much more from giraffes than just the delicate art of being a towering, but gentle, giant.

Due to their extreme height, the tallest animals on land, giraffes have long drawn the attention of scientists interested in how such a unique creature evolved. Evolution through natural selection acts through traits, like height or fur color. A trait is "selected for" if individuals with that trait are more likely to survive and have offspring than are individuals without the trait. It is easy then to see some of the evolutionary benefits giraffe's tall stature provide: they can easily access food (i.e. leaves) that other animals cannot and with their high vantage point they can better be on the lookout to avoid predators. But, as they say, there is no such thing as a free lunch. With the benefits that come with giraffe's height, also come some pretty serious drawbacks. Giraffes' necks are long enough to reach the tastiest leaves, but they are not long enough for giraffes to drink water on the ground while standing. Instead, giraffes must lower down onto bent front legs in order to get a drink of water. This puts them at increased risk of attacks from predators, not least of which because giraffes are relatively slow to rise due to how far messages must travel along neurons from their brain to their legs. Similarly, giraffe hearts have to pump blood significantly further and against higher pressure than other, shorter animals.

To better understand how giraffes have evolved to deal with the unique challenges they face, a recent study published in the journal Science Advances studied the genome of giraffes compared to fifty other related animals. An organism's genome is its total DNA sequence, containing all of the organism's genes and DNA sequences that control whether those genes are on or off. Genes are the instructions for making proteins, which are the molecules that do all of the work in our bodies, from making sure the heart pumps blood to digesting food. Different gene sequences create different proteins and other DNA sequences control when and how many proteins are made. Therefore, comparing the genome sequences of different animals can allow scientists to identify DNA regions that may explain physical differences between the animals, such as height. Understanding the differences in DNA that underlie differences between animals, in things like regulating blood pressure, can help develop new treatments for human diseases, such as hypertension.

In order to be able to find differences in the genomes of different animals, scientists must first "sequence" the genome of each animal. All life on earth uses the same DNA "code" of four letters, and the different arrangements and combinations of millions of these four letters, called base pairs, make up a genome. We can imagine a genome as a cookbook written in invisible ink. All of the instructions to make all of the cakes, croissants, and puddings of life are in the book, but until we can see the ink we cannot read any of the instructions. By sequencing a genome, scientists add lemon juice and heat to the cookbook so that the words (DNA basepairs) are revealed and we can read the instructions.

In this study, the first thing Chang Liu and colleagues did was sequence the giraffe genome. Once they had this readable "cookbook" for giraffe life, they could compare the recipes and instructions to those in the genomes, or cookbooks, of other related animals. The authors of this study compared the giraffe genome with that of closely related animals such as okapi and goat as well as that of the very distantly related sperm whale. By doing these comparisons, the authors can look for DNA sequences, or "recipe instructions", that are specific to giraffes. Such giraffe-specific sequences are then likely candidates for controlling giraffe-specific features, such as height or blood pressure.

The gene with the highest number of "recipe variations", called mutations (i.e. changes in the DNA sequence), in giraffes compared to the other animals was a gene called FGFRL1. Mice or humans with mutations in this gene have substantial issues in both their cardiovascular and skeletal systems. The authors therefore suspected that the giraffe-specific version of this gene may be important for giving giraffes their extreme height and insensitivity to high blood pressure. Although giraffes have much higher blood pressure than other animals due to how far up blood must travel to reach their head, this high blood pressure does not cause the damage to the heart, kidneys, and other organs that it does in other animals, including humans.

To test whether the giraffe-specific version of FGFRL1 is important in regulating height and blood pressure, the authors of the study used CRISPR to change the DNA sequence of the FGFRL1 gene in mice to the giraffe FGFRL1 sequence (Figure 1). At baseline, the mice with the giraffe FGFRL1 gene had the same blood pressure levels as mice with the normal mouse FGFRL1 gene. But, when the authors induced high blood pressure in the two groups of mice using a drug (angiotensin II) that constricts blood vessels and causes hypertension, the blood pressure levels of the mice with the giraffe gene did not increase! The mice who had the mouse version of FGFRL1 developed hypertension, and associated damage to their hearts and kidneys, but the mice with the giraffe FGFRL1 did not. In addition to FGFRL1, the authors identified numerous other genes which had giraffe-specific mutations that are likely involved in the giraffe's unique ability to tolerate high blood pressure. While many more experiments are needed to understand how giraffe-specific mutations in FGFRL1 and other genes enable giraffes to tolerate high blood pressure, these genes are potential candidates for hypertension therapy in humans.

