PLOS Science Wednesday: Hi Reddit, I'm Professor Damien Keating and I discovered gene RCAN1 is linked to blood sugar problems in both Type 2 Diabetes and Down Syndrome – Ask Me Anything!

PLOSScienceWednesday [1] and r/Science AMAs[1]

[1]Affiliation not available

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Abstract

Hi Reddit, My name is Professor Damien Keating and I am a cell physiologist at Flinders University in the School of Medicine. My research focuses on understanding how cells release chemical signals to each other and how this relates to certain diseases such as diabetes. I recently published a paper titled A Syntenic Cross Species Aneuploidy Genetic Screen Links RCAN1 Expression to β-Cell Mitochondrial Dysfunction in Type 2 Diabetes in PLOS Genetics. In this study we wanted to identify what might drive pancreatic beta cell dysfunction and reduced plasma insulin in type 2 diabetes (T2D). If those beta cell changes don't happen people don't develop T2D. We tackled this in a different way by looking at diabetes in Down syndrome (where chromosome 21 is triplicated), as beta cells in individuals with Down syndrome show the same defects observed in T2D beta cells. Using a screening approach combining Down syndrome mouse models and human T2D beta cells, we arrived at a single lead candidate, RCAN1. We then provided functional evidence that increased RCAN1 expression causes defects in beta cell mitochondrial function and insulin release that are observed in T2D beta cells. We hope this will provide a platform to examine whether affecting RCAN1 function or expression could have positive influences in T2D. I look forward to chatting with you all on this topic from Australia. I will be answering your questions at 1pm ET – Ask Me Anything! Don't forget to follow me on Twitter @dj_keating.

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PLOSSCIENCEWEDNESDAY R/SCIENCE

Hi Reddit,
My name is Professor Damien Keating and I am a cell physiologist at Flinders University in the School of Medicine. My research focuses on understanding how cells release chemical signals to each other and how this relates to certain diseases such as diabetes.
I recently published a paper titled A Syntenic Cross Species Aneuploidy Genetic Screen Links RCAN1 Expression to β-Cell Mitochondrial Dysfunction in Type 2 Diabetes in PLOS Genetics.
In this study we wanted to identify what might drive pancreatic beta cell dysfunction and reduced plasma insulin in type 2 diabetes (T2D). If those beta cell changes don't happen people don't develop T2D. We tackled this in a different way by looking at diabetes in Down syndrome (where chromosome 21 is triplicated), as beta cells in individuals with Down syndrome show the same defects observed in T2D beta cells. Using a screening approach combining Down syndrome mouse models and human T2D beta cells, we arrived at a single lead candidate, RCAN1. We then provided functional evidence that increased RCAN1 expression causes defects in beta cell mitochondrial function and insulin release that are observed in T2D beta cells. We hope this will provide a platform to examine whether affecting RCAN1 function or expression could have positive influences in T2D.
I look forward to chatting with you all on this topic from Australia. I will be answering your questions at 1pm ET -- Ask Me Anything!
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Hi Dr. Keating, and thank you for doing this AMA.

RCAN1 has always struck me as a very interesting gene in the context of human health and disease. As I am sure you are aware, there are a number of reports suggesting that heightened levels of RCAN1 expression lie at the heart of Down Syndrome. Some research suggests that more RCAN1 leads to sensitivity to oxidative stress. With this in mind, I have several questions about your recent study:

- How heavily expressed is RCAN1 in Beta cells. Looking at your paper, Fig2A suggests that expression is pretty weak in the islets (I am assuming that is Log2 of the RPKM value). You show some Western blots, which seem to suggest that protein can be detected, but these are hard to interpret without knowing more about how much was loaded or exposure times for the blot (I was also surprised by the lack of loading controls for these experiments - i.e. Fig 2F, 2H, 3D, 3F - especially in figure 3 where you are trying to draw conclusions about differences in expression in response to certain stimuli). How does islet expression of RCAN1 compare to neural tissue expression (where there is a defined pathological role for the gene)?

Anything!, *The Winnower* 3:e146659.99918 , 2016 , DOI: 10.15200/winn.146659.99918

- You note that overexpression of RCAN1 in mice resulted in hyperglycemia. I was somewhat surprised by this result as the literature suggests that overexpression of RCAN1 is embryonic lethal. How did your mouse model differ from previous reports? How does this impact your interpretation of these results?
- You suggest that RCAN1 may be a therapeutic target for T2D. Did you do any experiments to pursue this idea (i.e. knockdown of the gene in cell lines, heterozygous mice and glucose sensitivity etc.)?

