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Rare Alleles within the *CYP2E1* (MEOS System) Could be Associated with Better Short-Term Health Outcome after Acute Methanol Poisoning

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Abstract: Genetic polymorphisms influence the metabolism of ethanol and methanol, but the potential effects of genetic predisposition on the clinical course, outcome and short-term health sequelae of acute methanol poisoning are unknown. To evaluate the role of the MEOS system in methanol poisoning, we analysed the effect of three polymorphisms (RsaI – rs2031920; PstI – rs3813867; insertion/deletion I/D) within the CYP2E1 enzyme (MEOS system) in 50 adult survivors of methanol poisoning and compared their genotype frequencies with 460 controls. The minor allele frequencies of all three polymorphisms were below 5% in both groups. We did not detect significant differences in the genotype frequencies between survivors of methanol poisoning and controls ($p = 0.34$ for the RsaI variant; $p = 0.59$ for the PstI variant and $p = 0.21$ for the I/D polymorphism). The carriers of at least one minor allele in the *CYP2E1* gene had less severe clinical symptoms and better short-term outcome after acute poisoning. Variants within the *CYP2E1* gene are likely not significant genetic determinants of acute methanol poisoning (if survivors are analysed), but they may influence the severity of methanol poisoning and its visual/central nervous system (CNS) outcome.

Methanol poisoning is a rare condition, occurring mainly as a result of consuming adulterated liquor/spirit.

From September 2012 to December 2013, approximately 150 cases of acute methanol poisoning with 48 deaths were registered in the Czech Republic. The cause of these poisonings was the consumption of illegal spirits adulterated with methanol. However, the registered number of intoxicated individuals does not reflect the estimated number of bottles that have toxic contents (according to law enforcement, several thousand bottles may be poisoned), and the poisoning severity in many cases did not correspond to the dose of ingested toxic spirits [1]. Therefore, we suggest that there may be specific genetic backgrounds that are more sensitive (or more resistant) to the toxic effects of methanol and its metabolite formic acid and that may potentially influence the clinical course and outcome of acute methanol poisoning. To date, no study examining the potential role of such genetic (pre)dispositions has been published.

Approximately 30% of consumed methanol is excreted unchanged through the respiratory tract, and low amounts of methanol are excreted through sweat or urine [2]. In human beings, methanol is metabolized by the same pathways as ethanol due to its structural similarity to ethanol. The majority of methanol is oxidized by alcohol dehydrogenase [3]; however, this enzyme is monomorphic in poisoned Czech patients (Hubacek, unpublished results).

The second largest amount of consumed methanol (approximately 10%) is metabolized by the inducible hepatic MEOS (microsomal ethanol oxidizing system) [4]. The activity of this system is strongly increased in individuals who consume ethanol regularly (long-term ethanol abuse) and with higher ethanol blood concentrations (over 0.5‰).

The most important enzyme of this metabolic pathway is cytochrome CYP2E1 (OMIM acc. No.: 124040; ethanol-inducible P450). CYP2E1 enzyme is primarily involved in the biodegradation of numerous xenobiotics and drugs [5,6] but also participates in ethanol and methanol metabolism. *CYP2E1* variants are associated with different response levels to alcohol, and some authors associated these variants with alcoholism [7] and the predisposition to liver cirrhosis [8]. The possible relevance of *CYP2E1* variants to alcoholic liver disease has also been widely discussed, but with contradictory conclusions (reviewed by Lieber [6]). Increased CYP2E1

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activity caused by alcohol abuse can lead to the higher production of different toxic metabolites (dependent on the primary xenobiotics); therefore, certain variants of CYP2E1 enzymes have been extensively discussed as risk factors for the development of different types of cancer (for an example, see [9,10]).

