Case-control study of genotypes in multiple chemical sensitivity: CYP2D6, NAT1, NAT2, PON1, PON2 and MTHFR

Gail McKeown-Eyssen,[1,2] Cornelia Baines,[1] David EC Cole,[3,4,5] Nicole Riley,[1] Rachel F Tyndale,[6,7] Lynn Marshall[8,9] and Vartouhi Jazmaji[1]

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Background	Impaired metabolism of toxic chemicals is a postulated mechanism underlying multiple chemical sensitivity (MCS). Because genetic variation alters the rate of chemical metabolism, this study was designed to determine if MCS cases differed from controls for genetic polymorphisms in drug-metabolizing enzymes.
Methods	Female Caucasian participants (203 cases and 162 controls) were drawn from a larger case-control study based on a reproducible and validated case definition. Common polymorphisms for CYP2D6, NAT1, NAT2, PON1, and PON2 were genotyped.
Results	Comparing cases and controls, significant differences were found in genotype distributions for CYP2D6 ($P = 0.02$) and NAT2 ($P = 0.03$). Compared with the referent homozygous inactive (CYP2D6) or slow (NAT2) metabolizers, the odds for being CYP2D6 homozygous active (OR = 3.36, $P = 0.01$) and NAT2 rapid (OR = 4.14, $P = 0.01$) were significantly higher in cases than controls. The odds for being heterozygous for PON1-55 (OR = 2.05, $P = 0.04$) and PON1-192 (OR = 1.57, $P = 0.04$) were also significantly higher in cases.
Conclusions	A genetic predisposition for MCS may involve altered biotransformation of environmental chemicals. The CYP2D6 enzyme activates and inactivates toxins; the NAT2 enzyme bioactivates arylamines to protein-binding metabolites. A gene–gene interaction between CYP2D6 and NAT2 suggested that rapid metabolism for both enzymes may confer substantially elevated risk (OR = 18.7, $P = 0.002$). Our finding parallels others' observation of a link between PON1 heterozygosity and neurological symptoms in Gulf War syndrome. This first demonstration of genetic variation in drug-metabolizing enzymes in association with MCS requires replication. However, it suggests new research directions on genetically variable toxin pathways that might be important in MCS.
Keywords	Multiple chemical sensitivity, environmental intolerance, environmental hypersensitivity, unexplained symptoms, genotype

Departments of [1]Public Health Sciences and [2]Nutritional Sciences, [3]Laboratory Medicine and Pathology, [4]Medicine, [5]Pediatrics (Genetics), [7]Pharmacology, University of Toronto.

[6] Centre for Addiction and Mental Health.

[8] Environmental Health Clinic Unit, Women's College Hospital Toronto.

[9] Ambulatory Care Centre, Sunnybrook and Women's College Health Sciences Centre, Toronto.

Correspondence: Gail E McKeown-Eyssen, 12 Queen's Park Cres.W, Toronto, ON, Canada. M5S 1A8. E-mail: gail.eyssen@utoronto.ca

Multiple chemical sensitivity (MCS), also known as environmental sensitivity, environmental illness, and idiopathic environmental intolerance[1] has been described as disabling multi-organ symptoms triggered by multiple exposures,[2,3] a somatization disorder,[4] an irrational fear of chemicals,[5] and panic attacks paired with non-noxious stimuli.[6] An impaired ability to 'detoxify' has been postulated in MCS.[2,3] This study was designed to determine whether cases and controls differ

genetically for specific enzymes, primarily those possibly associated with chemical detoxification.[7,8]

The University of Toronto case-control study of MCS was designed to seek laboratory tests which would clarify the aetiology of MCS and differentiate people with MCS from those without. The background and methods have already been described along with case-control comparisons for haematological, biochemical, vitamins B6 and B12, and serum volatile organic compound measures (in press, *Journal of Occupational Medicine*).

Here, in order to determine whether there are genetic differences in xenobiotic metabolism, we describe our association analysis for CYP2D6, NAT1, NAT2, PON1-55, PON1-192, and PON2-148 polymorphisms comparing cases and controls. Additionally, MTHFR C677T (methylenetetrahydrofolate reductase) genotyping was performed. The rationale for each is given below.

