May 5th 2016

Professor Anne-Christine Ritschkoff
Executive Vice president, CTO

Dear professor
Your request for assistance in the evaluation of a paper published in JEM., your e-mail of March 1, 2016.

My evaluation is as followed:

In the paper *Dysregulation of lipid and amino acid metabolism precedes islet autoimmunity in children who later progress to type 1 diabetes* published in JEM December 2008 by the authors Orešicˇ M et al. they have described the metabolome preceding pancreatic islet cell autoimmunity in children who later progressed to type 1 diabetes sampled from a ongoing birth cohort study in Finland. Individuals developing diabetes had a metabolic profile associated to the inflammasome at the birth and preceded the seroconversion to autoantibody development. The lipid changes did not associate to the HLA-associated genetic risk. The authors conclude that the autoimmunity related to the disease is a rather late response compared to the early metabolomic disturbances.

The pathophysiological mechanisms regulating the autoimmunity towards pancreatic beta cells is rather complex and not fully understood. Therefore, performing this study is highly recommended. I will in the following make a scientific evaluation of the strength and the weakness of the study.

The strength of the study is at various levels.

1. This is a prospective ongoing cohort study (DIPP) from birth on were potential progression to type 1 diabetes are registered and matched controls of so-called non progressors. The possibility of longitudinal serial analyses of the metabolome is a great strength of this study.
2. The metabolomic analyses are performed by highly dedicated methods in mass spectrometry.
3. The presentation of the high amount of data from various groups and subgroups is hard to perform due to the complexity of various parameters and needs a great bioinformatic support. As presented especially in the figures 3-5 these figures communicate well despite its complexity. The case report presented in fig 2 is highly valuable for the understanding of the longitudinal changes.
4. The metabolome/lipidome profile preceding the autoantibody seroconversion and the relation to the specific autoantibodies are descriptive data but can be associated to pathological pathways and therefore to some extent functional data.

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5. The biochemical model of metabolomic changes as presented in fig 6 is comprehensible and especially for the "ordinar" reader of the paper.
6. The interpretation of the metabolomic changes reflecting immunological mechanisms (especially the innate immune response) in the Discussion is highly speculative and especially its potential relation to gut microbiome. However, some references are highly interesting.
7. The study is highly hypothesis generating

The weakness of the study is as followed.

1. The combined material of 117 subjects from DIPP and only apparent highly selective 12 subjects from the STRIP study may by the worse represents pitfalls. This is not enough addressed in the paper.
2. No statistical power estimated have been done.
3. Clustering presentations should have been performed?
4. Statistical evaluations

The concentrations compared were statistical evaluated by Wilcoxon rank-sum test and this model does not need normally distributed data. However, when comparing lipid class concentrations a linear mixed effect model as constant levels for each group, i.e., for progressors and nonprogressors, and the random effects were modeled as constant deviations from these constant group level trajectories". This model requires normal distribution of the t residuals of the model - may it was but I cannot see this statement. Moreover, to evaluate this model we need to know the exact equation used (such as metabolite [constant] ~ task*sex[variable], random = ~ 1|ID/task[random]), method="ML")- the equation used is not described of referred to.
Finally, paired comparisons need to come from the same of the two polulations studied – DIPP and STRIP- I assume that this was done.

In addition to the puplished paper the authors have sent a set of data- not all- presenting the paired longitudinal data for some of the most parameters of interest were significant differences were obtained. I cannot check that these paired comparisons were representative for the whole dataset, but data presented seem to fit well with the data presented in Results.

Concluding remarks

New and original data are presented – and potential biomarkers of early events of the autoimmune events in type 1 diabetes have been proposed. Whether these potential biomarkers will be documented in future reports from other research groups are an open question also as stated in the Conclusion. There is some minor weakness in the paper, but I would as a potential reviewer- proposed some minor corrections before acceptance.

I have no indications of any misconduct. By the worse, the biomarkers documented may not fit with other future reports, but this was especially taken into consideration by the authors.

This evaluation has been performed after discussion and evaluations also by specialists in mass spectrometry and bioinformatics- all evaluations have been confidential performed.

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