Brain and Behavior

Open Access

# Polymorphisms of GSTT1, GSTM1, and EPHX genotypes in patients with cryptogenic polyneuropathy: a case–control study

Jonas Lindh[1], Peter Söderkvist[2], Mats Fredrikson[3], Shahrzad Hosseininia[4], Martin Tondel[5], Bodil Persson[3] & Magnus Vrethem[6]

[1]Section of Neurology, Department of Internal Medicine, Ryhov County Hospital, Jönköping, Sweden
[2]Division of Cellular Biology, Department of Biomedicine and Surgery, Linköping University, Linköping, Sweden
[3]Department of Occupational and Environmental Medicine, University Hospital, Linköping, Sweden
[4]Department of Occupational and Environmental Medicine, Faculty of Medicine, University of Tehran, Tehran, Iran
[5]Occupational and Environmental Medicine, Department of Public Health and Community Medicine, University of Gothenburg, Gothenburg, Sweden
[6]Departments of Neurology and Neurophysiology, University Hospital of Linköping, Linköping, Sweden

**Keywords**
Chronic idiopathic axonal neuropathy, Glutathione S-Transferase, epoxide hydrolase, smoking, solvents.

**Correspondence**
Jonas Lindh, Section of Neurology, Department of Internal Medicine, Ryhov County Hospital, S-551 85 Jönköping, Sweden.
Tel: +4636322020; Fax: +4636322188;
E-mail: jonas.lindh@lj.se.

Funded by grants from FORSS (Medical Research Council of Southeast Sweden) and Futurum—the academy for healthcare, Jönköping County council.

Received: 14 June 2011; Revised: 02 September 2011; Accepted: 12 September 2011

doi: 10.1002/brb3.26

## Abstract

The aim of this study was to analyze whether polymorphisms for the null alleles of Glutathione S-Transferase Mu-1 (GSTM1), Glutathione S-Transferase Theta-1 (GSTT1), and a low-activity genetic variation of epoxide hydrolase exon three (EPHX*3) affect the risk of developing polyneuropathy. The enzymes of these genes are important in the metabolism of toxic compounds. Seventy-nine patients with cryptogenic polyneuropathy (equivalent to chronic idiopathic axonal neuropathy) and 398 controls were tested for the genetic polymorphism. Medical records were reviewed to collect data regarding clinical findings at diagnosis, and exposure data was collected via questionnaires. The odds ratios (ORs) for the null forms of GSTM1 and GSTT1 and the normal activity YY form of EPHX*3 were close to one except GSTT1, which reached 1.86. The highest risk of polyneuropathy was found in smokers with GSTT1 null, who had a 3.7 times increased risk. Interactions between genes were analyzed and confirmed the increased OR for GSTT1, which was strongest if the patients had the low-activity HH form of EPHX*3 (OR 2.37).Our hypothesis is that the GSTT1 null polymorphism may be related to an impaired metabolism of toxic substances that could lead to nerve damage in the peripheral nervous system.

## Introduction

Polyneuropathy is a neurological disorder that is common in middle and late adulthood. Estimates of its prevalence range from 2.4% to 8% and depend on the selection of patients, that is, general population or hospital series, and is even higher in individuals exposed to various toxins, or patients with diabetes mellitus (Beghi et al. 1995). Although there are several known causes of polyneuropathy, the etiology often remains unknown (Martyn and Hughes 1997) and is then labeled cryptogenic. The mechanism in those cases is often considered to be exposure to occupational or environmental agents, which we have previously investigated (Tondel et al. 2006). Axonopathy is the most common form of pathology in toxic neuropathies, but underlying pathologic mechanisms are unclear. It is believed that failure of axonal transport results in degeneration of vulnerable distal nerve segments (Spencer et al. 1979; Griffin and Watson 1988) and when the process continues, the degeneration proceeds proximally towards the cell body.