Cytochrome P4502D6 (CYP2D6) expresses the monoxygenase enzyme debrisoquine hydroxylase primarily in liver, but also at lower levels in brain and other tissues.[9–11] This enzyme metabolizes a wide variety of substances including therapeutic drugs, drugs of abuse, procarcinogens, and neurotoxins.[12] It is genetically polymorphic.[13,14] Thus the 5–10% of Caucasians who are CYP2D6 homozygous for two non-functional alleles display impaired metabolism of many centrally acting drugs and toxins such as tricyclic antidepressants, selective serotonin re-uptake inhibitors, monoamine oxidase inhibitors, amphetamines, codeine, neuroleptics, and neurotoxins.[12] The enzyme also metabolizes endogenous neurotransmitters[15,16] which may be related to the observation that poor metabolizers score higher on scales of anxiety and lower on socialization.[17] Although not all studies agree, a review has indicated evidence of a link between CYP2D6 genotype and Parkinson's disease, Alzheimer's disease, neuroleptic-induced extra-pyramidal side effects, and some cancers.[11,18]

The arylamine transferases expressed by NAT1 and NAT2 may play an important role in determining susceptibility to aromatic amine-induced toxicity.[19] NAT1 functional differences are controversial and may be a marker for a susceptibility in a nearby gene locus.[20]

The paraoxonase (PON) genes that have been linked to the Gulf War Syndrome[21] are yet another group whose protein products have important pharmacogenetic properties.[22] The PON1 peptide is a high density lipoprotein (HDL)-associated enzyme which reacts with toxic organophosphorus compounds, including insecticides (malathion) and nerve agents (sarin, somon, and diazinon).[23] PON1 also appears to be a cyclohydrolase that cleaves the homocysteine-thiolactone ring[24,25] and exhibits similar activity towards lipoxidation derivatives of low-density lipoprotein.[26] PON2 on the other hand was only recently identified by genetic means[27] and less is known about this isoenzyme.[28] Both PON1 and PON2 contain common genetic polymorphisms. In the PON1 gene, a leucine-to-methionine (L55M) mutation causes a decrease in enzyme activity with artificial ester substrate while a glutamine-to-arginine mutation (Q192R) causes a decrease in sensitivity to paraoxon, a toxic organophosphate, due to more rapid inactivation.[28,29] Both polymorphisms are in strong linkage disequilibrium (i.e. they are not independent of one another). There are also two common missense polymorphisms in PON2,

-G148A, and –C311S, that have been associated with disease phenotypes. Linkage disequilibrium between PON1-Q192R and PON2-C311S is essentially complete (i.e. there is perfect correlation) and only two haplotypes are observed, PON1-Q192/PON2-C311 and PON1-192R/PON2-311S.[28]

Because impaired Vitamin B12 metabolism contributes to neurological symptoms,[30] and because we had previously observed that serum vitamin B12 levels were higher in cases than in controls (in press, *Journal of Occupational Medicine*), we hypothesized it might be involved in MCS and sought case-control differences in MTHFR-C677T genotype.

Methods

Development of case definition

The University of Toronto Health Survey (UTHS) is a reproducible, self-administered questionnaire,[31] designed to assess 171 symptoms, 85 exposures linked to symptoms, and 9 specific 'features' described in six previously published MCS case definitions.[3,32–36] The UTHS was sent to 4126 individuals attending four practice types: family medicine (1337), allergy (751), occupational medicine (791), and environmental medicine (1247); 61.7% responded.[31] Our analysis of UTHS responses revealed that two features described by Nethercott *et al.*[34], namely 'symptoms linked to low-level exposure resolving with removal of the exposure' and 'symptom chronicity', differentiated female respondents attending environmental medicine practices (with a high likelihood of MCS) from those attending general practices (with a low likelihood).[37] Furthermore, combinations of four self-reported UTHS symptoms also discriminated between the two practice types. The four symptoms were a stronger sense of smell than others have, feeling dull or groggy, feeling spacey, and difficulty concentrating. To achieve discrimination required either a stronger sense of smell than others or, in the absence of that symptom, any two of the three remaining UTHS symptoms.[37]

Selection of cases and controls

Participants in the present study were drawn from female patients of any of the practices previously described who had participated in the initial UTHS survey so that eligibility criteria could be applied. Only previous responders who had volunteered to participate in future research studies were approached. Women were eligible if they were Toronto residents, age 30–64 years, and not pregnant. Women from any practice were considered to be cases if they fulfilled both Nethercott features and either of the two discriminatory combinations of UTHS symptoms as described above.[37] Severity of symptoms was not considered in defining cases. Controls, drawn only from family practices, were ineligible if they reported either of the two Nethercott features or a stronger sense of smell than others or more than one of the other three UTHS symptoms which defined a case.