Previous studies had suggested that the giraffe version of the FGFRL1 gene was responsible for the long necks of giraffes. Did these authors then end up with mouse-sized giraffes? Unfortunately, no, the mice with the giraffe FGFRL1 gene were the same size as the mice containing the mouse version of the gene. But the mice with the giraffe changes did have higher bone density than their non-edited counterpart mice. Although giraffes are the mammals with the fastest growing skeleton, which is usually related to weaker bones, they are also able to achieve strong, high density bones. The findings of this study that mice with the giraffe version of FGFRL1 had higher bone density suggests that this gene may play an important role in giraffes' ability to develop strong bones, even at their faster growth rate. While again, more study is still needed, this suggests FGFRL1 may also be a good potential target for human therapies against bone-related diseases such as osteoporosis.

By sequencing the giraffe genome (reading the cookbook of instructions for making a giraffe), scientists identified other giraffe-specific versions of genes. A number of genes that are involved in cardiovascular health, including regulating blood pressure, were found to have unique sequence changes in giraffes. Similarly, the scientists found many giraffe-specific changes in genes involved in controlling sleep, which main explain how giraffes can function on as few as three hours of sleep a day. Future investigations into these giraffe-specific changes in different genes will help scientists understand both how giraffes establish their unique body and potentially how mutations in these genes in humans can have impacts on health and disease.

Giraffes have long captured the attention of curious humans – from evolutionary biologists to mesmerized zoo-goers. By investigating the unique characteristics of giraffes at the DNA level, Liu and co-authors provide us with insight into how giraffes survive and thrive with their unique physiology. This insight is not only useful to any and all giraffe-lovers, but also stands to aid in our understanding of and treatment of human diseases including hypertension and osteoporosis.

