Preview

Functional screens refine height GWAS loci

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https://doi.org/10.1016/j.xgen.2023.100325

Genome-wide association studies (GWASs) have demonstrated the complexity of human height. Baronas et al.[1] used a high-throughput CRISPR screen to identify genes that participate in growth plate chondrocyte maturation as a functional follow-up and validation screen to refine loci and establish causality after GWASs.

The most recent genome-wide association study (GWAS) from the GIANT consortium has uncovered over 7,000 non-overlapping genomic segments associated with human height.[2] The lead single-nucleotide polymorphisms (SNPs) at many GWAS loci are found in untranslated regions of the genome, and they are often intergenic.[3] For this reason, multiple methods including computational and experimental manipulations have been employed to determine and characterize how identified causal variants impact the biology of the ultimate phenotype.[3] Assigning the correct causal gene to a locus is not straightforward.[3] Baronas et al. present a high-throughput CRISPR knockout (KO) screen in a mouse cell line model of growth plate-like chondrocytes (GPLCs, Figure 1A) to identify genes with significant GWAS associations that specifically impact growth plate chondrocyte maturation.[1] They use the findings from their screen to gain insight into and to expand upon the likely function of two height GWAS hits.[2,4] In doing so, they expand our understanding of the molecular mechanisms by which height is impacted.

Growth of long bones is mediated by the proliferation and differentiation of chondrocytes at the growth plate, which is located between the epiphyses and metaphyses. The growth plate is organized into three zones. The resting zone contains quiescent cells and is located on the epiphyseal side of the growth plate. Below the resting zone, moving toward the diaphyseal side of the growth plate, is the proliferative zone. Here chondrocytes flatten and arrange themselves in a columnar fashion while retaining the ability to divide. Below the proliferative zone,

moving even closer toward the diaphyseal side of the growth plate, is the hypertrophic zone, which contains non-proliferating pre-hypertrophic chondrocytes. These pre-hypertrophic cells can further differentiate into hypertrophic cells that increase in size or swell. Growth of the bone is mediated by the continuous flow of cells from the resting zone, their replication in the proliferative zone, and an increase in size of the hypertrophic cells.[5]

Previous studies using GPLC cells have shown that in a monolayer culture, these cells will mature and express many of the known markers defining maturation of the native growth plate chondrocytes.[6] Baronas et al. sought to identify genes that alter the expected trajectory of maturation (Figure 1B) and thus putatively impact the transition from immature, proliferating cells to more mature cells.[1] Specifically, they used a genome-wide CRISPR KO library to disrupt genes in GPLCs in a high-throughput fashion. By focusing on an early differentiation step (day 4 of culture) and late time point (15 days), these experiments identified KO cells that represented accelerated maturation or delayed maturation at each time point. Importantly, in a focused replication, the effect on maturation was validated by knocking out top target genes. Many expected genes were identified, such as suppressors of Indian hedgehog signaling (*Sufu* and *Ptch1*). Further, 17 of the CRISPR KO genes identified are listed in the Online Mendelian Inheritance in Man (OMIM) database as being causative for "disorders of skeletal growth." Collectively, these results indicate that this assay has biological relevance (Figure 1C).

The authors then mine this list of genes to determine what this information tells us about genetic determination of height. An enrichment was observed for the height GWAS-prioritized genes[2,4] among KO genes that alter GPLC maturation (Figure 1C). Specifically, the authors show that KOs that alter maturation at day 4 are more likely to be associated with GWAS-prioritized genes than those that affect later maturation. Furthermore, SNPs near the CRISPR KO genes have increased per SNP heritability. This biological screen helps explain how genes impact height in a manner not possible to glean solely from GWAS summary statistics and gene prioritization efforts. Excitingly, this study yields invaluable information about previously uncharacterized genes. The authors highlight *Det1*, a gene prioritized by Yengo et al.,[2] which lacked any prior association with chondrocyte biology. This study provides information on novel height GWAS genes, such as the transcription factor *Bptf*, which was not previously studied in chondrocytes but demonstrated effects in maturation at both day 4 and day 15. Further supporting a function in height, mutations in *BPTF* in humans are associated with neurodevelopmental disorder with dysmorphic facies and distal limb anomalies (NEDDFL), a rare disease that causes short stature, among other clinical features.[7]