SirT6

OK we are off to a flying start, great questions. RCAN1 expression was measured using the SYPRO Ruby Protein Gel Stain rather than a housekeeping protein comparison as loading control. Its expressions is a little lower in beta cells than neurons, but not much lower. It is in the top 13% of most highly expressed genes in human islets. RCAN1 overexpression was shown to be lethal in another paper but they used a different transgene promoter in which RCAN1 was (over)expressed in all cells. Ours used its endogenous promoter so that RCAN1 was only overexpressed in cells in which it is normally expressed in. This was they key difference. We are currently pursuing whether knockdown etc in T2D beta cells improves/rescues beta cell function. This will be a key test for its role in beta cell dysfunction in T2D. Thanks for the great questions.

Does this have any relation to the tendency for both of these populations to be overweight? Edit: too early, not enough coffee to make complete sense.

nyanXnyan

Hi nyanXnyan, while individuals with both T2D and DS do have an increase in central adiposity, we dont know if RCAN1 plays any role in this. We focused on insulin secretion and beta cell function in our paper, but these would be great to follow up on.

Hello Doctor. The pathophysiology of DM type II involves peripheral resistance to insulin, followed by an initial, compensatory increase in insulin secretion, and finally a later decrease in insulin due to pancreatic beta cell exhaustion.

How does RCAN1 fit in that sequence of events? Is it only involved in the latter stages when the beta cells are near exhausted? Or does it have any role in the the previous stage of insulin resistance?

FatherSpacetime

Yes another great question and your explanation of the process of T2D pathogenesis is exactly right. We hypothesise that RCAN1 expression is increased at a later stage of this process, possibly once the compensatory increase in insulin secretion has begun. What gene changes drive beta cell exhaustion/failure to cause T2D is the big unknown in the field. We need to test whether reducing RCAN1 in beta cells stops this but its difficult because mouse models arent perfect and human islets from T2D are so rare to obtain. This is a problem the whole field faces but such repositories exist in Edmonton, Canada and in Scandanavia to name two. We see no change in IR in our mice overexpresing RCAN1.

Does this mean if you are genetically predisposed to get t2d you are also more likely to have a child with Down syndrome or is that a whole different ball game?

caalko

No, those two things are completely unrelated to each other. The greatest risk factor for having a child with DS is mothers age.

How many years of research did it take you to discover this? It seems like research involves more failure than success.

grybreard

This work took ~3 years. It was a really big team of people across many countries involved that was difficult to organise. We started by screening a bunch of different DS mouse models to zoom in on a region of chr 21, these mice were from labs outside Australia. We then did a bunch of work on RCAN1 in beta cells in our lab and others. The patch clamp studies at the end are pretty slow going, but I am a cell physiologist so I needed to prove to myself (and the world) that the mitochondrial defects we observed actually had a functional consequence (ie they reduced ATP levels enough to effect beta cell function and explain the reduced insulin secretion we saw). The reviewers requested a bunch of experiments that took ~8 months of this time, but this was mainly due to the fact our lead person, Heshan Peiris, had moved from my lab to undertake a great post-doc opportunity at Stanford University with Prof Seung Kim.

Research certainly does involve failure, as does any worthwhile endeavour. If this stuff was easy it would have been done already, and the setbacks make you hungry for success and make the publication of your work that much more rewarding. Studying how nature works in health and disease is a great way to get paid, I try to remember that when things get tough :)

What qualifications does one need to become a cell physiologist and did you only ever do research?

brandon1810

I obtained a Bachelor of Science with Honours (1st Class). This was needed in order to get a scholarship to undertake my PhD in cell physiology. I trained with some really good people and went to Germany for my first post-doc and Melbourne for my second before getting an offer in Adelaide 10 years ago to start my own small group.

I had other jobs before deciding on this pathway including a raft of crummy jobs during my uni years. I worked in a mining lab for 6 months after completing my undergrad degree. I liked getting paid but this experience inspired me to go back and do Honours because I had loved research as an undergrad and was bored with a 9-5 job.

Hi Dr. Keating, and thank you for doing this AMA.

Is there any practical way diabetics of all types can use your research to improve their health and treatment.

grepnork

this is similar to the question from RefriedJean so I will answer both here. The answer at this stage is no, this is of no use right now to improving your health or reducing risk of T2D or treating it.

