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REDDIT

PLOS Science Wednesday: Hi reddit we're Graham and Richard, and we identified the molecular mechanism that creates genetic variations associated with increased risk of autoimmune diseases, such as celiac, ulcerative colitis etc. – Ask Us Anything!

PLOSSCIENCEWEDNESDAY R/SCIENCE

Hi Reddit,
My name is Graham Lord and I am the Professor of Medicine at King's College London. My research focuses on understanding the cellular and molecular mechanisms that cause inflammatory bowel diseases.
And my name is Richard Jenner and I am a Reader (Associate Professor) in Molecular Biology at University College London (UCL). My research focuses on understanding how gene transcription and chromatin modification regulate immune cell function in health and disease. We recently published a paper titled "Genetic variants alter T-bet binding and gene expression in mucosal inflammatory disease" in PLOS Genetics. We found that the transcription factor, T-bet bound to genetic variants associated with human mucosal inflammatory diseases, such as celiac disease, Crohn's disease and ulcerative colitis. We also developed a new technique called "OligoFlow" that allowed us to identify altered transcription factor binding to these variants.
We will be answering your questions at 1pm ET – Ask Us Anything!
Click here for more information about Dr. Jenner's lab.

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Hi Graham and Richard, and thank you for doing this AMA. Interesting paper, a few questions if you don't mind:

- I didn't see it mentioned in the text, but what was the effect size/odds ratio for the individual SNPs you investigated with regards to mucosal autoimmune disease? How does the effect size/p-value change if you create a quantitative trait for "T-bet" binders/non-binders?
- In the text, you argue that T-bet is important for specifying the Th1 lineage in CD4+ T-cells. My recollection, though, is that T-bet contributes to gene regulatory programs in multiple immune cell types - including CD8s, innate lymphoid cells, NK cells, and subsets of Tregs and Th17 cells. Have you investigated any of these cell populations? Or do you see this as a mostly Th1-driven phenomenon? To my knowledge, Th1 cells are not required for disease pathogenesis in EAE-type models, but T-bet is.
- What are the minor allele frequencies for rs1465321 and the other SNPs with the strongest evidence for impacting T-bet binding? Have you thought about what sort of evolutionary pressures may have been (or still are) driving selection on these sites? Maybe something to do with pathogenic immunity?

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SirT6

Hi. Thanks for your questions. In answer to point 1, Huang and colleagues have calculated posterior probabilities for causation for many of the variants in their recent publication in Nature (https://www.nature.com/nature/journal/v547/n7662/abs/nature22969.html). We cited the pre-print of this study in our paper. T-bet bound variants tended to have higher posterior probabilities than those that were not bound (Figure S1B in our paper). We haven't re-calculated probabilities for causation based on T-bet binding but this would be interesting to do.

Hi Graham and Richard, and thank you for doing this AMA. Interesting paper, a few questions if you don't mind:

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SirT6

Hi

In response to your second point - as you say T-bet is expressed in multiple immune cell lineages. We wrote a recent review about this and the mechanistic links to mucosal inflammation in IBD (Nature Review Immunology 2013). It is likely that pathogenic Th17 cells are "ex-Th1" cells and Tregs that express T-bet may also play a role in regulating pro-inflammatory responses. We focused on Th1 cells in the first instance as we were able to generate sufficient numbers of cells to perform ChIP-seq for T-bet and are now working to extend these findings to the other T-bet expressing cells that you mention. We are particularly interested in ILCs, where we have shown that T-bet plays a key role in mucosal inflammation in pre-clinical disease models (e.g. Immunity 2012, Gastro 2015) - in fact this may explain the mucosal-specificity we see in our fGWAS.

If I have an inflammatory bowel disease, say Crohn's, how would this newly-discovered mechanism benefit me and my treatment options short-term, long-term? Would CRISP-R technology permit the alteration or amelioration of affected loci down the road?

ejscarpa91

Hi there. In the short term, these findings may help to stratify treatment options depending on your genetic predisposition to disease. CRISPR technology is a long way from the clinic at the moment, but theoretically could be used to correct causal genetic variation in the future.

How can a computer scientist help to get the field forward? If there is much work to do, how is it likely for someone without academic biology knowledge to get accepted as a PhD student in labs similar to yours?

morteza_milani

Lots! Definitely possible to get a place as a PhD student in genomics or genetics without a biology background.

Hi and thanks for joining us today!

Given the importance of microbiome health, do you think neonatal antibiotic use is more damaging than we know?

Regarding immunomodulation by parasitic helminths, where do we stand in terms of therapeutic use?

It would seem fecal transplants are becoming mainstream, do you think this is a good thing?

PHealthy

Having a "healthy" microbiome is clearly important. The long term studies linking neonatal antibiotic use to health outcome later in life are ongoing, so it's probably too early to say. Helminths (or proteins derived from them) showed early promise but larger scale trials have been somewhat disappointing. Fecal transplants have their place in treating antibiotic resistant c diff for example, but more widespread use is not yet proven.

