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Metabolites, genomics, epigenomics, exposomics and health: Focus on serum bilirubin concentrations in subjects with metabolic syndrome from a Mediterranean population

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Introduction

Although metabolomics aims at the measurement of small molecules (metabolites) in a biological sample, this knowledge requires additional information on the related genetic variants, epigenetic regulators and environmental factors (diet, smoking, physical activity, etc.) in order to translate the knowledge into actionable therapeutic or preventive evidence for complex disease outcomes. We focused on serum bilirubin, a metabolite generated when heme oxygenase catalyzes the degradation of heme (Figure 1). This produces biliverdin, which is converted into bilirubin by biliverdin reductase. Bilirubin is further processed in hepatocytes, where unconjugated bilirubin is conjugated by uridine diphosphate-glucuronosyltransferase (UDP-GT) to a water-soluble form for excretion. For decades, increased serum bilirubin concentrations were considered a threatening sign of underlying liver disease and had been associated with neonatal jaundice. However, data from recent years show that bilirubin is a powerful antioxidant and suggest that slightly increased serum bilirubin concentrations are protective against oxidative stress-related diseases.

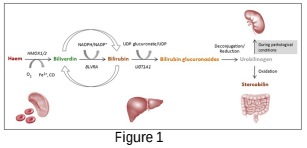


Figure 1

In subjects with Gilbert syndrome, the UDP-GT activity is reduced to 30% of the normal, resulting in hyperbilirubinemia. Several polymorphisms in the UDP-GT family 1 member A1 (UGT1A1) gene, on chromosome 2, have been associated with the Gilbert's syndrome, but the most common one is a TATA box polymorphism (rs8175347). The UGT1A cluster includes 9 similar protein-coding (Figure 2).

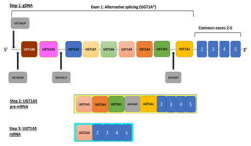


Figure 2: Human UGT1A cluster, with 9 protein-coding (UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9 and UGT1A10) genes and the 4 non-coding genes (pseudogenes)

AIMS:

To examine the genetic, epigenetic and environmental factors associated with serum bilirubin concentrations in subjects with metabolic syndrome from a Mediterranean population using genomics, epigenomics and exposomics approaches.

Methods

We analyzed subjects with metabolic syndrome from a Mediterranean population (n=430) recruited in the PREDIMED PLUS-Valencia trial. Table 1 shows the main characteristics of these participants.

Table 1. Demographic, clinical, lifestyle and genetic characteristics of the study participants at baseline according to sex.

	Total (n=430)	Men (n=188)	Women (n=242)	P
Age (years)	65.1±5.2	63.9±5.4	66.1±5.1	<0.001
Weight (Kg)	84.5±5.7	92.8±5.0	78.0±5.6	<0.001
BMI (Kg/m²)	32.4±5.2	32.3±5.2	32.5±5.2	0.629
Waist circumference (cm)	106.1±5.5	111.2±5.6	102.0±5.6	<0.001
SBP (mm Hg)	141.6±5.9	140.8±13	139.9±1.2	0.026
DBP (mm Hg)	81.0±5.5	82.6±8.7	79.7±5.6	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	125.8±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	0.256
Fasting glucose (mg/dL)	112.5±1.3	112.8±2.0	112.3±1.7	0.882
Bilirubin (mg/dL)	0.58±0.01	0.64±0.02	0.53±0.01	<0.001
AST (U/L)	26.4±0.4	27.7±0.7	25.4±0.5	0.005
ALT (U/L)	28.4±0.8	30.8±1.2	26.8±1.0	0.006
Type 2 diabetes: n, %	169 (39.3)	74 (39.4)	95 (39.3)	0.982
Current smokers: n, %	49 (11.4)	30 (16.0)	19 (7.9)	<0.001
Medications: n, %				
Antihypertensive drugs	339 (78.8)	148 (78.7)	191 (78.9)	0.959
Hypolipidemic drugs	278 (64.7)	125 (66.5)	153 (63.2)	0.482
Insulin	22 (5.1)	11 (5.9)	11 (4.5)	0.542
Physical Activity				
(MET.min/wk)	1715±77	1940±134	1539±89	0.227
Adherence to MedDiet (P17)*	7.97±0.13	7.79±0.20	8.12±0.18	0.010

Values are mean±SE for continuous variables and number (%) for categorical variables. BMI: indicates body mass index; MedDiet, Mediterranean diet; P-value for the comparisons (means or %) between male and female individuals; AST: Aspartate transaminase; ALT: Alanine transaminase; MET: Metabolic Equivalent. 1 MET is equivalent to kcal·kg-1·h-1; the oxygen cost of sitting quietly measured as 3.5 ml/kg/min; * Questionaire 17-item questionnaire for Adherence to Mediterranean diet (MedDiet).