In industrial settings and the general environment, some compounds are neurotoxic, and frequently affect the peripheral nerve (Spencer and Schaumburg 2000). One example is n-hexane, which accumulates in nerve tissue during chronic exposure (Feldman 1999). It is known to cause primary axonal degeneration with secondary demyelination (Chang et al. 1993).

Biotransformation of exogenous and endogenous compounds may play a role in individual susceptibility due to the genetic variability of enzymes involved in detoxification. The

© 2011 The Authors. Published by Wiley Periodicals, Inc. This is an open access article under the terms of the Creative Commons Attribution Non Commercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

metabolism of biotransformation can be divided into two phases. In phase 1, the original foreign molecule is altered by adding a functional group, which can then be conjugated in phase 2. The conjugated molecule can then be excreted (Timbrell 2000). Normally, these steps lead to a less toxic molecule, but in some cases, the opposite occurs.

Epoxide hydrolases (EPHXs) are an example of a phase 1 enzyme system that acts by adding water to the epoxide (Timbrell 2000). These enzymes play an important role in the metabolism of exogenous chemicals such as polycyclic aromatic hydrocarbons (PAHs) (Omiecinski et al. 1993). Epoxides can be detoxified partly by microsomal EPHX (mEPHX), which catalyzes their hydrolysis, thereby yielding the corresponding dihydrodiols (Oesch 1973). Although this hydrolysis is generally considered to represent a detoxification reaction because less toxic chemicals are produced, some dihydrodiols generated from PAHs are substrates for additional metabolic changes to highly toxic, mutagenic, and carcinogenic polycyclic hydrocarbon diol epoxides. Thus, EPHX*3 plays the same dual role in detoxification and activation of procarcinogens as found in some cytochrome P450s (Benhamou et al. 1998) and, as a consequence, may also play an important role in neurotoxicity (Guengerich 1982) and in drug-related adverse events. Two amino acid polymorphisms have been identified in the coding region of exon three (EPHX*3), the tyrosine 113 histidine (Y113H) exchange, resulting in a low activity form of the enzyme (Hassett et al. 1994), which may influence epoxide deactivation in the cell. Patients with Leber's Hereditary Optic Neuropathy, who were homozygous for histidine 113 developed the disease earlier than those without this genotype (Ishikawa et al. 2005). The polymorphism in exon four, histidine 139 arginine (H139R, rs2234922), has been suggested as a high-activity isoform of mEPHX (Smith and Harrison 1997; Benhamou et al. 1998).

The glutathione S-transferases (GST) are a family of phase 2 enzymes responsible for the metabolism of a broad range of xenobiotics and carcinogens (Mannervik 1985). These enzymes catalyze the conjugation of glutathione with a wide variety of organic compounds to form thioethers, a reaction that is sometimes a step in a detoxification process leading to mercapturic acid formation, a classical excretion product of xenobiotics (Mannervik 1985). The GST enzymes have been shown to protect organisms from reactive oxygen compound damage through their ability to bind with glutathione (Hayes and Strange 2000). Based on amino acid sequence similarities and antibody cross-reactivity, the GSTs are divided into several classes, including mu and theta. Glutathione S-Transferase Mu-1 (GSTM1) and Glutathione S-Transferase Theta-1 (GSTT1) are both polymorphic in humans and deletions in the genes result in virtual absence of enzyme activity, particularly with simultaneous deletions in both GSTM1 and GSTT1 genes (Abu-Amero et al. 2009).The genetic variations can change an individual's susceptibility to carcinogens and toxins as well as affect the toxicity and efficacy of certain drugs (Ginsberg et al. 2009). The genes encoding the mu class of enzymes are known to be highly polymorphic (Xu et al. 1998). They are involved in the detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins, and products of oxidative stress, by conjugation with glutathione. It has been reported that individuals with GSTM1 null genotype and high exposure to solvents are at increased risk of developing solvent-induced chronic toxic encephalopathy (Söderkvist et al. 1996) and Parkinson's disease (Dick et al. 2007). The GSTT1 gene is situated on chromosome 22. For both GSTT1 and GSTM1, the null genotype has been associated with an increased risk of optic neuropathies (Abu-Amero et al. 2009) and adverse events to drugs, including cognitive impairment after therapy in patients with medulloblastoma (Barahmani et al. 2009), but not to Leber's Hereditary Optic Neuropathy (Ishikawa et al. 2005) or neuropathy in patients receiving oxaliplatin-based chemotherapy (Lecomte et al. 2006).