Two functional variants (in strong linkage disequilibrium) have been described in the 5′ flanking region of this gene. These variants are recognized by the restriction enzymes PstI (rs3813867) and RsaI (rs2031920). The minor allele of this enzyme lacks the RsaI restriction site (mostly associated with the presence of the PstI restriction site) and is associated with higher transcriptional activity, protein levels and enzyme activity compared with the major allele [11–13]. This allele is relatively common in Asian populations, but in Caucasians, its maximal frequency is approximately 5% [14,15]. Previously, it was demonstrated that the *CYP2E1* locus may contribute to the determination of early hepatic alcohol metabolism [16]. Furthermore, a potentially functional insertion/deletion of the 96-base pairs (consisting of two almost identical 48 base-pair repeats) within the promoter region of this gene was previously described by Fritsche *et al.* [17].

Different enzyme activities are associated with the presence of these different alleles and may affect methanol metabolism. Therefore, these alleles may influence susceptibility to methanol intoxication, as well as potential later health sequelae.

In our study, we examined the potential effects of three different *CYP2E1* variants on the clinical course and outcome of acute methanol poisoning and short-term (3–8 months) visual/central nervous system health sequelae in individuals who survived acute methanol intoxication during the Czech mass methanol outbreak in 2012/2013.

Material and Methods

Study participants. Surviving victims (n = 80) of methanol poisoning (121 hospitalized in total) were invited to participate in this study twice in writing and, if no response was received, twice by phone. Fifty patients who survived acute methanol poisoning during the Czech methanol outbreak, with a median age of 48 years (range 23–73), including 41 males and nine females, agreed to participate in the study and were examined (for detailed characteristics, see table 1) [18]. As a control population, 460 healthy individuals (aged between 26 and 65 years, median age 49 years, 280 males and 180 females) were genotyped. They represent a portion (18%) of the 3-year cohort of the selected 1% Czech population sample [19]. The control individuals were recruited in nine districts in 1997/1998 and re-invited to participate in 2000/2001 according to a WHO protocol ('*MONItoring of CArdiovascular risk factors Project*'. Manual WHO/MNC 82.2, Nov-1983).

The discharge reports of 50 hospitalized patients with a confirmed diagnosis and the results of neurological and ophthalmological examinations at admission, during hospitalization and at discharge were collected and analysed in the Czech Toxicological Information Centre [20]. Laboratory investigations at admission included serum methanol, ethanol, formate, lactate, electrolytes and bicarbonate levels, as well as arterial blood gas analysis, pH, base deficiency, anion and osmolar gaps, glucose, complete renal and hepatic tests, complete hemogram and serum proteins. The clinical examination protocol included a complete ocular examination with standard ophthalmic tests (visual acuity, visual fields, colour vision, contrast sensibility and fundoscopy), computed tomography of the brain and a standard neurological examination.

Table 1.
Basic characteristics of the survivors of methanol poisoning.

Age (years)	48 (23–73)
Sex (male/female)	41/9
Alcoholism (Yes/No)	32/18
Dose of toxic spirit (mL)	300 (100–1000)
Time to treatment (hr)	24 (2–96)
Antidote (fomepizole/ethanol)	10/37
Folate (Yes/No)	37/13
Haemodialysis (intermittent/continuous veno-venous)	21/17
HCO_3 (mmol/L)	11.4 (2.5–23.7)
Methanol (mmol/L)	28.7 (2.7–228.1)
Ethanol (mmol/L)	0.1 (0–96.8)
Formate (mmol/L)	11.7 (0–31.1)
Lactate (mmol/L)	1.9 (0.7–17.1)
Anion gap (mmol/L)	24.5 (11.1–54.8)
Base deficit (mmol/L)	16.1 (0.1–38.1)
Visual sequelae[1]	6
CNS sequelae[1]	8
Visual and CNS sequelae[1]	14
Without any sequelae[1]	22

Data are given as mean (minimum–maximum), and age as median (minimum–maximum).
[1]3–8 months after acute methanol poisoning.