As to the future, I have commented elsewhere that we would like to identify drugs that effect RCAN1 expression or function, thats the natural progression of our basic science discovery.

As for how the DNA changes; we believe that epigenetic (ie; a chemical modification) changes in the RCAN1 gene are driving its increased expression in T2D beta cells. In this case it is a reduction in DNA methylation at 3 different sites (Fig 2C-E) that seems to drive this. We dont know why that methylation would change but reduced DNA methylation commonly results in higher gene expression.

Hello Dr. Keating, in T1D is there any possibility that the immune system may have targeted those beta cells as a result of a strange expression of RCAN1?

loneadii

we dont think this is a possibility as we know that transient overexpression of RCAN1 in beta cells also reduced insulin secretion in response to glucose. So a direct effect occurs.

Can this also be linked to insulinemia and/or metabolic syndrome? How will this discovery affect research on treatments for Type 2 Diabetes?

gracesw

as I said above, we would like to screen for chemical modifiers of RCAN1 as none exist. If we can obtain such drugs, we would then test them in a T2D setting to see if they improve the profile of disease such as increasing beta cell numbers and plasma insulin. We have a target (potentially), we now need to target it.

I know very little about Metformin I was told it protects beta cells and lowers blood sugar slightly. How does the use of Metformin and insulin affect the expression of RCAN1 if at all? Does any modern drug do this?

madbot4525

Metformin is usually the first drug prescribed in T2D. It has been around a very long time for this use and is throught to work on sites other than beta cells, typically targeting the improvement of how our body responds to insulin, rather than affecting how much insulin we make.

We know that RCAN1 expression is induced by chronic high glucose and oxidative stress in beta cells, so any treatment that brings blood glucose down and helps control our diabetes would likely reduce beta cell RCAN1 expression secondarily. No drug currently directly targets RCAN1.

What is also worth remembering in this regard is that only the symptoms, not the cause, of T2D is treated with existing drug approaches. So reversal of T2D is not possible with current drugs. Identifying the causes of β-cell dysfunction is the first step in finding treatments for it. We still haven't done this but this is a good first step.

I know this is a relatively new paper, has it been peer-reviewed and independently tested yet, and what are your contemporaries and critics saying?

Do you believe that this can be used to identify people who are more susceptible for developing T2 Diabetes, specifically those who are not in the weight/diet risk categories?

Do you believe that this is actually going to be useful as a method for treating T2 Diabetes?

galorin

This paper was certainly peer-reviewed :) This was well accepted when we presented it publicly. Our finding wont be relevant, we dont think, for screening for people with T2D or at risk of getting it. Thats because its not a mutation in all our cells thats associated here, but rather we think that its specific to beta cells in susceptible people so that when the beta cells are overworked and under stress, RCAN1 expression increases (its a stress-induced gene) which then causes beta cell failure. As far as our work being an avenue for T2D treatment; we would like to screen for novel small molecules that effect RCAN1 expression and see if they are beneficial, but this is someway off. Its certainly where we want to take this research though with tratment in mind. Thats the sort of thing that drives a lot of medical researchers on, even basic sscientists like myself, that hope of impacting on human health.

Thank you for doing this AMA. Is RCAN1 expression hereditary? genetic or epicgenetic? How can this expression be affected or controlled?

amrutanargundkar

I think I mentioned above a few things about this. We show in beta cells that RCAN1 expression is controlled epigenetically and by high glucose and oxidative stress. Whether glucose and oxidative stress cause the epigenetic modifications to drive up RCAN1 expression is something we dont know but would like to know.

RCAN1 expression is dynamic and changes in response to the cells environment (especially stress). So that components isnt really hereditary. However we dont know it you inherit some susceptibility for beta cell failure or larger changes in RCAN1 expression.

Why do some people get fat and others not on similar intakes of food? Is there a metabolic difference between people? Is this related in any way to the gene you discovered?

oohhhhcanada

This isnt really related to our paper but it is a really great question that goes to the heart of a lot of T2D and obesity research. Its not easily answered in this form I am afraid but we do know that genetic differences in humans can account for altered obesity susceptibility. This susceptibility can increase if either your mum or dad is obese at the time of coneption (Margaret Morris in Sydney had a great paper in Nature a few years ago on this) or if mum is obese during pregnancy. But risk genes may also be inherited regardless of these also.

