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The polymorphism rs2480258 within CYP2E1 is associated with different rates of acrylamide metabolism in vivo in humans

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Abstract

In a recent study, we demonstrated that the variant allele of rs2480258 within intron VIII of CYP2E1 is associated with reduced levels of mRNA, protein, and enzyme activity. CYP2E1 is the most important enzyme in the metabolism of acrylamide (AA) by operating its oxidation into glycidamide (GA). AA occurs in food, is neurotoxic and classified as a probable human carcinogen. The goal of the present study was to further assess the role of rs2480258 by measuring the rate of AA>GA biotransformation in vivo. In blood samples from a cohort of 120 volunteers, the internal doses of AA and GA were assessed by AA and GA adducts to hemoglobin (Hb) measured by mass spectrometry. The rate of biotransformation was assessed by calculating the GA-Hb/AA-Hb ratio. To maximize the statistical power, 60 TT was compared to 60 CChomozygotes and the results showed that TT homozygotes had a statistically significant reduced rate of biotransformation. Present results reinforced the notion that T-allele of rs2480258 is a marker of low functional activity of CYP2E1. Moreover, we studied the role of polymorphisms (SNPs) within glutathione-S-transferases (GSTs) enzymes and epoxide hydrolase (EPHX), verifying previous findings that SNPs within GSTs and EPHX influence the metabolism rate.

Introduction

Recently, we showed for the first time that carriers of the T-allele of rs2480258 within CYP2E1 had reduced levels of CYP2E1–mRNA, protein, and enzyme activity (Pellé et al. 2016). A similar result was also found as eQTL in GTex portal, strongly supporting the association between genotypes of this single nucleotide polymorphism (SNP) and the functional activity. CYP2E1 is the main enzyme involved in acrylamide (AA) metabolism by catalysing its oxidation into glycidamide (GA) (Sumner et al. 1999). Acrylamide is ingested at relatively high doses with foods (Tareke et al. 2002; EFSA 2015), and it is considered a probable carcinogen (IARC 1994). It was shown to be able to induce mammary gland tumors and thyroid carcinoma in animal studies (Beland et al. 2013). GA is also carcinogenic in animal studies (Beland et al. 2015) and shown to be genotoxic (Rice 2005). Perhaps this explains why rs2480258 genotypes were associated with cytogenetic damages and risk of differentiated thyroid carcinoma (DTC) in human studies (Merlo et al. 2014; Pellè et al. 2016). AA and GA are detoxified through conjugation with glutathione (GSH), forming mercapturic acids, N-acetyl-S-(2-carbamoylethyl)- cysteine and N-acetyl-S-(2-hydroxy-2-carbamoylethyl)- cysteine (Fennell and Friedman 2005; Fuhr et al. 2006). GA is also detoxified by epoxide hydrolase (EPHX1) to glyceramide (Fennell and Friedman 2005). As conceivable, polymorphisms within EPHX1 or glutathione-S-transferases (GSTs) have been found associated with the amount of excreted GSH conjugates (Huang et al. 2011, 2012), with the levels of AA and GA adducts to hemoglobin (Hb), as well as their ratio (GA-Hb/AA-Hb ratio), which reflects the metabolic transformation of AA to GA (Duale et al. 2009). Unfortunately, previous studies could not evaluate the role of polymorphisms within CYP2E1 because they focused mainly on the known PstI and RsaI variants, whose minor alleles are too rare among Caucasians (qGA biotransformation in vivo.

Materials and methods

Patients diagnosed with differentiated thyroid carcinoma (DTC) during 2008–2013 were recruited by the Department of Endocrinology, whereas healthy controls by the Occupational Medicine department (University Hospital of Pisa). Following blood withdrawal, volunteers filled in selfadministered questionnaires and gave informed consent to the study. To maximize the statistical power of the analysis for rs2480258-CYP2E1, 60 TT- and 60 CC-homozygotes, paired for health status, sex, age (within 5 years), Body Mass Index (within 1 unit), and all non-smokers were selected. DNA was extracted (with standard methods) from buffy coats and genotyped with TaqMan® Assay (Life Technologies, USA) or PCR-electrophoresis (for GSTM1 and

