ALLERGY, GENETICS

HOPE DIES LAST

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Maybe the Nature editors should have read their own writings as the “roadmap for regulation” (19 Feb 2015) states correctly about gene methylation

> Some major caveats should be noted. These studies are based on analysis of cell populations, and therefore miss potentially crucial aspects of cellular variability within populations. When tissues are examined, enhancer landscapes represent the composite of the cell types that make up that tissue, not a pure cell population.

Given that fact, is there any sense of a “blood” methylation analysis published on the same day? With erythrocytes, reticulocytes megakaryocyte, monocytes (classical, non classical, intermediate), macrophages (M1, M2), Langerhans and dendritic cells (CD11c+ myeloid DCs, CD141+ myeloid DCs, CD303+ plasmacytoid DCs), neutrophil, eosinophil and basophil granulocytes, mast cell, helper, suppressor, cytotoxic and natural killer T cells, B cells (precursors, B1, B2) just to name a few? At least, the editors thinks so by publishing an article about “An epigenome-wide association study of total serum immunoglobulin E concentration”

> We … surveyed epigenetic associations between serum IgE concentrations and methylation at loci concentrated in CpG islands genome wide in 95 nuclear pedigrees, using DNA from peripheral blood leukocytes … The panel is enriched for genomic regions regulating expression, but does not cover all functionally important CpG sites.

Not unexpectedly there are 36 undermethylated (“hyperactive”) loci associated to IgE. But everything looks preliminary in this study: low number of study participants, insufficient genomic coverage, poor sample prep, questionable statistical models. Biological readouts are not being reported (although available) — it is just an association legitimated by some p--values.
The authors may have felt this insufficiency by studying additionally eosinophils of 8 asth-

matics with high, 8 with low serum IgE and 8 controls (the paper was held backmore than a year). And yeah, the lowest levels of IL5RA methylation (a selectively receptor of eosinophil progenitors) or PAF (an eosinophil stimulus) is found in subjects with high Ig E who have concomitant high eosinophil numbers. So all the methylation signal reported is not necessarily an IgE effect, it may reflect the collateral change of eosinophils in the blood pool. The authors acknowledge this

> Lineage commitment to particular cell types is accompanied by spe-cific methylation changes … fitting regression models that included differential white cell counts … we identified partial associations with eosinophil numbers for all IgE-associated loci, consistent with independent effects on IgE from the numbers of eosinophils.

which is a bit lame. I have general doubts if there is any statistical method that can definitely disentangle the blood pool. There are even more doubts given the earlier dispute of these authors with an eminent statistician about their methods.

> Our regression models found that the top IgE associations were not accounted for by concomitant correlation with lymphocyte count … Surrogate CpG markers that identify lymphocyte subsets can be used as an alternative to white cell counts in association models. We also applied these methods to our data.

This looks like desperately trying to makes sense of a study design. (Un)fortunately there appeared another paper in JACI that used also PBMCs but DNA + RNA from 97 asthma/high E kids showing 81 loci at the 450K Illumina Infinium Human Methylation Kit:

> CS444397 SKI ACOT7 ACOT7 LOC391005 Intron;utr3 PAX7 ALPL ALPL RUNX3 MAP3K6 TCTEX1D4 EPS8L3 ATP8B2 KCTD3 AX748369 LINC00299 LINC00299 C2orf16 MEIS1 NMUR1 AK097934 VGLL4 ITIH1 TIGIT AK125775 GRAMD3 IL13 EXOC2 HIST1H3F TRIM27 SLC44A4 C2;ZBTB12 MICAL1 GET4;SUN1 MAD1; MAD1L1 AMZ1 TCRg;TRGV9 POM121C TRMT12 FAM84B LARP5 KLF6 KLF6 PRF1 CPN1 FAM53B PWWP2B CAT FLJ14213 SNHG1 SF1 APLP2 PITPNM2 PR133 TMCO3 RASA3 BCL11B INF2 BLM KIAA0182 KIAA0182 BC041439 ZFPM1 ZFPM1 CNP NBR2 ABI3 SLC9A3R1 TSPAN10 HEXDC TBCD MIDN ATG4D MAST3 SHANK1 KIR2DL4; KIR2DS2; KIR3DL1 C20orf118 RBM38 PCNT TTC38 (Jaci)

In contrast the Nature paper used 355 DNAs (age 2-61 years) in the discovery sample tested by the Illumina HumanMethylation27 array:

LPCAT2 IL5RA ZNF22 L2HGDH IL4 SLC25A33 RB1 SERPINC1 TFF1 SLC17A4 L2HGDH TMEM86B COL15A1 CEL SPINK4 ADARB1 MFSD6 TMEM52B FAM112A SLC7A11 KEL PIK3CB TMEM41A IL5RA PDE6H SEPT12 KLF1 ITGA2B PRG3 SLMAP PRG2 EFNA3 SLC43A3 CLC ALDH3B2 GATA1 (Nature)

Is there any overlap in the results? Again no, there is no dominant inheritance, no maternal inheritance, no epidemic related to infection, and no association with FcE RIß in all these painstaking studies. So we are left alone with the last sentence in the Nature paper

Our findings suggest the presence of novel therapeutically tractable pathways underlying IgE production.

Hope dies last.