Hi Professor Keating, I worked on a different gene outside of insulin and its relation to T2D, CDKAL1, for therapeutic targeting. I was wondering if you were thinking of RCAN1 as a possible therapeutic target? Also thank you for your contribution to T2D research. In my experience it's a small group!

AreEnAy

We will try and test properly whether RCAN1 really is a viable target for therapy in T2D. CDKAL1 is also a really interesting target and I wish you all the best with that work. As for it being a small group, T2D is one of the biggest growth problems in human health in modern society that is associated with all the other big killers like hypertension, CVD, stroke, obesity. There are plenty of people/companies looking for cures. I guess the number focusing on beta cells however is small in comparison to other target sites.

Your research is very interesting. Diabetes is a complex disease as you know and I was wondering

whether you anticipate finding other genes llke RCAN1? Secondly, do you see personalized medicine being important for treating diabetes in the future?

butt_typist

We certainly dont think RCAN1 would be the only gene important for beta cell failure in T2D. As for personalised medicine; while technological advances in medical science have allowed us to do so much more (eg; tech advances in genomics have outstripped those in computer science in the past 10 years, that blows my mind), I dont think we are yet at a stage in which personalised medicine is clinically relevant. A lot of sham companies will tell you (or sell you) thats not the case, but maybe it will be in the not too distant future. We are not there yet though.

[deleted]

[deleted]

Awesome to hear from someone working in the area, thanks so much. Certainly other chr 21 genes could contribute to the hyperglycemia we cant rule that out. What we concluded however, was that only 5 of the 38 genes are overexpressed in human T2D islets, with RCAN1 being the only gene of these that is known to affect mitochondria and secretion. So we chased that lead up further in our paper.

We havent looked beyond the DS models in the paper but would have liked to. Your are right, DS is a complex gentic disease and multiple gene interactions are likely to contribute. We used the DS approach to screen for relevant T2D genes, so we dont really answer a lot related to DS.

Is diabetes increased in DS: the data around this is a bit sketchy. There is increased rate of diabetes in young DS individuals. This is often diagnosed as T1D due to early onset, but its not all due to the typical autoantibodies associated with diagnosing T1D. So genetics factors are thought to contribute. The rate of T2D in the DS population is not well studied, but doesn't seem to be higher than the non-DS population. Whats really interesting here is that low insulin typically occurs, but not diabetes. Our study indicates that there are likely some chromosome 21 genes that, when overexpressed, improve insulin sensitivity. This might explain why diabetes incidence doesn't really increase in DS.

Your trisomic mouse models were all maintained on a mixed background (B6C3) but your RCAN1 mice were on B6 which has been previously determined to have a genetic predisposition to T2D. Have you accounted for this natural bias in your models? Dp(16)1Yey and Ts1Rhr can both be maintained on B6. Retesting with these backgrounds on mice may lead to further confirmation. Ts65Dn cannot be inbred on B6, but can get to 75% B6 for research purposes.

sdxben

to clarify, the RCAN1 mice are on a mixed background also (C57BL/6 x CBA) to reduce any such disease susceptibility.

Hi. Non doctor but patient here. My question isn't strictly about RCAN1 or diabetes, but polycystic kidney disease. Can your approach be used to prevent this disease? I was told PKD is a genetic disorder, and inheritable. I'm affected by this disease and lost a kidney last year, so I know it can be a huge problem when mixed with high blood pressure.

BorisUlianov

sorry to hear that. I am afraid our research doesnt really cross over to PKD. Best of luck with it.

After identifying RCAN1 as the expressive gene, what do you think the next step in research is? Further, when (if at all) do you think it will be come common to screen fetuses for genetic diseases and/or be able to manipulate the gene to have a desired effect (i.e eliminate problems, enhance growth, strength, etc)? I'm thinking on the lines of Gattaca the movie and building the "perfect" human. Thanks so much for your time and contributions to science.

LethalDildo

fetal/ambryonic screening has been occurring for a long time already through sampling from an amniocentesis, for example (although this has its own risks). DS, for example, can be diagnosed with such an approach. I dont think we will ever manipulate the genome of humans for any desired effect. I just think it is an ethical minefield.

Have you tried knocking this gene out in any nice yet? Is rcan1 known to serve a function in any other vital life processes?

Strider_91

We have knocked it out and are in the process on publishing some work around that. Cant give too much away but they are largely normal.