GSTT1) (Bottari et al. 2006). Samples were re-genotyped for quality control. Erythrocytes were frozen for adduct measurements. Blood samples were prepared for analysis according to the adduct FIRE procedure (Rydberg 2009) for LC/MS/ MS analysis of adducts from AA and GA to the N-terminal valines of Hb, detached as fluorescein thiohydantoin (FTH) derivatives (for details on the procedure, see: von Stedingk et al. 2011). Samples were analyzed in batches of 10 cases/matched-control pairs. Hardy–Weinberg Equilibrium (HWE) was evaluated with the chi-square test. Statistical analyses were carried out on Loge-transformed adduct level and the GA-Hb/AA-Hb ratio. Comparisons among groups were carried out with the analysis of variance (ANOVA). To analyze the combined effects, genotypes were first converted into predicted phenotypes: GST=0, GSTM1+GSTT1 null-genotypes, GST=1, one null-genotype, GST=2 no null-genotypes; EPHX1: low/intermediate/high activity according to Benhamou et al. (1998); CYP2E1: low/high activity according to our previous results. Then, expected phenotypes were included in a multivariate regression analysis (MRA). The statistical threshold of significance was 0.05 in two-tailed tests. Repetition assured the quality of the genotyping. Genotypes followed the HWE (when applicable) and allele frequencies matched those present in SNP databases for Caucasians. The study was approved by the Regional Ethics Review Board in Stockholm and Pisa.

Results

The demography is summarized in Table 1. Patients and controls did not show any difference in the AA-Hb (average ± standard deviation: 46.3 ± 19.9 vs 39.4 ± 14.9; p=0.06) and GA-Hb (23.1±10.8 vs 20.9±9.11; p=0.30) adducts levels or ratio (0.51±0.16 vs 0.54±0.13; p=0.21). This allowed combining the two groups to study the influence of rs2480258-CYP2E1 on the adduct ratio rather than evaluating them separately. Females had higher levels than males of both AA-Hb (46.9±18.5 vs 36.2±13.2; pp<0.001) and GA-Hb (26.1±10.7 vs 17.7±6.69; p<0.001) adducts, however the GA-Hb/AA-Hb ratio did not show any statistical difference (0.55±0.16 vs 0.50±0.11). The rs2480258-CYP2E1 was associated with the GA-Hb/AA-Hb ratio; homozygotes CC showed a 15% increased average than TT and this difference was statistically significant (Table 2, p=10−3). Also GSTM1 and EPHX1 genotypes were associated with the ratio (Table 2). The combination of the predicted phenotypes in a multivariate model (MRA) showed that CYP2E1 and GSTs are associated, as independent factors, with GA-Hb/AA-Hb ratio (p=0.003 and p=0.009, respectively).

Discussion

Although animal studies showed AA to induce thyroid carcinoma (Beland et al. 2013; Rice 2005), the present results could not support a role of AA exposure/metabolism in the etiology of DTC. In addition, we found that females had increased internal dose of AA compared to males. Similar results were reported in rodents, suggesting that the hormone status or the specific distribution of body fat in females could play a role (Sánchez et al. 2008). However, the rate of biotransformation to GA was independent by gender. We also found associations between polymorphisms within GSTs or EPHX1 and AA metabolism, in part confirming previous findings (reviewed by Hogervorst et al. 2016). Of note, this work showed for the first time that homozygotes for T-rs2480258 allele had reduced rates of AA>GA biotransformation compared to CC-homozygotes in vivo. We previously showed in vitro that the same allele was associated with reduced activity of CYP2E1, thus present data reinforce the hypothesis that T-rs2480258 is a marker of low CYP2E1 activity. This SNP is in almost complete linkage disequilibrium with rs2480256 and rs2480257 (r2= 0.99) that affect the binding of hsa-miR-570 to the CYP2E1 3'UTR (Nakano et al. 2015). Therefore, our findings could be explained by an altered regulation of CYP2E1 gene expression. The importance of these SNPs should be stressed as, unlike other commonly studied variants, the frequency of their rare alleles is high enough to allow adequate statistical power in epidemiological studies.