DNA was isolated from blood. Genome-wide genotyping was carried out using the Infinium OmniExpress genotyping array and both genome-wide association analyses (GWAS) and candidate gene analyses were undertaken. Epigenome-wide methylation analysis (EWAS) was carried out using the Infinium MethylationEPIC array (in a subsample). Serum total bilirubin levels were measured by a colorimetric method using the timed-endpoint diazo method with 2,5-dichlorophenyl diazonium. Adherence to the Mediterranean diet (MedDiet), smoking and physical activity were analyzed as environmental factors. Statistical analysis, including adjustment for covariates were carried out with PLINK, Partek Genomics Suite and R.

Results

Total serum bilirubin concentrations were higher in men than in women, but in both groups, the mean value of bilirubin concentrations was within the normal limit (<=1 mg/dL generally proposed as reference).

Figure 3 shows adjusted means for total serum bilirubin concentrations in men and women after adjustment for age, diabetes, BMI, medications, smoking, physical activity and adherence to the MedDiet.

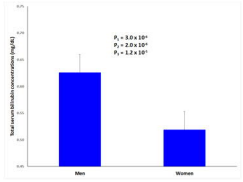


Figure 3: Sex-differences in serum bilirubin concentrations in men and women

Table 2 shows GWAS results for total bilirubin in the whole sample including both men and women. Included in the Table are the 59 top-ranked SNPs with P-values for association with bilirubin concentrations at the genome-wide level (P<5x10-8) o significance in the crude model and/or in the model adjusted for sex and age.

Table 2. GWAS results (the top-ranked SNPs) for the association between bilirubin concentrations in the whole sample.