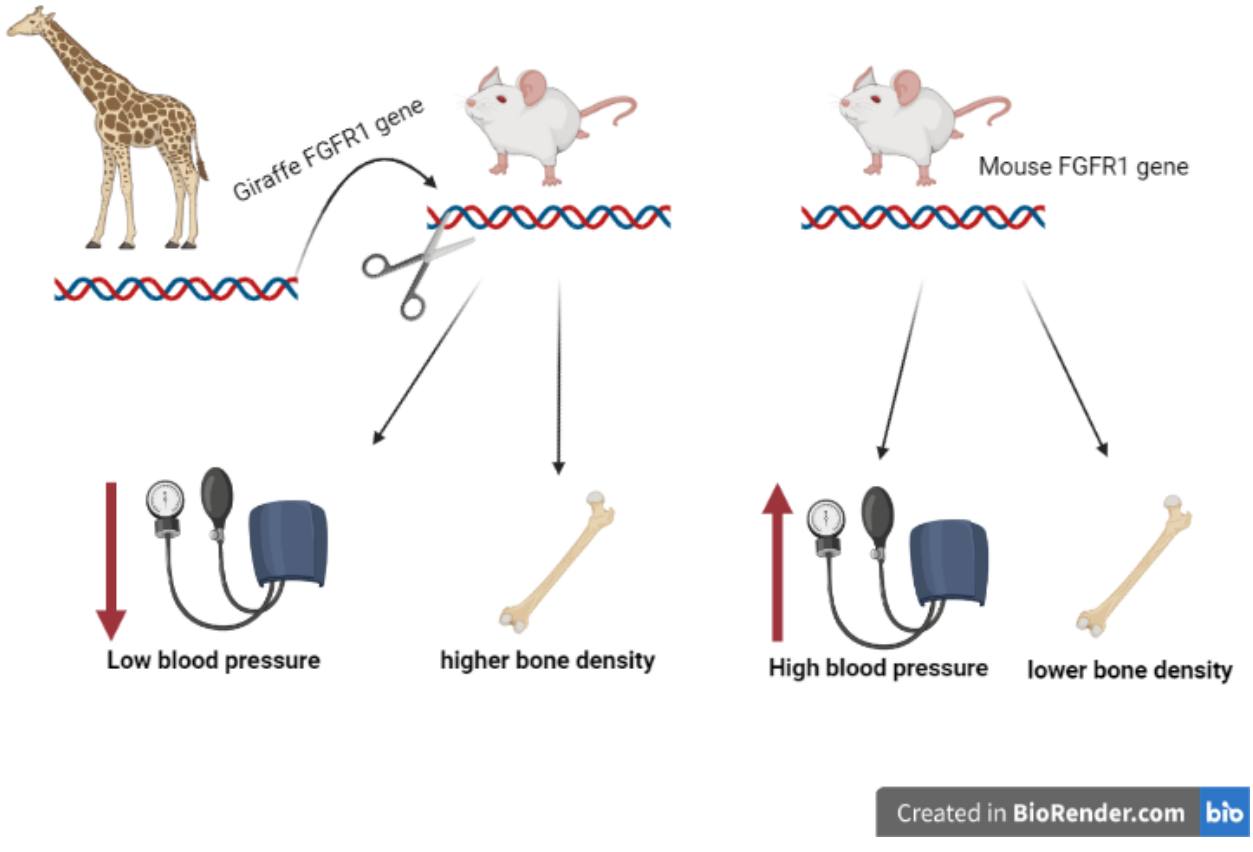

Figure 1 – Mice with giraffe FGFR1 gene maintain lower blood pressure and higher bone density. The authors of the study used CRISPR to edit the FGFR1 gene in mice to the DNA

sequence found in giraffes. The mice with the giraffe FGFR1 gene did not develop high blood pressure or related tissue damage when given a drug to induce hypertension, compared to normal mice with the mouse FGFR1 gene who did. Additionally, the mice with the giraffe FGFR1 gene had higher bone density as adults than did the mice with the mouse FGFR1 gene.

Citations:

https://giraffeconservation.org/facts/13-fascinating-giraffe-facts/

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Scientific Summary

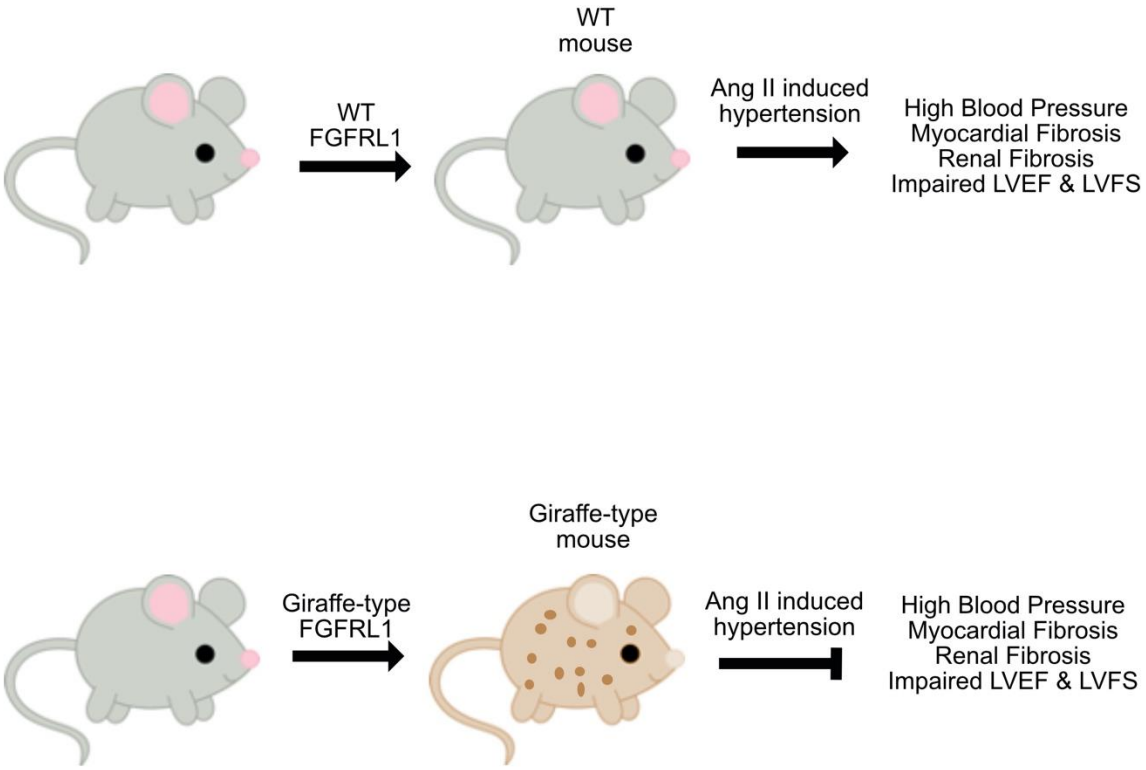

Figure 1. Introduction of Giraffe-type FGFRL1 receptor into mice alleviate the consequences of Angiotensin II induced hypertension.

Giraffes are easily identifiable terrestrial mammals associated with their towering stature and long necks. This unusual anatomy has been thought to provide giraffes with a variety of advantages in the wild. Not only are giraffes capable of reaching food sources normally thought to be out of reach (Cameron and du Toit, 2007) but their tall stature allows them to easily scan the area surrounding them in order to detect predators, competitors, or other sources of food (Coimbra et al., 2013). However, this incredible anatomy is not without consequence. According to Mitchell and Skinner (2009) the giraffes cardiovascular system has to tolerate twofold higher systemic blood pressure compared to other mammals. Not only this, but giraffes have had to adapt to sudden changes in blood pressure related to raising and lowering of their heads (Brondum et al., 2009) as well as neuromotor delays due to the large length of the giraffe neural networks (More et al., 2013). Together this makes the giraffe an interesting animal to study the evolution of several different traits that are associated with such a body plan, which is what Liu et al. sought to do.

In 2016 the first giraffe and okapi (a member of the Giraffidae family) draft genomes were published (Agaba et al., 2016), however these genomes were largely fragmented and did not provide the full picture of the giraffe's genome. Comparative analysis using these draft genomes was also limited due to alignment with cattle reference transcripts, thus limiting the ability to explore unique features of the giraffe genome. The authors here report an improved chromosome level genome assembly of a Rothschild's giraffe. Using this new high-quality genome along with their previously published whole genome dataset of approximately 50 ruminant species (Chen et al., 2019) allowed the authors to identify giraffe specific mutations at a higher level of accuracy than previously reported.

The first thing that the authors sought to do is generate a high-quality genome assembly of a giraffe. To do so the authors sequenced the genome of a male Rothschild's giraffe using three techniques. The authors combined single-molecule real-time sequencing, paired-end sequencing, and Hi-C sequencing. Using the real-time sequencing data along with the paired-end data the authors were then able to anchor that data to the giraffe chromosomes using the data generated from Hi-C. After a series of quality checks the authors believed that they had produced a high-quality genome assembly of the Rothschild's giraffe. One interesting finding from this initial sequencing is that the giraffe has many fewer chromosomes (diploid of 30) than the putative ancestral karyotype (diploid of 58 to 60) (Liu et al., 2021). Using the genome data of other ruminant species, the authors were able to determine that at a minimum four fissions and 17 fusions occurred between the pecoran ancestor and the giraffe, resulting in the decrease in chromosome number (Liu et al., 2021), however the functional significance of these fission and fusion events remains unknown to the authors.