Given how growth plate chondrocytes mediate growth *in vivo*, cellular maturation is a logical phenotype to test. Although it reveals the relative importance of early versus late maturation to height, this screen could not identify a putatively causative gene for all of the loci identified in
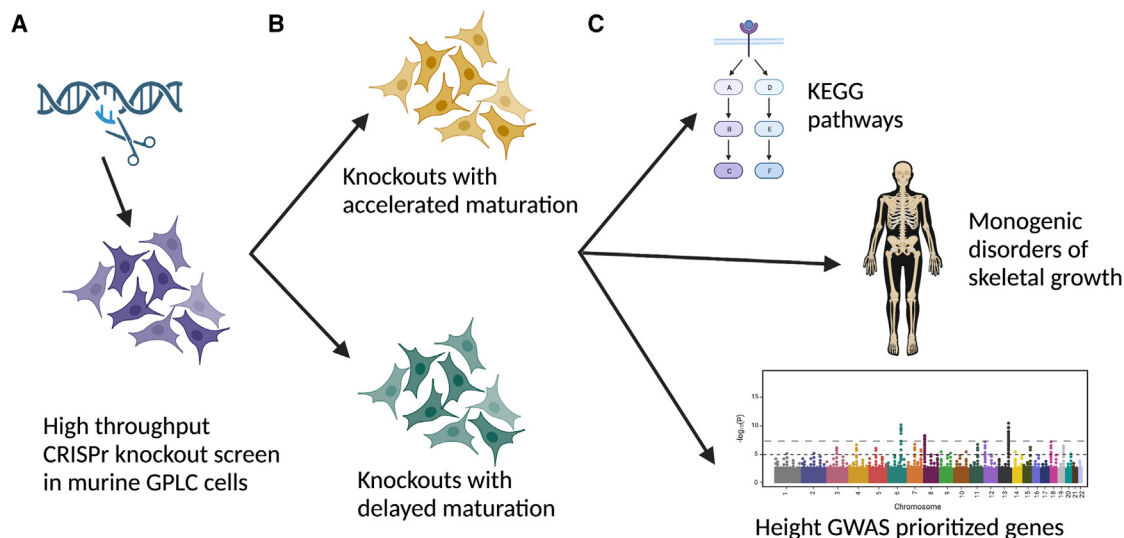
Figure 1. Flowthrough from CRISPR-induced mutations to functional contextualization
(A) A high-throughput protocol was used in a mouse cell line model of growth plate-like chondrocytes (GPLCs) in which genome-wide CRISPR knockout (KO) genes could be studied.
(B) The KO genes with the most accelerated or suppressed GPLC maturation were identified.
(C) The genes most affecting maturation showed enrichment for Kyoto Encyclopedia for Genes and Genomes (KEGG) pathways that regulate chondrocyte maturation, were overrepresented among GWAS prioritized genes, and were responsible for rare Mendelian syndromes affecting height.

height GWASs,[2,4] as the study is not designed to follow every part of growth plate biology. One limitation is that Baronas et al. did not complete any other *in vivo* or mechanistic follow-up for any of their genes as part of this study.[1] Explorations of public databases such as the Mouse Genome Informatics database (MGI)[8] and the International Mouse Phenotype Consortium (IMPC) portal[9] were not helpful, as the primary phenotype available in the IMPC is nose-to-anus length, which is not a surrogate for height. However, these investigations provide a strong foundation from which to launch new studies focused on other aspects of growth and for the development of new hypotheses regarding specific genes or pathways. The authors provide a searchable web portal for the community to use for just such a purpose (https://chondrocyte.shinyapps.io/). Most excitingly, this study provides additional proof of the value biological screens bring to GWASs for human phenotypes and disease.

DECLARATION OF INTERESTS

C.L.A.-B. serves on and or has served on the editorial board for the *Journal of Bone and Mineral Research*, *JBMR Plus*, *Bone*, and *Bone Reports*. She also is on the board of reviewing editors for *eLife*.

REFERENCES

1. Baronas, J.M., Bartell, E., Eliasen, A., Doench, J.G., Yengo, L., Vedantam, S., Marouli, E., Kronenberg, H.M., Hirschhorn, J.N., and Renthal, N.E. (2023). Genome-wide CRISPR screening of chondrocyte maturation newly Implicates multiple genes in Longitudinal skeletal growth and height-GWAS associated loci. Cell Genomics *3*, 100299.

2. Yengo, L., Vedantam, S., Marouli, E., Sidorenko, J., Bartell, E., Sakaue, S., Graff, M., Eliasen, A.U., Jiang, Y., Raghavan, S., et al. (2022). A saturated map of common genetic variants associated with human height. Nature *610*, 704–712.

3. Li, B., and Ritchie, M.D. (2021). From GWAS to gene: Transcriptome-wide association studies and other methods to functionally Understand GWAS Discoveries. Front. Genet. *12*, 713230.

4. Yengo, L., Sidorenko, J., Kemper, K.E., Zheng, Z., Wood, A.R., Weedon, M.N., Frayling, T.M., Hirschhorn, J., Yang, J., and Visscher, P.M.; GIANT Consortium (2018). Meta-analysis of genome-wide association studies for height and body mass index in approximately 700000 individuals of European ancestry. Hum. Mol. Genet. *27*, 3641–3649.

5. Hallett, S.A., Ono, W., and Ono, N. (2021). The hypertrophic chondrocyte: to be or not to be. Histol. Histopathol. *36*, 1021–1036.

6. Renthal, N.E., Nakka, P., Baronas, J.M., Kronenberg, H.M., and Hirschhorn, J.N. (2021). Genes with specificity for expression in the round cell layer of the growth plate are enriched in genomewide association study (GWAS) of human height. J. Bone Miner. Res. *36*, 2300–2308.

7. Wu, W., and Chen, R. (2023). The effect of growth hormone treatment in children with novel BPTF gene variants: a report of two cases and literature review. Mol. Genet. Genomic Med. *11*, e2066.

8. Blake, J.A., Baldarelli, R., Kadin, J.A., Richardson, J.E., Smith, C.L., and Bult, C.J.; Mouse Genome Database Group (2021). Mouse genome database (MGD): Knowledgebase for mouse-human comparative biology. Nucleic Acids Res. *49*, D981–D987.

9. Groza, T., Gomez, F.L., Mashhadi, H.H., Munoz-Fuentes, V., Gunes, O., Wilson, R., Cacheiro, P., Frost, A., Keskivali-Bond, P., Vardal, B., et al. (2023). The International Mouse Phenotyping Consortium: comprehensive knockout phenotyping underpinning the study of human disease. Nucleic Acids Res. *51*, D1038–D1045.