Since activity of these xenobiotic-metabolizing enzymes generally is necessary to promote efficient detoxification, thereby protecting the body from injury caused by exposures, we analyzed whether polymorphisms for the null alleles of GSTM1 and GSTT1 and a genetic variation of mEPHX (low activity) affect the risk of developing polyneuropathy.

## Materials and Methods

In a previous study of patients with cryptogenic neuropathy, 168 consecutive outpatients from departments of neurology at three hospitals in two neighboring counties in Sweden (Östergötland County, University Hospital, Linköping and Motala Hospital and Jönköping County, Ryhov County Hospital, Jönköping) between 40 and 79 years of age at the time of diagnosis were studied (Lindh et al. 2005). Ethics committee approval was obtained to re-review records and contact these subjects. Medical records were reexamined with a predetermined study protocol including symptoms, signs, and laboratory tests, in order to confirm the correct diagnosis in each case. Patients with a dominantly demyelinating neuropathy, hereditary neuropathy, or any other identified cause of neuropathy were excluded (Lindh et al. 2005).

Of the initial cohort of 168 patients, 158 were still alive, and they were asked to participate in the study. Blood samples were collected from the 79 patients (response rate 50%) who agreed to participate. There were 54 men and 25 women with polyneuropathy (mean age 71.0 and 68.5, respectively). The 89 patients who did not participate were slightly older (72.5 vs. 70.2 years old), had higher clinical (1.6 vs. 1.4) and neurophysiological severity (2.0 vs. 1.8), but the differences did


© 2011 The Authors. Published by Wiley Periodicals, Inc.

not reach statistical significance, and there was no difference in sex distribution.

The control group was 398 persons from a population-based control group from the Swedish part of a Parkinson's disease study of the same genetic polymorphisms living in the same geographic area (Dick et al. 2007). The controls consisted of 198 men and 200 women (mean age 67.4 and 67.5, respectively). The mean age was slightly higher among cases than controls (70.2 vs. 67.5 years, $P < 0.05$).

Cases and controls completed questionnaires about smoking status and other exposures including solvent or pesticide exposure, generalized anesthesia, and drinking water from private wells as previously described (Tondel et al. 2006; Dick et al. 2007).

The clinical severity of the neurologic condition was graded based on the functional deficit. Grade 1 (mild) clinical severity was defined as minor motor and/or sensory symptoms without functional deficit. Grade 3 was defined as severe symptoms with functional deficit, including slight ataxia or at least some need for assistance. Grade 2 or moderate severity was defined as those symptoms and deficits that were in between Grades 1 and 3. In the same way, patients were regarded as having grade 1 (mild) neurophysiological findings if neurography and EMG (electromyography) at diagnosis showed a slight decrease of Compound Motor Axonal Potentials (CMAP), Sensory Nerve Axonal Potentials (SNAP), or Conduction Velocity (CV) in at least two nerves. Grade 3 (severe) neurophysiological findings were defined as loss of sensory or motor responses in at least two nerves as judged in a previous study and Grade 2 (moderate) as those neurophysiological findings in between Grades 1 and 3 (Lindh et al. 2005).