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References

Beland FA, Mellick PW, Olson GR et al (2013) Carcinogenicity of acrylamide in B6C3F(1) mice and F344/N rats from a 2-year drinking water exposure. Food Chem Toxicol 51:149–159.

Beland FA, Olson GR, Mendoza MC et al (2015) Carcinogenicity of glycidamide in B6C3F1 mice and F344/N rats from a two-year drinking water exposure. Food Chem Toxicol 86:104–15.

Benhamou S, Reinikainen M, Bouchardy C et al (1998) Association between lung cancer and microsomal epoxide hydrolase geno- types. Cancer Res 58:5291–5293

Bottari F, Landi S, Gemignani F (2006) Single tube genotyping of GSTM1, GSTT1 and TP53 polymorphisms by multiplex PCR. DNA Seq 17:396–399

Duale N, Bjellaas T, Alexander J et al (2009) Biomarkers of human exposure to acrylamide and relation to polymorphisms in metabo- lizing genes. Toxicol Sci 108:90–99

EFSA (2015) EFSA panel on contaminants in the food chain. Scientific opinion on acrylamide in food. EFSA J 13(6):4104. https://doi. org/10.2903/j.efsa.2015.4104

Fennell TR, Friedman MA (2005) Comparison of acrylamide metabo- lism in humans and rodents. Adv Exp Med Biol 561:109–116.

Fuhr U, Boettcher MI, Kinzig-Schippers M et al (2006) Toxicokinet- ics of acrylamide in humans after ingestion of a defi dose in a test meal to improve risk assessment for acrylamide carcino- genicity. Cancer Epidemiol Biomark Prev 15:266–271.

Hogervorst JG, van den Brandt PA, Godschalk RW et al (2016) The infl of single nucleotide polymorphisms on the association between dietary acrylamide intake and endometrial cancer risk. Sci Rep 7:6:34902.

Huang Y-F, Chen M-L, Liou S-H et al (2011) Association of CYP2E1, GST and mEH genetic polymorphisms with urinary acrylamide metabolites in workers exposed to acrylamide. Toxicol Lett 203:118–126.

Huang Y-F, Chiang S-Y, Liou S-H et al (2012) The modifying eff

of CYP2E1, GST, and mEH genotypes on the formation of hemo- globin adducts of acrylamide and glycidamide in workers exposed to acrylamide. Toxicol Lett 215:92–99.

IARC (1994) Acrylamide, IARC Monographs on the Evaluation of Car- cinogenic Risks to Humans, Some Industrial Chemicals, vol 60. International Agency for Research on Cancer, Lyon, pp 389–433.

Merlo DF, Agramunt S, Anna L et al (2014) Micronuclei in cord blood lymphocytes and associations with biomarkers of exposure to car- cinogens and hormonally active factors, gene polymorphisms, and gene expression: the NewGeneris cohort. Environ Health Perspect 122:193–200.

Nakano M, Mohri T, Fukami T et al (2015) Single-nucleotide poly- morphisms in cytochrome P450 2E1 (CYP2E1) 3'-untranslated region affect the regulation of CYP2E1 by miR-570. Drug Metab Dispos 43:1450–1457.

Pellé L, Cipollini M, Tremmel R et al (2016) Association between CYP2E1 polymorphisms and risk of diff entiated thyroid carci- noma. Arch Toxicol 90:3099–3109.

Rice JM (2005) The carcinogenicity of acrylamide. Mutat Res 580:3– 20.

Rydberg P (2009) European Patent EP1738177. The patent belongs to Adduct Analys AB, Enebyberg Sánchez J, Cabrer JM, Rosselló CA et al (2008) Formation of hemo- globin adducts of acrylamide after its ingestion in rats is depend- ent on age and sex. J Agric Food Chem 56:5096–5101.