Potential cases (493) and controls (481) received invitation letters requesting they notify study investigators if unwilling to participate. Otherwise recipients were telephoned (8 attempts) and invited to enrol. Ultimately 223 cases and 194 controls were recruited, a 64% and 50% response respectively of those

reached and eligible. Forty-six controls reported one UTHS symptom and the remainder none. However, because the frequency of enzyme variants differs profoundly among ethnic groups, eligibility for the genotype analysis was further limited to those reporting four Caucasian grandparents[38,39] leaving 203 cases and 162 controls with 37 controls reporting one symptom and the remainder none.

Study conduct

The case-control study was conducted in the fall and winter of 1997–1998 in the environmental health clinic (EHC) at Women's College Hospital, a teaching hospital affiliated with the University of Toronto, Canada. Hospital and University ethics review boards approved the study protocol.

Sample Collection and Analysis

MedChem Health Care Ltd (Toronto) was responsible for collection and preparation of all blood samples for this study. With participants in a fasting state, 7 ml blood were drawn for genomic DNA isolation. Samples for genomic DNA were kept on ice and taken daily to the Toronto General Hospital Genetic Repository. Genotyping was performed only for CYP2D6 *3, *4 and *5 non-functional alleles,[13,14] NAT1 and NAT2,[40,41] PON1-55, PON1-192 and PON2-148,[42] and MTHFR-677.[43]

Analysis

The frequency distribution of alleles and genotypes in cases and controls was determined. Chi-square tests determined whether there were case-control differences. On the assumption that no

Table 1 Case-control comparison: demographic characteristics, Caucasians only

Variable	(n)	Cases	Missing values	(n)	Controls	Missing values	P
Mean age (SD)	(203)	47.5 (8.2)	0	(162)	48.2 (8.4)	0	0.42*
Marital status							
Ever married	(157)	78%	2	(129)	80%	1	0.62†
Never married	(41)	20%		(28)	17%		
Other	(3)	1%		(4)	2%		
Education							
Primary only	(3)	1%	2	(1)	1%	0	0.49†
Secondary only	(30)	15%		(22)	14%		
College only	(55)	27%		(36)	22%		
University	(113)	56%		(103)	64%		
Smoking							
Smoke daily							
Yes	(15)	7%	0	(17)	10%	0	0.30‡
No	(188)	93%		(145)	90%		
Mean no. cigarettes/day							
(SD)	(15)	9.8 (5.2)		(17)	20.2 (10.0)		0.001*
Log transformed		2.15 (0.53)			2.89 (0.50)		0.0003*
Passive smoke daily							
Yes	(41)	20%	0	(35)	22%	0	0.74‡
No	(162)	80%		(127)	78%		
Place of birth							
Canada	(152)	75%	0	(96)	60%	2	0.02‡
Western Europe	(30)	15%		(37)	23%		
South/Central America	(0)	0%		(4)	2%		
USA	(7)	3%		(9)	6%		
Africa	(1)	—		(3)	2%		
Asia	(3)	1%		(2)	1%		
Other	(10)	5%		(9)	6%		
Vitamin supplements in last 24 hours							
Yes	(135)	67%	3	(84)	52%	1	0.003‡
No	(65)	33%		(77)	48%		

* = T Test; † =Fisher's Exact; ‡ = Chi-square.

related subjects were included in the groups, Chi-square or Fisher's exact tests were performed to test whether genotypes were in agreement with the Hardy-Weinberg law. Both univariate and multivariate odds ratios (OR) were calculated to describe the association between genotype and the likelihood of being a case.[44] Univariate odds ratios (OR) are reported because adjustment for potential confounders (age, smoking, birth in Canada, and recent vitamin supplement use) had no substantial effect. Because PON2-C311S was in complete disequilibrium (i.e. completely correlated) with PON1-192 in both cases and controls, all results for PON1-192 also apply to PON2-311.