Whole blood was collected and leukocyte DNA was isolated with Wizard Genome DNA purification kit (Promega Inc., Madison, Wisconsin). The GSTM1 and GSTT1 null genotypes were assessed in a multiplex polymerase chain reaction (PCR) with $\beta$-globin as an internal control gene for a successful PCR amplification (Arand et al. 1996). The amino acid polymorphisms in the mEPHX gene (EPHX1 exon 3) were determined by a PCR-RFLP (restriction fragment length polymorphism) assay (Lancaster et al. 1996; Smith and Harrison 1997). For exon 3, there are three possible genotypes: YY, YH, and HH. The wild-type normal activity allele is YY and the low-activity genotype is HH.

The ethics committee at the Faculty of Health Sciences at Linköping University approved the project.

### Statistical methods

The statistical analysis was performed using SPSS version 15. Because neither the controls nor the polyneuropathy patients were normally distributed regarding age, statistical analyses were performed using a nonparametric method; the Kruskal–Wallis test followed by Mann–Whitney U test for post hoc analysis (using Bonferoni's correction for multiple analyses). The chi-square test was used for categorical variables. For groups with less than five respondents, the analysis was performed with Fischer's exact test. Relative risk was expressed as odds ratio (OR) with 95% confidence intervals (CI). Comparisons were considered significant if *P*-values were <0.05. The polymorphisms were analyzed independent of sex, as the genes are located on the autosomes.

Multiple logistic regression analyses were used to obtain estimates of ORs for genetic factors adjusting for the following prespecified covariates: age, sex, ever used tobacco, solvent or pesticide exposure, generalized anesthesia, and drinking water from private wells. Interaction between genes was analyzed with Stata version 11.0.

## Results

In total, 79 cases with cryptogenic polyneuropathy and 398 controls were tested for genetic polymorphisms in the GSTM1, GSTT1, and mEPHX genes. The frequencies of the different genetic polymorphisms are presented in Table 1. Among the controls there were significantly more persons with GSTT1 null in women than in men, ($P = 0.04$), and the homozygous HH variant in mEPHX was more common in men ($P < 0.01$). The other variants did not differ between men and women. There were no statistically significant differences between cases and controls in any group.

The OR for the null forms of GSTM1 and GSTT1 and the YY form of EPHX*3 were close to one for all polymorphisms except GSTT1, which reached 1.86. When men and women were analyzed separately, we found that the OR of EPHX*3 YH and HH versus YY was 0.7 in men, whereas in women it was 2.1, almost reaching significance (Table 2).

Regarding clinical findings, 24 patients were considered to have mild findings and 39 patients had severe findings. No significant differences were found between the groups in clinical or neurophysiological severity at diagnosis except a tendency for GSTM1 null to have more severe clinical findings than GSTM1 positive cases (mean 1.55 vs. 1.31, $P = 0.064$). Axonal neuropathy was observed in 41 patients and combined axonal and demyelinating neuropathy in 19 patients. Regarding neurophysiological findings, two patients had pure motor neuropathy, 13 patients had pure sensory neuropathy, and 64 patients had a mixed sensorimotor neuropathy. Genetic polymorphisms were not significantly related to these neurographic findings.

We also investigated the effects of different exposures. In the control group, there were 189 (47%) smokers or previous smokers compared with 43 (54%) smokers among the cryptogenic polyneuropathy patients. Exposure to solvents during work or leisure time was reported by 24 (30%) of the patients with cryptogenic polyneuropathy and 132 (33%) of the

© 2011 The Authors. Published by Wiley Periodicals, Inc. 