Sumner SC, Fennell TR, Moore TA et al (1999) Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. Chem Res Toxicol 12:1110–1116

Tareke E, Rydberg P, Karlsson P et al (2002) Analysis of acrylamide, a carcinogen formed in heated foodstuff J Agric Food Chem 50:4998–5006

von Stedingk H, Vikström AC, Rydberg P et al (2011) Analysis of hemoglobin adducts from acrylamide, glycidamide, and ethylene oxide in paired mother/cord blood samples from Denmark. Chem Res Toxicol 24:1957–1965.

Table 1 Characteristics of the study population

CYP2E1 rs2480258	C/C	T/T
Status/gender		
Cases, females	15	15
Males	15	15
Controls, females	15	15
Males	15	15
Age (years)		
Mean ± SD	42.4 ± 10.8	41.0 ± 12.6
Median	43.5	40
Body Mass Index		
Mean ± SD	24.2 ± 3.1	24.5 ± 3.9
Median	28.0	24.2
Total	60	60
GSTT1		
Positive	44 (78.6%)	44 (77.2%)
Null	12 (21.4%)	13 (22.8%)
GSTM1		
Positive	28 (51.8%)	23 (46.9%)
Null	26 (48.2%)	26 (53.1%)
GSTP1 rs1695		
A/A	25 (42.4%)	32 (54.2%)
A/G	19 (32.2%)	21 (35.6%)
G/G	15 (25.4%)	6 (10.2%)
EPHX1 rs2234922		
A/A	44 (73.3%)	34 (61.8%)
A/G	10 (16.7%)	19 (34.5%)
G/G	6 (10%)	2 (3.6%)
EPHX1 rs1051740		
A/A	31 (51.7%)	29 (49.1%)
A/G	25 (41.7%)	24 (40.7%)
G/G	4 (6.6%)	6 (10.2%)

When numbers within groups do not sum up to 60, the genotyping did not pass quality control in spite of repetitions and the samples were excluded

Table 2 Association of genotypes with acrylamide (AA), glycidamide (GA) hemoglobin adducts, or GA/AA ratio

Genotype	No of subject	Mean of AA-Hb (pmol/g globin)±SE	*p* value	Mean of GA-Hb (pmol/g globin)±SE	*p* value	Mean ratio of GA-Hb/AA-Hb ± SE	*p* value
CYP2E1 rs2480258							
C/C	58	41.7±2.32		23.1±1.22		0.56±0.02	
T/T	57	44.1±2.40	0.43	20.8±1.23	0.22	0.48±0.02	**10^{-3}**
GSTT1							
Positive	85	43.5±1.96		21.9±1.07		0.51±0.02	
Null	25	39.3±3.44	0.24	21.8±2.08	0.83	0.56±0.03	0.15
GSTM1							
Positive	49	42.0±2.59		20.2±1.36		0.49±0.02	
Null	50	44.9±2.66	0.46	24.3±1.50	**0.04**	0.56±0.02	**0.03**
GSTP1 rs1695 (p.Ile105Val)							
A/A	55	42.0±2.16		21.1±1.33		0.51±0.02	
A/G	39	42.3±2.96		22.0±1.57		0.53±0.02	
G/G	20	44.0±4.70	0.89	23.4±2.19	0.57	0.53±0.02	0.33
A/A + A/G vs G/G			0.68		0.36		0.42
EPHX1 rs2234922 (p.His139Arg)							
A/A	75	41.8±2.05		22.1±1.21		0.53±0.02	
A/G	33	46.5±3.32		21.7±1.67		0.47±0.02	
G/G	7	37.7±3.93	0.33	22.6±2.91	0.89	0.60±0.04	**0.02**
A/A + A/G vs G/G			0.58		0.64		0.11
EPHX1 rs1051740 (p.Tyr113His)							
A/A	59	43.2±19.2		22.5±10.9		0.53±0.13	
A/G	46	42.2±15.6		20.6±7.8		0.51±0.15	
G/G	10	44.2±21.3	0.99	25.5±13.2	0.51	0.57±0.09	0.22
A/A + A/G vs G/G			0.93		0.36		0.17

When numbers within groups do not sum up to 120, the analysis did not pass quality control in spite of repetitions and the samples were excluded

SE standard errors

p values from ANOVA

Statistically significant results at $p < 0.05$ are highlighted in bold