EMBO | EMBL Symposia

Multiomics to Mechanisms - Challenges in Data Integration. September 11th – 13th 2019
European Molecular Biology Laboratory. Heidelberg, Germany

Genome-wide association analyses of weight loss in a randomized controlled trial of lifestyle intervention, and combined transcriptome-wide associations in a Mediterranean population

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Background and aims

Although large-scale genome-wide association studies (GWAS) for obesity traits have identified more than 400 associated loci from observational studies (Figure 1), we highlight the fact that currently the number of GWAS for intentional weight change in randomized controlled trials (RCT) of lifestyle interventions is very scarce.

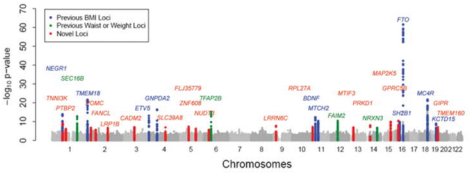


Figure 1: Genes associated with weight and obesity-related measures in GWAS from observational studies (Speliotes et al, Nat Genet. 2010; 42: 937–948)

Only a few RCT on weight loss have been carried out and recently a GWAS including 2 populations (a Canadian RCT and the Diogenes RCT) was been published (Valsesia et al, Nat Communications, 2019). Likely, at the transcriptome level, there is a scarcity of transcriptome-wide association studies (TWAS) of weight loss in RCT. Moreover, this scarcity is higher for studies including subjects from the Mediterranean countries.

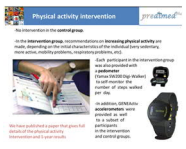
Therefore, our first aim was to undertake a GWAS in overweight/obese subjects from a Mediterranean population (Spain) after 1-year lifestyle intervention (including an energy restricted Mediterranean diet plus physical activity) in a RCT to identify genetic variants associated with weight loss and related outcomes. In addition, as a second aim, we carried out a TWAS in a subsample of subjects for the same intervention to identify changes in gene expression and related pathways.

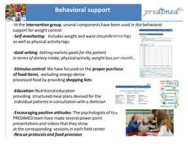
Methods

1 Study participants

We have analyzed participants recruited from the PREDIMED Plus-Valencia study (located on the eastern Mediterranean coast, Spain). This is one of the field centers of the multi-center PREDIMED Plus study, which is an ongoing trial. This RCT was registered at https://doi.org/10.1186/ISRCTN89898870. Eligible participants, recruited from several primary care health facilities in the Valencia field center, were community-dwelling adults (men, 55–75 years, women, 60–75 years) with a body-mass index (BMI) in the overweight or obesity range ((BMI) ≥ 27 and <40 kg/m2) and had at least three components of the metabolic syndrome [58]. In the Valencia field center (located on the eastern Mediterranean coast), the total number of randomized participants included in the PREDIMED Plus trial was 465.

Study participants were randomized 1:1 to the intervention group or the control group. A Computer-generated random allocation was centrally elaborated in blocks of six subjects and stratified by sex, age (<65, 65–70, >70) and center. The randomization procedure was internet-based and blinded to all staff and to the principal investigators of each center. In the intervention group we evaluated the effect of an intensive weight loss intervention based on an energy-restricted traditional Mediterranean diet, physical activity promotion and behavioral support as compared to a usual care intervention consisted only on energy-unrestricted MedDiet recommendations (control group).

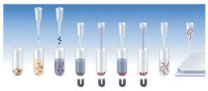


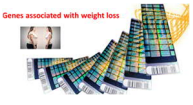


Anthropometric variables were determined by trained staff and follow the PREDIMED Plus operations protocol detailed in the study Web site (http://www.predimedplus.com/). Weight and height were measured with calibrated scales and a wall-mounted stadiometer, respectively. BMI was calculated as the weight in kilograms divided by the height in meters squared. The waist circumference was measured midway between the lowest rib and the iliac crest after normal expiration, using an anthropometric tape. We calculated the weight loss after 1-year intervention in the intensive intervention group and in the control group. Stratified GWAS were carried out to identify the genes associated with weight loss.

2. Genomic analyses and GWAS

DNA was isolated from blood (buffy-coats), and high-density genotyping was performed at the University of Valencia using the Infinium OmniExpress-24 v1.2 BeadChip genotyping array (Illumina Inc., San Diego, CA, USA), according to the manufacturer's protocol with appropriate quality standards.