Results

There were significant case-control differences for mean number of cigarettes smoked per day (10 versus 20, $P = 0.001$), birth in Canada (75% versus 60%, $P = 0.02$), and use of vitamin supplements in the 24 hours preceding venepuncture (67% versus 52%, $P = 0.003$) respectively (Table 1). However, there was no relationship between these potential confounders and genotype (data not shown).

Significant case-control differences in allele frequency were observed (Table 2) for CYP2D6 ($P = 0.02$) and there were also marginally significant differences for NAT2 ($P = 0.07$). No differences between cases and controls were observed in the allele frequencies of the NAT1, PON1, or PON2 genes (Table 2).

The genotype distributions of each gene in both cases and controls (Table 3) did not differ significantly from those predicted by the Hardy-Weinberg law (data not shown), indicating no evidence of non-random selection.

Cases and controls differed significantly on the distribution of genotypes (Table 3) for CYP2D6 ($P = 0.02$) and NAT2 ($P = 0.03$). Cases were significantly more likely than controls to be CYP2D6 homozygous active (OR compared with homozygous inactive = 3.36, $P = 0.01$). Cases were significantly more likely than controls to be NAT2 rapid (OR compared with NAT2 slow = 4.14, $P = 0.01$). Cases were more likely to be PON1-55 heterozygous (OR compared with MM = 2.05, $P = 0.04$). And cases were more likely to be PON1-192 heterozygous (OR compared with QQ = 1.57, $P = 0.04$). However, the PON1-55 and PON1-192 genotypes are in strong linkage disequilibrium (i.e. strongly associated with each other) in both cases and controls (χ^2 with 1 d.f. = 70.9, $P < 0.001$). There is a weaker disequilibrium between PON1-55 and PON2-148 (χ^2 with 1 d.f. = 12.2, $P < 0.001$).

MTHFR-C677T genotype was not associated with case-control status, nor was there a significant interaction between genotype and case-control differences for serum vitamin B12, folate or homocysteine (data not shown).

Discussion

The CYP2D6 enzyme is known to activate and inactivate toxins and endogenous neurochemicals. Our results suggest that individuals with higher CYP2D6 activity (homozygous active) are at increased risk for MCS compared with individuals with two non-functional alleles (Table 3). In addition, a significant gene–dose effect exists ($\chi^2 = 6.72$, $P < 0.001$) with CYP2D6 heterozygotes being at intermediate risk of MCS. Although

Table 2 Case-control comparisons: allele frequencies, Caucasians only

Gene (Allele)	Cases		Controls		P
	n*	(%)	n	(%)	(χ^2)
CYP 2D6 (#124030)					
1	328	(82)	237	(73)	0.02[†]
3	3	(1)	3	(1)	
4	62	(15)	67	(21)	
5	9	(2)	17	(5)	
NAT-1 (#108345)					
10	88	(22)	65	(20)	0.87[†]
11	10	(2)	10	(3)	
14	6	(2)	4	(1)	
15	3	(1)	1	(0)	
4	291	(73)	244	(75)	
NAT-2 (#243400)					
4	105	(26)	62	(19)	0.07
5	173	(43)	162	(50)	
6	110	(28)	85	(26)	
7	12	(3)	15	(5)	
PON1-55 (#168820)					
M	135	(34)	117	(36)	0.51
L	265	(66)	207	(64)	
PON1-192 (#168820)					
Q	277	(69)	236	(73)	0.29
R	123	(31)	88	(27)	
PON2-148 (#602447)					
A	311	(78)	246	(76)	0.56
G	89	(22)	78	(24)	

* = Number of alleles varies slightly due to missing values.

† = Fisher's Exact.

numbers for each mutation are small, these relationships are in the same direction for each individual variant tested (Table 3). These findings combine to support a direct role of the enzyme function being associated with risk of MCS, rather than CYP2D6 being a marker for a nearby gene. However, in view of the small numbers, such an association must be viewed cautiously. If chemical(s) in the environment are metabolized to toxins by the CYP2D6 enzyme, it would be consistent with the concept that MCS is associated with toxins. It is also possible that the enzyme is metabolizing an endogenous compound(s) and that the decreased level of the parent compound, or increased level of the metabolite, enhances the relative risk for MCS.