How were the genetic variants you focus on originally identified? Do these pop up in GWAS, or were they initially candidates based on molecular function? Do you think this work could lead to improved therapy?

p1percub

They were all identified through GWAS studies. The work could lead to improved understanding of disease mechanisms, which could lead to improved therapies. The work could also help stratify patients for personalized therapies in the future.

Hello, thanks for doing the AMA.

I'm intrigued by oligoFlow, and I have a couple questions about it.

- Do you see this as a replacement for ChIP-seq, even for small scale experiments?
- Do you think it would be possible to assess TF binding strength via competition with flourescent oligos?
- Why do you think the variation in relative intensity was so much higher for the significant variants than for the rest?
- What do you think of *in silico* prediction of transcription factor binding sites generally? The whole thing seems pretty wishy-washy at the moment to me, and your paper seems to support that.

Thanks, and have a good day!

avematthew

OligoFlow identifies the degree to which a protein binds to a DNA sequence in vitro so is complementary to ChIP, which tells you if a protein binds to a specific genomic region in cells, but can't

replace it.

I think it could be possible to assess relative binding strength of a protein to an oligo bound to the bead vs a competitor soluble oligo.

The greater variation for the significant variants is due to more replicates being performed for these.

In silico prediction is certainly not ideal. Many predicted sites are not bound and sites lacking canonical motifs are often also bound. The binding preference of a transcription factor is also often altered by other factors.

Hi, thank you for doing this AMA!

Could this mechanism be linked to type 1 diabetes?

And more generally, how does it change the way we see such diseases?

Hafornin

great question!

T-bet has been associated with T1DM in disease models - indeed one of the SNPs we are interested in (in the IL2 receptor, CD25) is implicated in IBD and T1DM and is bound by T-bet - the effect is in the opposite direction though - so we are performing comparative studies across the 2 diseases. Studies like this are starting to link genetic variation in individual to actual cellular and molecular mechanisms that we hope will one day lead to more personalised treatment of disease.

Hello! I have Crohn's disease, which manifests very differently from individual to individual. I react well to Humira, but a gastroenterologist told me that there is consistently ~33% of patients with Crohn's who react well, ~33% that have no beneficial effect, and ~33% who react badly. This indicates to me that there may be multiple genetic or environmental variants to each auto-immune disease. How does your new technique help with more precise diagnosis and treatment? Thank you for all your work.

Sherlockiana

What an interesting question - we are currently looking at patients that respond differently to humira and other treatments to see if we can stratify groups of patients that may benefit from certain drugs. Rather than looking at small individual changes in single genes, we hope that binding of T-bet (or other factors) to large numbers of these single genes may give us a more powerful tool in the search fro the best drug for each group of patient.

Is diet involved in the process?

freethinker78

It probably is - but we are not quite sure of all of the details of this yet!

Thanks for taking the time to share your work with us. I'll admit this is a bit difficult for me to understand.

If I understand it correctly, your new technique will help counteract certain autoimmune diseases. How

generalizable is that that technique?

Also, I hope it's not too much to ask, but could you provide a bit simpler of an overview of your work? Thanks!

PapaNachos

The technique should work for all autoimmune diseases, so we hope the scientific community will take it forward.

In terms of a simple analogy, the genetics of a specific autoimmune disease show us the shape of the "keyhole". For IBD, we have found one sort of "key" that fits into this keyhole to explain some of the disease mechanism. Hope that makes sense!

How much difference is there in how T-Bet binds to the different mucosal inflammatory diseases? How does celiacs distinguish itself from Crohn's or ulcerative colitis when using your technique?

kerovon

we have not looked at this yet, but we are hoping to do a study into this soon.

Your study focused on the most likely relevant tissue (T cells), but how applicable is this approach when the relevant tissue isn't necessarily known or available? Numerous studies have demonstrated strong correlation between locally-regulated expression of a gene across different tissues. Given that, can other tissues be used as proxy?

quattro

Good question! The answer is yes. We used peripheral blood CD4 T cells and activated these in vitro, so although T cells are a strong contender for the pathological cell type in IBD, the cells were not taken directly from the inflamed tissue. However, genetic variants that disrupt the binding of a transcription factor in one cell type or condition are also likely to disrupt binding in another cell type or condition, although course this won't always be the case. Indeed, it is possible that the alteration in T-bet binding in another cell type, such as innate lymphoid cells, also contributes to IBD pathology.

Thanks for coming to talk with us! Do your insights suggest different basic strategies for treating these diseases?

asbruckman

Potentially yes - if we can identify which key transcription factors are critical determinants of disease causation (and in which immune cell type they are having their major effect), then directly targetting that pathway may be more selective in defined patient strata.