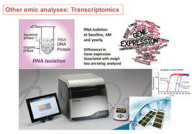




This array captures 713,599 markers. Allele detection and genotype calling were performed in the GenomeStudio genotyping module (Illumina, Inc., San Diego, CA, USA). Data cleaning was performed using standard analysis pipelines implemented in the Phyton programing language using the Numpy library modules combined with the PLINK. From the initial full set, those SNPs not mapped on autosomal chromosomes were filtered out.
In addition, SNPs with a minor allele frequency (MAF) < 0.01 or those that deviated from expected Hardy-Weinberg equilibrium (p < 1.0 × 10−5) were removed. A total of 622,468 SNPs that passed the quality filter remained for further analysis. The total number of subjects with GWAS genotyping was 448. For GWAS, genetic association analyses were performed using PLINK v1.9. To evaluate the association of 1-year weight loss with each SNP, using PLINK, an additive genetic model was fitted (0, 1, or 2 copies of the variant allele). Coefficients for the minor allele were estimated. Unadjusted and adjusted (for sex and age or for additional variables) general linear models were fitted. Stratified GWAS analyses by intervention group were carried out. We used the conventional threshold of p < 5 × 10−8 for genome-wide statistical significance. Since this threshold is very conservative for a small sample size, SNPs with p-values below 1 × 10−5 were also considered suggestive of genome-wide significance. SNPs were rank-ordered according to the minimum p-value in the genetic models.

3. RNA isolation and TWAs

RNA was isolated from fresh blood at baseline and after 1-y of follow-up with the Promega device and kit. RNA integrity was assayed by means of the 2100 Bioanalyzer with Eukaryote total RNA Nano Assay (Agilent Technologies, Santa Clara, CA, USA). RNA integrity number (RIN) served as RNA integrity parameter (selection criteria RIN ≥ 9.0).Microarray experiments were performed at the Central Research Unit (University of Valencia). GeneChip Human Gene 2.0 ST Array containing over 41,000 transcripts and represent over 36,000 well-characterized human genes (Affymetrix, Santa Clara, CA, USA) was used for microarray analysis. The fragmentation of biotinylated cRNA derived from 150 ng of total RNA was used to hybridize to GeneChips. The hybridization cocktail was incubated overnight at 45°C while rotating in a hybridization oven. After 16h of hybridization, the cocktail was removed and the arrays were washed and stained in an Affymetrix GeneChip Fluidics Station 450, according to the Affymetrix's protocol. The distribution of fluorescent material on the array was obtained using GeneChip Scanner 3000 7G (Affymetrix, Santa Clara, CA, USA). GeneChip Operating Software supplied by Affymetrix was used to generate.CEL files. Partek Genomic suite was used for gene expression analysis. Multivariate models were used to adjust for potential confounders including batch effect, sex, age, leukocyte counts, etc. TWAS analyses were carried out in a subsample of individual (n=48). In the intervention group (n=36), we selected subjects with a higher weight loss (mean 5 kg), and in the control group (n=we selected subjects paired by age and sex with the intervention group but without losing weight.



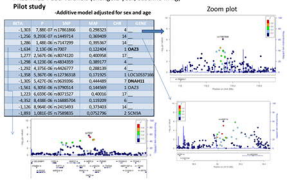
Results

Table 1 shows the main characteristics of the Predimed-Valencia study participants at baseline.

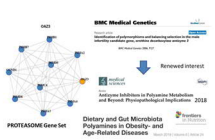
Table 1: General features of the Predimed-Valencia study participants

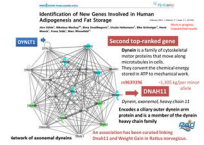
	Total (n=465)	Men (n=198)	Women (n=267)	P
Age (years)	65.1±0.2	63.9±0.4	66.1±0.3	<0.001
Weight (Kg)	84.5±0.7	92.8±1.0	78.0±0.6	<0.001
BMI (Kg/m^2)	32.4±0.2	32.3±0.2	32.5±0.2	0.629
Waist circumference (cm)	106.1±0.5	111.2±0.6	102.0±0.6	<0.001
SBP (mm Hg)	141.6±0.9	143.8±1.3	139.9±1.2	0.026
DBP (mm Hg)	81.0±0.5	82.6±0.7	79.7±0.6	0.002
Total cholesterol (mg/dL)	196.4±1.8	188.3±2.8	202.6±2.3	<0.001
LDL-C (mg/dL)	125.0±1.5	121.6±2.4	127.7±1.9	0.044
HDL-C (mg/dL)	51.5±0.5	47.5±0.8	54.7±0.7	<0.001
Triglycerides (mg/dL)	141.6±2.9	138.2±3.8	144.3±4.2	0.296
Fasting glucose (mg/dL)	112.5±1.3	112.8±2.0	112.3±1.7	0.862

These subjects were randomized 1:1 to the intervention group and to the control group. After 1-y of follow-up, mean weight loss in the intervention group (n=238) was -2.81+/-2.7 Kg versus -0.41+/-2.7 Kg in the control group (n=225). There were large inter-individual differences in weight loss despite the same intervention. So in the intervention group, the maximum weight loss reached was -13 kg. We first analyzed the genes associated with weight loss in the whole population (intervention+control group). The top-ranked gene was the SLC24A2 (Solute Carrier Family 24 Member 2) at the suggestive GWAS level. However, taking into account that our main interest was the in the intervention group, we present here the results for the intensive intervention group.
For the intensive intervention group, the top-ranked SNPs associated with 1-y weight loss in an additive model adjusted for sex and age are presented in Table X. The first three SNPs were intergenic (the corresponding zoom plots are presented), and the first SNP within a gene was the rs7007-OAZ3 (ornithine decarboxylase antizyme 3) at P=3x10E-07 (crude) and 2.1x10E-6 (adjusted).