For NAT1, there were no significant differences between cases and controls in allele frequency (Table 2) or in genotype (Table 3). However, NAT2 did differ for cases and controls. While differences in NAT2 allele distribution were only marginally significant (Table 2), when alleles combine into genotype, rapid acetylators were found to be at increased risk (Table 3). The over-representation of the rapid acetylator genotype (*4/*4) could be associated with the role the NAT2 enzyme plays in bioactivating arylamines to protein-binding metabolites. This might result in cellular

Table 3 Case-control comparisons: genotype distributions, Caucasians only

Gene (genotype)	Cases		Controls		P[†]	OR (CI)	P[‡]
	n**	(%)	n**	(%)			
CYP 2D6 (#124030)[#]							
Homozygous (inactive)					0.02	1.0	
*3/*5	0	(0)	1	(1)			
*4/*4	4	(2)	8	(5)			
*4/*5	2	(1)	5	(3)			
*5/*5	1	(1)	2	(1)			
Heterozygous (intermediate)						2.49 (0.95, 6.51)	0.06
*1/*3	3	(1)	2	(1)			
*1/*4	52	(26)	46	(28)			
*1/*5	5	(2)	7	(4)			
Homozygous (active)						3.36 (1.33, 8.50)	0.01
*1/*1	134	(67)	91	(56)			
NAT-1 (#108345)							
Intermediate					0.48	1.0	
*10/*14	5	(3)	3	(2)			
*4/*14	1	(1)	1	(1)			
*4/*15	3	(2)	1	(1)			
Rapid						0.67 (0.22, 2.05)	0.48
*10/*10	13	(6)	15	(9)			
*10/*11	2	(1)	2	(1)			
*4/*10	55	(28)	30	(19)			
*4/*11	8	(4)	8	(5)			
*4/*4	112	(56)	102	(63)			
NAT-1 (#108345)							
*Not *10*					0.18	1.0	
*4/*11	8	(4)	8	(5)			
*4/*14	1	(1)	1	(1)			
*4/*15	3	(2)	1	(1)			
*4/*4	112	(56)	102	(63)			
*10						1.35 (0.87, 2.10)	0.17
*10/*10	13	(7)	15	(9)			
*10/*11	2	(1)	2	(1)			
*10/*14	5	(3)	3	(2)			
*4/*10	55	(28)	30	(19)			
NAT-2 (#243400)							
Slow					0.03	1.0	
*5/*5	44	(22)	44	(27)			
*5/*6	44	(22)	38	(23)			
*5/*7	7	(4)	5	(3)			
*6/*6	15	(8)	10	(6)			
*6/*7	3	(2)	7	(4)			
Intermediate						1.18 (0.75, 1.83)	0.47
*4/*5	34	(17)	31	(19)			
*4/*6	33	(16)	20	(12)			
*4/*7	2	(1)	3	(2)			

Table 3 continued

Gene (genotype)	Cases		Controls		P†	OR (CI)	P‡
	n**	(%)	n**	(%)			
Rapid						4.14 (1.36, 12.64)	0.01
*4/*4	18	(9)	4	(2)			
PON1- (#168820)							
PON1-55					0.11		
MM	18	(9)	25	(15)		1.0	
ML	99	(50)	67	(41)		2.05 (1.04, 4.05)	0.04
LL	83	(41)	70	(43)		1.65 (0.83, 3.26)	0.15
PON1-192					0.11		
QQ	93	(46)	90	(56)		1.0	
QR	91	(46)	56	(34)		1.57 (1.01, 2.45)	0.04
RR	16	(8)	16	(10)		0.97 (0.46, 2.05)	0.93
PON2-148 (#602447)					0.84		
AA	122	(61)	94	(58)		1.0	
AG	67	(33)	58	(36)		0.89 (0.57, 1.39)	0.61
GG	11	(6)	10	(6)		0.85 (0.35, 2.08)	0.72

** Number of genotypes varies slightly due to missing values.

† Chi square.

‡ unadjusted regression analysis.

= Numbers in parentheses refer to the catalogue number for Online Mendelian Inheritance in Man: *http://www3.ncbi.nlm.nih.gov/omim/*.

derangements leading to MCS. In view of the small numbers, such an association must be viewed cautiously.