The patients were again examined 3–8 months after hospital discharge to determine the long-term health sequelae of acute methanol poisoning. The clinical examination protocol included a complete ocular examination and standard ophthalmic tests, optical coherence tomography (OCT) with retinal nerve fibre layer estimation (RNFL), visual evoked potentials (VEP), magnetic resonance imaging (MRI) of the head, neurological and neuropsychological examination, biochemical tests (electrolytes, glucose, glycohaemoglobin, albumin, pre-albumin, renal and hepatic tests, cholesterol, lipids, thyroid stimulating hormone, vitamin B_{12}, carbohydrate-deficient transferrin, complete hemogram and haematocrit level), ethyl glucuronide in urine and questionnaire forms (circumstances of poisoning, anamnesis, comorbidity, etc.).

The patients were considered to have long-term visual sequelae due to acute methanol poisoning if toxic neuropathy symptoms in the optic nerve were documented at admission/during hospitalization, with pathological findings for visual acuity, visual fields, colour vision, contrast sensitivity, persisting lesions observed during fundoscopy and with other symptoms of visual damage after hospital discharge and pathological findings during the examination 3–8 months after discharge (pathological RNFL, pathological VEP in at least one eye with pathological fundus findings, perimeter, colour vision and contrast sensitivity).

The patients were considered to have long-term central nervous system (CNS) sequelae due to methanol poisoning if symmetrical necrosis and haemorrhages of basal ganglia were present on the brain computed tomogram obtained during hospitalization for acute methanol poisoning and pathological findings were present on the brain MRI 3–8 months after hospital discharge (symmetrical necrosis of the putamen, globus pallidus, lemniscus medialis).

DNA analysis. DNA was isolated using a slightly modified version of the method described by Miller *et al.* [21]. Genotyping was performed with polymerase chain reaction – restriction fragment length polymorphism analysis. Briefly, oligonucleotides (5′ CCA GTC GAG

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TCT ACA TTG TCA and 5′TTC ATT CTG TCT TCT AAC TGG) were used to amplify a product that, after digestion with RsaI or PstI, can distinguish the rs2031920 and rs3813867 variants. Additional oligonucleotides (5′GTG ATG GAA GCC TGA AGA ACA and 5′ TTT GGT GGG GTG AGA ACA G) were used to genotype the insertion–deletion (I/D) polymorphism. The chemicals used were purchased from Fermentas International Inc., Burlington, Ontario, Canada, and the PCRs were performed with the DYAD Disciple PCR machine from MJ Research (Waltham, MA, USA).

All participants were ethnic Caucasians. Written informed consent was provided by all individuals. The study was approved by the institutional ethics committee and was conducted according to good clinical practice guidelines.

Statistical analyses. Deviations from Hardy–Weinberg equilibrium were evaluated using the www tools [22]. Because the minor allele frequency carriers were in all groups below 5%, we pooled the individuals into groups denoted as 'carriers of the common alleles only' (n = 47) and 'carriers of at least one minor allele' of the *CYP2E1* gene (n = 3) for the following analyses. Because of the low numbers of variant allele carriers, a comparison of two Poisson distributions on the basis of the F distribution was used for the statistical analysis.

Results

For each polymorphism, we successfully genotyped all the study patients and between 95.4% and 98.9% of controls. In the control subjects, the genotype frequencies were in accordance with previously available data [7,17] (the PstI and RsaI polymorphisms were not in complete linkage disequilibrium) and were in Hardy–Weinberg equilibrium (p values between 0.72 and 0.81).

The minor allele frequencies were low in all three groups (see table 2), and homozygous minor alleles were not observed in the patients or control subjects. There were no differences in genotype frequencies between the methanol-poisoned individuals and the controls ($p = 0.34$ for the RsaI variant; $p = 0.59$ for the PstI variant; $p = 0.21$ for the I/D polymorphism).

Finally, we compared the groups 'carriers of at least one minor allele' *versus* 'common homozygotes only' (3:47 *versus* 27:412). In this analysis, the frequencies were almost identical and were not significantly different between the groups ($p = 0.48$).