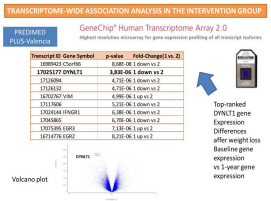


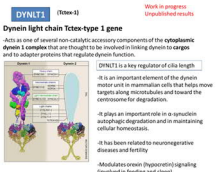
Ornithine decarboxylase (ODC) antizyme protein that negatively regulates ODC activity and intracellular polyamine biosynthesis and uptake in response to increased intracellular polyamine levels. Currenty this gene as well as the polyamines are presenting a renewed interest.





The Second top-ranked gene was the DNAH11 (Dynein, axonemal, heavy chain 11). Encodes a ciliary outer dynein arm protein and is a member of the dynein heavy chain family. In the TWAS, we obtained as the top-ranked gene differentially expressed from baseline to 1-y follow-up in the intervention group, the DYNLT1 gene.





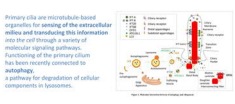
Although these results are still preliminary, we have obtained as top-ranked the DYNLT1 both in the GWAS and in the TWAS analysis. More work is needed in order to integrate genomics and transcriptomics results, but these preliminary results are suggesting a role of dyneins and the cilia in weight loos. In addition, when we carried out a pathway analysis in the TWAS, we obtained that several pathways related with the autophagy were among the top-ranked.

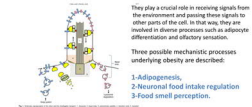
GENE EXPRESSION ANALYSIS: PATHWAY ENRICHMENT ANALYSIS*

Pathway name	Database	Enrichment Score	Enrichment p-value
Mitophagy - animal	**kegg**	**19,3744**	**3,85E-09**
Lysosome	**kegg**	**5,13117**	**5,91E-03**
RNA degradation	kegg	5,00893	6,68E-03
Salmonella infection	kegg	4,76605	8,51E-03
Non-homologous end-joining	kegg	4,60108	1,00E-02
PD-L1 expression and PD-1 checkpoint pathway in canc	kegg	4,19665	1,50E-02
IL-17 signaling pathway	kegg	4,00812	1,82E-02
Cocaine addiction	kegg	3,95399	1,92E-02
Human T-cell leukemia virus 1 infection	kegg	3,52517	2,94E-02
Cellular senescence	kegg	3,4208	3,27E-02
C-type lectin receptor signaling pathway	kegg	3,40907	3,31E-02
Legionellosis	kegg	3,33606	3,56E-02
Hepatitis B	kegg	3,28268	3,75E-02
cGMP-PKG signaling pathway	kegg	3,18353	4,14E-02
Lysine degradation	kegg	3,11475	4,34E-02
Yersinia infection	kegg	2,69626	6,75E-02
Terpenoid backbone biosynthesis	kegg	2,62381	7,25E-02
Amphetamine addiction	kegg	2,56441	7,70E-02
Mannose type O-glycan biosynthesis	kegg	2,49507	8,25E-02
FoxO signaling pathway	kegg	2,36321	9,41E-02
Epstein-Barr virus infection	kegg	2,29381	1,01E-01

*: Including 336 Transcript Ids with P-values <0.001 for differences in gene expression after 1 year of weight lost in the Intervention group.

There are several works showing the connection between cilia and autophagy and cilia and obesity.





Conclusions

Future work will include further omics integration and increasing sample size to better characterize our novel findings.

Acknowledgements

This study was partially funded, by the Spanish Ministry of Health (Instituto de Salud Carlos III) and the Ministerio de Economía y Competitividad-Fondo Europeo de Desarrollo Regional (FEDER, "A way of making Europe") (grants CIBER 06/03, PRX17/00500, SAF2016–80532-R) and Premio Rei Jaume I (Valencia, Spain); the University Jaume I (grants P1–1B2013–54, COGRUP/2016/06 and UJI-B2018-69); the Fundació La Marató de TV3 (grant 538/U/2016); and the Generalitat Valenciana (grant PROMETEO2017/017).