CHR	SNP	BETA	SE	SI	P	PI	MA	MAF	Gene
2	rs4148325	0.149	0.016	0.179	1.97E-19	9.25E-24	C	0.354	UGT1A1
2	rs6742078	0.148	0.016	0.173	2.84E-19	1.29E-23	T	0.348	UGT1A1
2	rs887829	0.148	0.016	0.171	3.72E-19	1.39E-23	T	0.354	UGT1A1
2	rs4148324	0.147	0.016	0.170	4.62E-19	9.46E-24	G	0.353	UGT1A1
2	rs7583787	0.142	0.016	0.157	1.40E-17	4.70E-20	G	0.353	UGT1A1
2	rs17692675	0.146	0.017	0.152	4.46E-17	1.98E-20	A	0.295	UGT1A6
2	rs10731341	0.143	0.017	0.150	8.64E-17	2.65E-20	A	0.330	UGT1A1
2	rs6742394	0.141	0.017	0.144	4.39E-16	4.09E-18	T	0.390	UGT1A6
2	rs887836	0.136	0.017	0.132	8.14E-15	1.83E-18	G	0.324	UGT1A1
2	rs2070959	0.127	0.016	0.127	2.47E-14	3.78E-16	G	0.278	UGT1A1
2	rs1183879	0.121	0.016	0.123	8.14E-14	1.99E-15	C	0.325	UGT1A1
2	rs1328680	0.120	0.016	0.120	1.55E-13	3.30E-15	G	0.343	UGT1A1
2	rs2741045	0.119	0.017	0.109	2.50E-12	7.03E-14	T	0.159	UGT1A10
2	rs7582278	0.111	0.016	0.105	7.21E-12	1.87E-13	T	0.391	UGT1A6
2	rs2032985	0.111	0.016	0.103	1.19E-11	3.46E-14	G	0.461	UGT1A6
2	rs6735478	0.110	0.016	0.102	1.27E-11	5.42E-13	T	0.398	UGT1A6
2	rs7604125	0.106	0.016	0.098	3.28E-10	2.09E-12	T	0.350	UGT1A6
2	rs3755339	0.102	0.016	0.098	7.53E-10	7.28E-12	A	0.450	UGT1A1
2	rs1481326	0.101	0.016	0.095	8.03E-10	8.20E-12	T	0.460	UGT1A1
2	rs4953985	0.101	0.016	0.094	1.00E-09	1.10E-11	T	0.415	UGT1A6
2	rs6124974	0.100	0.016	0.093	1.04E-09	9.01E-12	T	0.412	UGT1A6
2	rs2058595	0.101	0.016	0.093	1.06E-09	1.67E-11	C	0.412	UGT1A6
2	rs1875263	0.102	0.016	0.094	1.10E-09	1.03E-10	T	0.417	UGT1A6
2	rs6431639	0.100	0.016	0.093	1.20E-09	1.30E-11	A	0.410	UGT1A6
2	rs2741027	0.102	0.016	0.093	1.32E-09	9.63E-12	A	0.177	
2	rs10179091	0.100	0.016	0.093	1.34E-09	1.01E-11	T	0.462	UGT1A6
2	rs4953333	0.100	0.016	0.093	1.35E-09	1.38E-11	G	0.461	UGT1A6
2	rs4953363	0.100	0.016	0.093	1.35E-09	1.35E-11	T	0.420	UGT1A6
2	rs873114	0.100	0.016	0.092	1.37E-09	1.82E-11	T	0.428	UGT1A6
2	rs10197460	0.099	0.016	0.093	1.42E-09	2.32E-10	T	0.298	UGT1A10
2	rs2741029	0.102	0.017	0.091	1.79E-09	1.07E-11	G	0.377	UGT1A8
2	rs4288719	0.099	0.016	0.091	1.89E-09	1.93E-11	T	0.415	UGT1A6
2	rs2692373	0.102	0.017	0.090	2.16E-09	1.51E-11	C	0.377	UGT1A10
2	rs2692374	0.105	0.017	0.090	2.16E-09	2.36E-11	T	0.373	UGT1A10
2	rs2436969	0.098	0.016	0.089	3.15E-09	4.78E-11	A	0.405	UGT1A6
18	rs559195	0.286	0.047	0.078	3.33E-09	2.92E-08	A	0.282	CBLN2
2	rs10179084	0.098	0.016	0.078	3.75E-09	5.34E-10	A	0.297	UGT1A10
2	rs2741012	0.098	0.016	0.078	4.11E-09	3.22E-11	T	0.191	
2	rs6734485	0.095	0.016	0.077	4.50E-09	1.12E-09	A	0.358	UGT1A10
2	rs12988520	-0.098	0.016	0.077	5.01E-09	6.94E-10	C	0.470	UGT1A6
2	rs13033720	0.096	0.016	0.077	5.06E-09	6.86E-10	A	0.302	UGT1A10
2	rs7572563	-0.098	0.016	0.077	5.43E-09	1.03E-09	G	0.403	UGT1A6
2	rs4351716	0.095	0.016	0.076	6.40E-09	2.45E-09	T	0.358	UGT1A10
2	rs17692666	-0.097	0.016	0.076	6.86E-09	1.12E-09	A	0.402	UGT1A6
2	rs873741	0.094	0.016	0.075	7.24E-09	1.69E-09	A	0.362	UGT1A10
2	rs6738508	0.094	0.016	0.075	7.25E-09	1.69E-09	A	0.362	UGT1A10
2	rs12622171	0.094	0.016	0.075	7.85E-09	1.75E-09	G	0.362	UGT1A6
2	rs10168105	0.094	0.016	0.075	7.87E-09	1.69E-09	T	0.362	UGT1A10
2	rs6753133	0.093	0.016	0.074	1.03E-08	2.30E-09	C	0.363	UGT1A10
2	rs7583561	0.093	0.016	0.074	1.03E-08	2.30E-09	G	0.362	UGT1A10
2	rs4953933	0.093	0.016	0.074	1.05E-08	2.42E-09	T	0.358	UGT1A10
2	rs6753569	0.093	0.016	0.074	1.06E-08	2.39E-09	C	0.362	UGT1A10
2	rs6734532	0.093	0.016	0.074	1.13E-08	2.52E-09	C	0.358	UGT1A10
2	rs10180460	0.092	0.016	0.072	1.73E-08	3.94E-09	C	0.362	UGT1A10
2	rs715576	0.093	0.017	0.069	3.51E-08	6.99E-10	G	0.497	UGT 1A6
7	rs12540583	0.096	0.017	0.069	3.63E-08	1.17E-08	C	0.292	MROH2A
2	rs10180766	0.096	0.017	0.067	6.31E-08	7.05E-09	T	0.281	MROH2A
18	rs559213	0.252	0.046	0.066	7.47E-08	5.81E-07	T	0.291	CBLN2
2	rs6430106	0.409	0.077	0.062	1.79E-07	2.14E-08	T	0.221	

Figure 4 shows the corresponding Manhattan plot for the GWAS analysis.

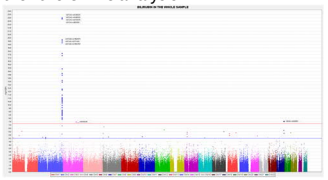
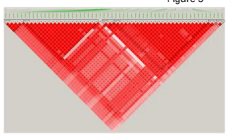


Figure 4: Manhattan plot

Figure 5 shows linkage disequilibrium among SPNs in the UGT1-cluster.