The authors next wanted to focus on the adaptive divergence between the giraffe and other mammals within coding regions. The authors detected 101 positively selected genes (PSGs) and 359 rapidly evolving genes (REGs) within the giraffe genome (Liu et al., 2021). This is a large increase compared to the previous data set published by Agaba et al. (2016), in which the authors identified 17 PSGs and 53 genes with adaptive divergence in giraffe. Seven of the 17 previously identified PSGs overlapped with the findings from Liu et al. The remaining 10

showed no positive selection based on the new analysis, this is believed to be due to the fact that many additional ruminant species were used as background. Similar to the PSGs only 15 of the 53 previously identified adaptive divergence genes showed up in the new analysis. With the improved genome assembly as well as the higher number of accessible ruminant reference genomes allowed the authors to decrease both the false positive and false negative signals of genes undergoing adaptive evolution in the giraffe.

The authors next performed Geno Ontology (GO) enrichment analysis and saw that 460 PSGs and REGs identified in this study were primarily related to growth and development, nervous and visual systems, circadian rhythm, and blood pressure regulation (Liu et al., 2021). KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis showed that rapidly evolving pathway in giraffe compared to okapi are related to metabolic, circulatory, and immune systems (Liu et al., 2021).

The authors next wanted to take a deep dive into which giraffe specific genes or mutations could be responsible for the extreme body plan of the giraffe compared to that of other ruminant species. One gene in particular that caught the attention of the authors was the giraffe fibroblast growth factor receptor-like protein 1 (*FGFRL1*). This gene was previously identified by Agaba et al. (2016) as a potential target for selection in the giraffe. The giraffe *FGFRL1* contains a cluster of seven nonsynonymous mutations in its key FGF binding domain when compared to the sequences of other ruminants or outgroup mammals. Due to the fact that the authors had such an expanded set of background group genome assemblies the authors were able to confirm that these seven mutations are unique to the giraffe version of this gene. On top of this the giraffe *FGFRL1* contains more unique substitutions than any other gene identified in giraffes. Mutations within *FGFRL1* in humans and mice cause severe cardiovascular and skeletal defects (Catela et al., 2009 and Engbers et al., 2009). The authors thus hypothesized that *FGFRL1* may be associated with the cardiovascular and skeletal adaptations that are seen in the giraffe. In order to investigate the in vivo consequences of these giraffe specific mutations on *FGFRL1* gene the authors utilized CRISPER-Cas9 technology. Using CRISPER-Cas9 the authors were able to introduce these seven giraffe specific mutations into the *FGFRL1* gene of mice creating a giraffe-type *FGFRL1* mouse. In contrast the mice that have a targeted deletion of *FGFRL1* Catela et al., 2009) the giraffe-type *FGFRL1* mice were viable and fertile.

The giraffe is characterized by exceptionally high blood pressure without any of the related organ damage. This is in contrast to the detrimental effects of hypertension observed in other animals and humans (Mitchell and Skinner 2009). Since *FGFRL1* is known to be involved in the cardiovascular system the authors hypothesized that the vascular adaptations in the giraffe may only be apparent in a state of hypertension. To test this the authors induced high blood pressure in both wild-type (WT) and mutant *FGFRL1* mice. To achieve this the authors infused the mice with angiotensin II (Ang II), which is known to induce hypertension by vasoconstriction and sodium retention. The giraffe-type *FGFRL1* mice showed no signs of congenital heart defects or any obvious alterations in the heart rate compared to the WT control mice (Liu et al., 2021). The authors did note that baseline blood pressure was slightly higher in giraffe-type *FGFRL1* mice compared to the WT although this difference was not significant. This