No case-control differences were observed in allelic frequencies for either PON1 or PON2 (Table 2). However, when examining genotype frequencies (Table 3), cases were significantly more likely than controls to be PON1 heterozygotes. This finding is of interest because it is consistent with Haley *et al.*[21] who observed that more neurological symptoms were reported by Gulf War veterans who were PON1-192 heterozygotes (QR). We and others have found neurological symptoms are a feature of MCS.[45] However, our findings differ from those of Haley *et al.* who also observed an association of Gulf War Syndrome with the homozygous R genotype, as we found no corresponding significant association with MCS for the homozygous L genotype (LL) of PON1-55 or for the homozygous R genotype of PON1-192. Neither were there differences between cases and controls in the frequency of the 55L allele (seen in 66.3% of chromosomes for cases and 63.8% for controls in Table 2, *P* = 0.52 from Fisher's exact test) or of the 192R allele (30.8% for cases and 27.1% for controls in Table 2, *P* = 0.32, Fisher's exact test). Since heterosis is a well-established genetic phenomenon, the finding of an association with MCS only in heterozygotes cannot be entirely dismissed. It is more likely that effects at contiguous, linked sites within the PON cluster account for this unusual effect. The small sample size makes it impossible to assess whether the heterozygote association is biologically significant. Further research is warranted to determine whether the association between PON genes and MCS demonstrates a gene–dose effect for variants of all three PON gene sequences which were not measured in this study but which are known to exist.[29]

Impaired Vitamin B12 metabolism can be related to non-specific neurological symptoms,[30] such as those seen in MCS.[45] Our previous findings (in press, *Journal of Occupational Medicine*) show that serum vitamin B12 levels were higher in cases than in controls (*P* = 0.02). We hypothesized that a gene involved in vitamin B12 and folate metabolism, MTHFR, might differ between case and controls. However, we found no association between MTHFR genotype and MCS and therefore conclude that differences between cases and controls are not mediated by the MTHFR-C677T polymorphism. It therefore seems likely that this gene does not contribute to the neurobehavioural and cognitive symptoms of MCS.

Building on our findings that MCS is associated with CYP2D6 and NAT2, genes involved in the metabolism (activation or inactivation, depending on the chemical) of endogenous and exogenous compounds, future studies should focus on identifying potentially important and genetically variable pathways within the body. For example, investigations might explore the role in MCS of genetically variable metabolism by brain or hepatically expressed CYP2D6 of centrally active neurosteroids, neurotransmitters, or neurotoxins (reviewed in ref. 46). Alternatively, both of these enzymes metabolize many exogenous drugs, toxins, and chemicals, and it is plausible that specific chemicals metabolized by these enzymes influence the risk for MCS. In addition, both of these genes have many additional genetic variants that were not examined in this first study. The CYP2D6 gene, for instance, has 46 alleles identified to date (http://www.imm.ki.se/CYPalleles/cyp2d6.htm); these alleles have increased, decreased, altered or no activity. Some alleles have altered activity towards some, but not all substrates. Gaining a greater understanding of the role of genetic variation

in CYP2D6 and NAT2, and the resulting metabolic impact, may aid in focusing the search for chemical exposures associated with MCS.

While not one of our original hypotheses, the potential for gene–gene interactions arose in response to reviews of this manuscript. Therefore, we examined the impact of being a rapid metabolizer for both NAT2 and CYP2D6. The OR for the rapid/rapid versus slow/slow combination of CYP2D6 and NAT2 is 18.7 with confidence limits that, although wide, exclude unity (95% CI: 2.9, 122.5). This suggests that NAT2 and CYP2D6 enzymes may interact together on exogenous chemicals or endogenous pathways to substantially increase risk for MCS beyond the risk that is observed for each gene alone. While highly speculative at this stage, this observation warrants further examination in other data sets.

We believe that our results, based as they are on a validated and reproducible case definition for MCS, together with preliminary evidence from Binkley *et al.*[47] that MCS patients displayed a higher prevalence of the cholecystokinin B receptor allele 7 compared with control subjects, demonstrate a potentially important association between risk for MCS and genetic variation in biologically plausible candidate genes. Nevertheless, the findings require replication in other settings. They also provide direction for research focusing on the search for toxin pathways which might contribute to the risk of MCS.

Acknowledgements

KEY MESSAGES

- Multiple chemical sensitivity (MCS) cases differ from controls for genetic polymorphisms in drug-metabolizing enzymes.

- Significant case-control differences in genotype distribution for CYP2D6 and NAT2 were identified and a gene–gene interaction between these genotypes elevated substantially the risk for MCS

- A useful symptom-based, reproducible, and validated case definition for MCS was applied and will be useful for future research into the role of genetics in MCS.

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