**Table 1.** Distribution of genetic polymorphisms in cryptogenic polyneuropathy patients and controls.

| | Total number of subjects | | | | Men | | | | Women | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pnp | | Controls | | Pnp | | Controls | | Pnp | | Controls | |
| | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % |
| GSTM1 null | 40 | 50.6 | 201 | 50.5 | 30 | 55.6 | 100 | 50.5 | 10 | 40.0 | 101 | 50.5 |
| GSTM1 pos | 39 | 49.4 | 197 | 49.5 | 24 | 44.4 | 98 | 49.5 | 15 | 60.0 | 99 | 49.5 |
| GSTT1 null | 7 | 8.9 | 61 | 15.3 | 3 | 5.6 | 23 | 11.6 | 4 | 16.0 | 38 | 19.0 |
| GSTT1 pos | 72 | 91.1 | 337 | 84.7 | 51 | 94.4 | 175 | 88.4 | 21 | 84.0 | 162 | 81.0 |
| EPHX*3 YH | 30 | 38.0 | 142 | 35.7 | 18 | 33.3 | 69 | 34.8 | 12 | 48.0 | 73 | 36.5 |
| EPHX*3 YY | 39 | 49.4 | 202 | 50.8 | 30 | 55.6 | 93 | 47.0 | 9 | 36.0 | 109 | 54.5 |
| EPHX*3 HH | 10 | 12.7 | 54 | 13.6 | 6 | 11.1 | 36 | 18.2 | 4 | 16.0 | 18 | 9.0 |

In total, 79 cases with polyneuropathy and 398 controls were tested for genetic polymorphisms. In the control population, there were significantly more cases with GSTT1 null in women than in men, and the homozygous HH variant was more common in men. The other polymorphisms did not differ between men and women. There were no statistically significant differences between cases and controls in any group. Pnp = Polyneuropathy.

**Table 2.** Analysis of genetic factors by case–control status (cases vs. controls).

| Genetic polymorphism | Reference group | Studied groups | All subjects | | Men | | Women | |
|---|---|---|---|---|---|---|---|---|
| | | | OR | *P* Value | OR | *P* Value | OR | *P* Value |
| GSTM1 | Null | Positive | 0.99 (0.61–1.61) | 1 | 0.82 (0.45–1.50) | 0.54 | 1.53 (0.66–3.57) | 0.40 |
| GSTT1 | Null | Positive | 1.86 (0.82–4.24) | 0.16 | 2.23 (0.64–7.74) | 0.31 | 1.23 (0.40–3.80) | 1 |
| EPHX*3 | YY | YH | 1.09 (0.65–1.84) | 0.79 | 0.81 (0.42–1.57) | 0.62 | 1.99 (0.80–4.50) | 0.16 |
| | YY | HH | 0.96 (0.45–2.05) | 1 | 0.52 (0.20–1.35) | 0.20 | 2.69 (0.75–9.67) | 0.12 |
| | YY | YH/HH | 1.06 (0.65–1.71) | 0.90 | 0.71 (0.39–1.30) | 0.29 | 2.13 (0.90–5.05) | 0.09 |

Odds ratios with 95% confidence intervals for polymorphisms of GSTM1, GSTT1, and EPHX3 were analyzed in 79 cases of cryptogenic polyneuropathy (54 men, 25 women) and 398 controls from the general population (198 men, 200 women). The chi-square test was used and the analysis was completed with Fischer's exact test in groups with less than five respondents. Comparisons were considered significant if *P*-values were $<0.05$.

controls. Exposure to pesticides was reported by eight (10%) of the patients with cryptogenic polyneuropathy and 29 (7%) of the controls. A total of 59 (74%) of the patients with cryptogenic polyneuropathy and 312 (78%) of the controls had been exposed to generalized anesthesia, and 51 (65%) of the patients with cryptogenic polyneuropathy and 29 (71%) of the controls had private water supply for at least a period in their life. The OR for cryptogenic polyneuropathy among exposed individuals are shown in Table 3. GSTT1 null among smokers reached the highest OR (3.72, $P = 0.08$), and EPHX*3 HH versus YY among solvent exposed had the lowest OR (0.30, $P = 0.14$). A logistic regression analysis for the different polymorphisms, sex, age, and exposures did not show any confounding effects except that increasing age and male sex increased the risk of cryptogenic polyneuropathy. Interactions between genes were analyzed and confirmed the increased OR for GSTT1, which was strongest if the patients had the HH form of EPHX*3 (OR 2.37).