Figure 5



We found that the UGT1A gene cluster was the locus most significantly associated with bilirubin levels (the rs4148325-UGT1A1 SNP; P=9.25×10-24, being the top ranked). We also found the MROH2A at the GWAS level. Other candidate SNPs (SLCO1B1 [solute carrier organic anion transporter family member 1B1] and HMOX1 [heme oxygenase (decycling) 1]), were associated at the nominal P-value. In the exposomic analysis, we found an interaction between adherence to MedDiet and the genotype at the GWAS level. The SNP involved was in the IL17B gene (P=3.14x10-8). Table 3 shows the gene*diet interaction results (top-ranked SNPs).

Table 3. GWAS results for the interaction term between Mediterranean diet adherence and the corresponding SPN (at P<1x10-5) for bilirubin concentrations in the whole sample.

		Low Adherence Mediterranean Diet¹		High Adherence Mediterranean Diet²		P gene-diet interaction			
CHR	SNP	BETA1	SE1	BETA2	SE2	P	MA	MAF	GENE
5	rs9887452	0.065	0.016	-0.087	0.018	3.14E-08	G	0.397	IL17B
10	rs4585154	-0.001	0.016	0.063	0.019	5.86E⁻⁰⁶	A	0.395	—
6	rs777370	-0.046	0.024	0.140	0.030	1.52E⁻⁰⁶	A	0.112	LAMA2
17	rs1242482	0.035	0.015	-0.074	0.018	2.78E⁻⁰⁶	C	0.249	—
4	rs13139021	0.052	0.017	-0.069	0.020	2.96E⁻⁰⁶	A	0.228	—
10	rs10806342	0.059	0.017	-0.067	0.022	4.18E⁻⁰⁶	A	0.277	—
18	rs12964365	-0.048	0.017	0.073	0.020	4.59E⁻⁰⁶	T	0.150	LOC107985179
6	rs9894783	-0.046	0.024	0.138	0.030	5.32E⁻⁰⁶	C	0.126	LAMA2
5	rs907195	0.054	0.017	-0.070	0.021	5.91E⁻⁰⁶	C	0.253	—
4	rs13020895	0.061	0.020	-0.076	0.023	6.04E⁻⁰⁶	G	0.341	EDNRA
1	rs12385512	0.037	0.016	-0.075	0.019	6.36E⁻⁰⁶	T	0.417	—
5	rs7443185	-0.046	0.019	0.089	0.024	8.62E⁻⁰⁶	A	0.277	—
11	rs4642983	-0.060	0.015	0.043	0.018	9.39E⁻⁰⁶	A	0.455	—
18	rs887862	-0.043	0.017	0.074	0.020	9.70E⁻⁰⁶	A	0.220	LINC01541
5	rs10771013	0.11	0.027	-0.072	0.031	9.70E⁻⁰⁶	G	0.081	LINC01331

We also analyzed the interaction between smoking and the genome on serum bilirubin at the GWAS level. We obtained an interaction with the rs17688326 (intergenic in chromosome 17 at P = 8.21E-9). The following top-ranked SPNs were located at NAPEPLD, ZNF154, TASP1, ITSN1 genes (Table 4).

Table 4: GWAS results for the interaction between smoking and the corresponding SNP for bilirubin concentrations (top-ranked interactions)

			Non-smokers			Current smokers				
Chr	SNP	Position	N	Beta1	SE1	N2	Beta2	SE2	P GXE	Gene name
17	rs17688326	62109123	379	-0.024	0.018	49	0.2015	0.035	8.21E-09	intergenic
7	rs12540583	102760511	380	-0.0184	0.013	49	0.3405	0.067	4.35E-07	NAPEPLD
19	rs10409458	53815467	381	-0.0525	0.022	49	0.2149	0.048	4.46E-07	ZNF154
20	rs12106004	13502940	380	-0.0182	0.045	49	0.4879	0.099	5.21E-07	TASP1
21	rs9781194	35254293	380	-0.0424	0.027	48	0.2814	0.059	5.86E-07	ITSN1

In the EWAS analysis we detected significant associations between methylation in several CpGs in candidate genes for bilirubin and bilirubin concentrations (i.e.: cg23938283 in SLCO1B1; cg21620495 in UGT1A10; cg05357229 in UGT1A6; and cg01764553 in UGT1A1). However, these CpGs were not among the top-ranked methylated signals in the EWAS for bilirubin concentrations. Finally, in a pilot study, we explored the interactions between genomics, epigenomics and environmental factors on serum bilirubin, revealing several modulations. Our results show the multiomics contribution to bilirubin and support the integration of metabolomics with other omics to better understand the associations between metabolites and health.

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