A detailed analysis of these three patients, carriers of one minor allele, revealed that they had been admitted to the hospital 24–36 hr after the end of alcoholic drink consumption, with a zero level of 'protective' serum ethanol and with very high serum levels of formic acid (314 mg/L, 699 mg/L and even 1400 mg/L, table 3). It is known that serum levels of formic acid from 300 to 400 mg/L are associated with visual toxicity clinical symptoms [23,24]. These participants' serum methanol levels at admission ranged between 400 and 806 mg/L; however, none of the participants were comatose at admission (their Glasgow coma scale was 15). The participants admitted had consumed toxic spirits concomitantly with other alcoholic beverages during two (one patient) and even three (two patients) consecutive days. The estimated dose of toxic spirit consumption ranged between 250 and 1000 mL. Despite high serum levels of formic acid in all three cases, only one participant (with formate 699 mg/L) had moderate metabolic acidosis at admission, with pH 7.17, base deficit −21 mmol/L and serum HCO_3 6.8 mmol/L; this patient reported visual disturbances and dizziness at admission. Two other patients with only mild metabolic acidosis reported a 'strange hangover'. None of these patients had typical dyspnoea or gastrointestinal symptoms during acute methanol poisoning. All patients had normal serum lactate and glucose levels at admission. In the hospital, ethanol was used as an antidote, and intermittent haemodialysis was applied in all three cases. Their neurological examinations during admission and discharge were normal, and these three patients were discharged from the hospital without visual or CNS sequelae due to methanol poisoning. The detailed clinical examination performed 3–6 months after discharge detected no symptoms of CNS damage or visual impairment (table 4). The patients had normal retinal nerve fibre layers as evaluated by OCT, normal visual evoked potentials (one 58-year-old patient had minor pathological VEP), normal colour vision, acuity, contrast vision and perimeter vision. Brain MR examinations in these patients revealed no basal ganglia damage.

Table 2.
CYP2E1 variants in the survivors of methanol poisoning and unaffected controls.

	Patients				Controls				
	MH		Het		MH		Het		
CYP2E1 variant	n	%	n	%	n	%	n	%	p
RsaI	49	98.0	1	2.0	430	96.6	15	3.4	0.34
PstI	49	98.0	1	2.0	442	97.1	13	2.9	0.59
I/D	48	96.0	2	4.0	429	97.7	10	2.3	0.21

MH, major homozygotes – presence of the RsaI restriction site, absence of the PstI restriction site and presence of the deletion; Het, heterozygotes (No minor allele homozygotes were detected).
No differences between the minor allele carriers were detected between the patients and controls.

Discussion

To our best knowledge, our study is the first study worldwide to examine (i) potential genetic predispositions to acute methanol poisoning; and (ii) the effects of selected polymorphisms on outcome among methanol poisoning survivors. Although this study is the largest methanol poisoning study conducted to date and included the most numerous group of methanol-poisoned individuals, its power was much smaller than recent typical genetic association studies. However, the unique characteristic of our patients prohibits the collection of thousands of patients or the inclusion of a confirmatory study. Unfortunately, samples from the non-survivors were not available, and the potential effect on mortality cannot be studied.

In comparison with other countries, self-reported alcohol consumption is relatively high in the Czech Republic [25],

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Table 3.
The laboratory and clinical parameters on admission in three patients with minor alleles within the *CYP2E1* gene.

ID	Sex/Age	MetOH	Formate	pH	Lactate	Glucose	HCO_{3-}	BD	GCS	Clinical features	Dose of toxic spirit, mL
1	M33	806	1400	7.36	1.7	6.7	15.6	−8	15	No	1000
2	M42	400	314	7.29	1.5	6.4	17.8	−7.5	15	F	350
3	M58	406	699	7.17	2.0	7.6	6.8	−21	15	VD, Dz	240

MetOH, serum methanol; Formate, serum formate (both in mg/L); Lactate, serum lactate; Glucose, serum glucose; HCO_{3-}, serum bicarbonate (all in mmol/L); BD, base deficit; GCS, Glasgow coma scale; F, fatigue; VD, visual disturbances; Dz, dizziness.