## Discussion

In this epidemiological case–control study of patients with cryptogenic polyneuropathy, we examined the association of GSTM1 and GSTT1 null polymorphisms and EPHX1 exon 3 HH polymorphism in relation to several environmental and chemical exposures. Although we did not find any statistically significant increased risk, the GSTT1 null genotype was associated with an almost twofold increased risk of polyneuropathy. Our hypothesis is that the GSTT1 null polymorphism may be related to an impaired metabolism of toxic substances and reactive oxygen that could lead to nerve damage, involving multiple sites along motor and sensory axons in the peripheral nervous system. This may result in axonal atrophy or axonal swelling, leading to progressive distal axonal degeneration. The myelin sheath may break down concomitantly with the axon. This could contribute to, or directly result in, an axonal or combined axonal-demyelinating neuropathy.

Components of cigarette smoke are examples of exogenous substrates that are toxic and, furthermore, are subject to bioactivation and may both directly and indirectly be neurotoxic. We found a nearly fourfold increased risk of polyneuropathy in GSTT1 null smokers that almost achieved statistical significance. Teunissen and co-authors reported an OR of 2.1 for current smoking in patients with chronic idiopathic axonal polyneuropathy (Teunissen et al. 2002), and it has


© 2011 The Authors. Published by Wiley Periodicals, Inc.

**Table 3.** Effects of genetic polymorphisms in different exposures (exposed cases and controls).

| Genetic polymorphisms | Studied groups | Exposure | Odds ratio | *P* Value |
|---|---|---|---|---|
| GSTM1 | Null versus positive | Smoking | 1.08 (0.56–2.10) | 0.87 |
| GSTT1 | Null versus positive | Smoking | 3.72 (0.85–16.2) | 0.08 |
| EPHX*3 | YH versus YY | Smoking | 1.40 (0.69–2.82) | 0.38 |
| | HH versus YY | Smoking | 0.57 (0.18–1.82) | 0.44 |
| | YH/HH versus YY | Smoking | 1.14 (0.58–2.22) | 0.74 |
| GSTM1 | Null versus positive | Solvent | 0.46 (0.18–1.14) | 0.12 |
| GSTT1 | Null versus positive | Solvent | 1.31 (0.28–6.1) | 1 |
| EPHX*3 | YH versus YY | Solvent | 0.72 (0.27–1.91) | 0.63 |
| | HH versus YY | Solvent | 0.30 (0.06–1.40) | 0.14 |
| | YH/HH versus YY | Solvent | 0.55 (0.22–1.34) | 0.27 |
| GSTM1 | Null versus positive | Pesticides | 0.98 (0.19–4.94) | 1 |
| GSTT1 | Null versus positive | Pesticides | 0.25 (0.01–4.51) | 0.39 |
| EPHX*3 | YH versus YY | Pesticides | 1.33 (0.24–7.28) | 1 |
| | HH versus YY | Pesticides | 0.80 (0.07–9.67) | 1 |
| | YH/HH versus YY | Pesticides | 1.18 (0.23–5.89) | 1 |
| GSTM1 | Null versus positive | Generalized anesthesia | 1.09 (0.62–1.90) | 0.78 |
| GSTT1 | Null versus positive | Generalized anesthesia | 1.05 (0.94–1.17) | 0.55 |
| EPHX*3 | YH versus YY | Generalized anesthesia | 1.10 (0.60–2.11) | 0.76 |
| | HH versus YY | Generalized anesthesia | 0.95 (0.39–2.32) | 1 |
| | YH/HH versus YY | Generalized anesthesia | 1.06 (0.61–1.85) | 0.89 |
| GSTM1 | Null versus positive | Private water | 0.78 (0.43–1.43) | 0.45 |
| GSTT1 | Null versus positive | Private water | 1.66 (0.57–4.91) | 0.48 |
| EPHX*3 | YH versus YY | Private water | 1.11 (0.58–2.14) | 1 |
| | HH versus YY | Private water | 1.01 (0.41–2.50) | 1 |
| | YH/HH versus YY | Private water | 1.09 (0.60–1.97) | 0.88 |