was not the case after Ang II infusion for 28 days. After Ang II infusion the authors saw that average systolic and diastolic blood pressure in WT controls were significantly increased (158.97 and 94.54 respectively). This confirmed to the authors that Ang II infusion was successful in inducing hypertension in these mice. To the authors surprise Ang II induced hypertension was not seen in the giraffe-type *FGFRL1* mice. On average these mice systolic and diastolic pressures were recorded as 125.30 and 83.43 respectively (Liu et al. 2021), showing no significant difference over vehicle-controlled giraffe-type *FGFRL1* mice. The authors also noted that in the giraffe-type *FGFRL1* mice there was significantly less myocardial and renal fibrosis compared to the WT mice. Impaired heart function in the WT mice due to the Ang II induced hypertension was also absent in the giraffe-type *FGFRL1* mice as seen by the improved left ventricular ejection fractions (LVEF) and fractional shortening (LVFS) (Liu et al. 2021). Taken together the authors were able to show that giraffe-type *FGFRL1* has little effect on the cardiac development in mice, however giraffe-type *FGFRL1* was able to prevent Ang II induced hypertension. Despite the differences in cardiovascular structure and physiology between mice and human, there is still potential that *FGFRL1* might be a useful therapeutic target for the prevention or treatment of hypertension and cardiovascular disease in humans. Especially since the giraffe-type *FGFRL1* mice were able to avoid the detrimental effects of hypertension such as the myocardial fibrosis.

Along with the effect on the cardiovascular system, the authors noticed that postnatal day 0 (P0) giraffe-type *FGFRL1* mice showed prenatal hypoplasia of skeletal elements, a smaller body size, delayed craniofacial development, shortened axial/appendicular skeletons, and smaller vertebral lengths compared to P0 WT mice (Liu et al., 2021). This is in contrast to adult giraffe-type *FGFRL1* mice that showed no discernable skeletal phenotype compared to WT or any significant differences in body size, weight, limb length, or vertebral height. This shows that giraffe-type *FGFRL1* associated postnatal bone growth can compensate for the prenatal effects of giraffe-type *FGFRL1*. Because of these observed effects the authors wanted to check the bone ultrastructure of giraffe-type *FGFRL1*. Giraffe-type *FGFRL1* mice showed significantly higher bone mineral density (BMD), bone volume/total volume ration, and average trabeculae thickness in vertebrae and distal femur (Liu et al., 2021). Along with this date the authors have found that not only does giraffe-type *FGFRL1* enhance hypertension resistance but also achieves normal bone strength, despite the accelerated bone growth.

Previous analysis has revealed that multiple giraffe organs are involved with the adaptations of the cardiovascular system. The results from Liu et al. have revealed that several pathways related to tissues that are influenced by high blood pressure have significantly diverged between giraffe and other ruminant species. For example, the platelet activation pathway which plays an important role in hypertension associated thrombosis (Maxwell et al., 2006). The authors found that three REGs and a number of genes with giraffe specific amino acid variations are involved the two major platelet activations paths. Furthermore, a set of PSGs and REGs that participate in the phosphatidylinositol metabolism may also be involved with platelet activation (Liu et al., 2021). Along with the platelet activation pathway some other pathways that showed strong divergence from giraffe and other ruminant species include the adrenergic signaling in cardiomyocytes, proximal tubule bicarbonate reclamation, and endocrine calcium reabsorption. The last two pathways both of which occur in the kidney. These findings

by Liu et al of multiple genes involved in several phenotypic traits that share evolutionary constraints due to the stature of the giraffe suggests that pleiotropy may play an important role in the evolution of such a body plan.

For herbivores that are subject to predation gathering visual information and activating a muscular response is paramount to the survival of these animals. As such, the authors investigated the other sensory systems of the giraffe based on the genome analysis. The authors found several PSGs and REGs that contribute to eye development and vision in the genome assembly. Not only this but the authors also found a number of genes that are related to Usher syndrome in humans which affects vision, hearing, and balance (Mathur and Yang (2015). Similar to other ruminant species the authors only found two opsin genes (which are related to vision of colors). Since only two genes were found the authors are not able to conclude as to if giraffes se color. Going beyond vision the authors found indications that the giraffe's sense of smell may have degenerated. Comparing to the okapi, the giraffe has lost at least 53 olfactory related genes, 50 of which encode for olfactory receptors (Liu et al., 2021). Overall, this type of genetic analysis conducted by Liu et al. highlights the fact that due to the stature of the giraffe several sensory systems had to undergo necessary changes and adaptations.

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