Table 4.
The results of clinical examination 3–6 months after discharge in three patients with minor alleles within the *CYP2E1* gene.

ID	VEP OD/OS	RNFL OD/OS	Visual acuity	Colour vision	Contrast sensitivity	Fundus n. opticus	Perimeter	Brain CT/MRI
1	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N
2	N/N	N/N	N/N	N/N	N/N	N/N	Sc	N
3	A1/A1	N/N	N/N	N/N	N/N	N/N	N/N	N

VEP, visual evoked potentials; RNFL, retinal nerve fibre layer; CT, computer tomography; MRI, magnetic resonance imaging; OD, oculus dexter; OS, oculus sinister; N, normal findings; A1, minor abnormal findings.

and the wide availability of strong alcoholic beverages in small shops and open-air markets allows easier distribution of illegal (and possibly methanol-adulterated) alcoholic beverages.

Methanol, *per se*, has relatively low toxicity and it is its metabolic products, primarily formic acid, that are responsible for the later toxic effects of methanol consumption [26]. There is substantial variability in the clinical symptoms, severity of poisoning and treatment outcome in different individuals after the consumption of comparable methanol doses. This variability may be due to different factors, such as concomitant consumption of other ethanol-containing beverages, different latency periods, time to diagnosis, body-weights, food co-ingestion, as well as certain genetic predispositions. There are many thousands of the chemicals (mainly industrial chemicals, pesticides and drugs) with potential toxicity for human beings [27]. Generally, xenobiotic metabolism within the human body is significantly influenced by genetic polymorphisms within various enzymes and transporters [15,28,29]. Therefore, genetic variants of the enzymes involved in methanol metabolism could potentially determine also the interindividual differences in the severity of acute methanol poisoning. The metabolism of methanol and ethanol is significantly affected by variants within the couple of genes (alcohol dehydrogenase, *CYP2E1*, etc.).

The consequences of ethanol consumption may be affected by different genetic variants, primarily the tagging variant rs1229984 in the alcohol dehydrogenase gene. The potential interaction of this gene with ethanol consumption, cardiovascular disease [30] or some types of cancer [31] has been widely discussed. There have also been discussions of this variant and its effect on the response to alcohol intake [32]. Therefore, it is expected that certain genetic variants may also affect the outcome of methanol poisoning because polymorphisms can affect xenobiotic toxicity [33].

The 50 patients in this study had detailed clinical examinations 3–8 months after acute methanol poisoning to determine potential long-term health sequelae [1,18,34]. Despite the relatively low number of examined patients, the observation that the genotype frequencies of the analysed polymorphisms were almost identical in affected and control individuals allows us to suggest that *CYP2E1* variants are not useful genetic predisposition markers for acute methanol poisoning, which could be used as a clinical practice for individual risk estimations. However, our data suggest that the minor alleles of *CYP2E1* may represent certain protective genetic predispositions most likely alleviating the inhibitory effect of formic acid on mitochondrial C oxidase and preventing severe lactate acidosis in patients with high formate serum levels. Serum lactate accumulation plays a significant role during acidosis, in addition to the amount of formate [35], resulting in 'the circulus hypoxicus' [36]. This potential protective effect may be responsible for less severe clinical courses and better visual/central nervous system outcome after acute methanol poisoning in the carriers of one *CYP2E1* minor allele compared with other methanol poisoning patients that had comparable or even lower formic acid serum levels.

We conclude that variants within the *CYP2E1* gene are not significant genetic predictors of acute methanol poisoning (if survivors are analysed); however, *CYP2E1* variants may influence the severity of methanol poisoning and its visual/central nervous system outcome.

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Declaration of Interest

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