Odds ratios with 95% confidence intervals for polymorphisms of GSTM1, GSTT1, and EPHX3 are given for exposed cases of cryptogenic polyneuropathy and exposed controls from the general population. The chi-square test was used and the analysis was completed with Fischer's exact test in groups with less than five respondents. Comparisons were considered significant if *P*-values were $<0.05$.

also been found that tobacco use may predispose to earlier development and more severe symptoms of diabetic neuropathy (Tesfaye et al. 2005). Our data indicate that this risk might be explained by smokers carrying certain genetic polymorphisms leading to impaired detoxification of the toxic compounds in cigarette smoke. In a study of solvent-induced chronic toxic encephalopathy, an increased risk ratio of 2.5 for the GSTM1 null genotype was found in smokers and a risk ratio of 1.5 for the GSTT1 null genotype in the overall population. In nonsmokers, the GSTM1 null genotype did not confer any risk for chronic toxic encephalopathy. None of the studied mEPHX polymorphisms were associated with an increased risk. The mechanism for the toxicity of cigarette smoke on nerves is not known, but it has been speculated that it is mediated by chemicals in the smoke where PAHs are regarded as the most important component. Impaired breakdown of PAHs in persons with the null genotype of GSTs may lead to increased exposure on nerves and thereby increased loss of nerves. n-Hexane is another toxic substance that is present in cigarette smoke and is well known to cause polyneuropathy (Zhang et al. 2006). However, in our study, smoking did not increase the risk of polyneuropathy. An enhanced negative association between cigarette smoking and GSTM1 activity has been described in Parkinson's disease, which may be mediated by neuroprotective effects of nicotine on the dopaminergic system (De Palma et al. 2010).

The frequency of GSTM1 null is about 42–60% in Caucasians (Garte et al. 2001). The frequency of homozygous null GSTT1 varies greatly with ethnicity and is 10–20% in Caucasians (Rebbeck 1997). The EPHX*3 gene can be found in three different forms, the wild-type/normal activity variant (YY), heterozygous (YH), or homozygous/low-activity (HH) genotypes. In a Caucasian population, about 40% of subjects are heterozygous and 12% are homozygous for the HH genotype (Garte et al. 2001). The frequency of these polymorphisms in our study population was similar. No differences in allele frequencies by age or sex were seen in large studies (Garte et al. 2001). Unfortunately we had an imbalance in our control group with 19% of the women and 12% of the men having the GSTT1 null polymorphism and 9% of the women and 18% of the men having the EPHX*3 HH polymorphism. Thus, it is not possible to draw any conclusions about differences in risks of cryptogenic polyneuropathy among men and women separately.

© 2011 The Authors. Published by Wiley Periodicals, Inc.

Individuals carrying genes that code for proteins with lost or impaired function have an impaired metabolic ability to eliminate toxic compounds and may therefore be at increased risk of polyneuropathy. This type of mutation often has an OR of 2–3 for increased risk for cancer. In our group of polyneuropathy patients, we found a trend toward lower OR for the EPHX*3 gene compared to controls. The total risk of polyneuropathy probably results from complex interactions of multiple genetic and environmental factors over time. We have previously confirmed that occupational exposures to Stoddard solvent, petrol exhausts, herbicides, or hand and foot vibrations generated significantly increased risk of polyneuropathy and new determinants were also indicated, that is, sulphur dioxide, xylene, and methyl ethyl ketone (Tondel et al. 2006).

We did not find any significant correlation between clinical or neurophysiological severity and genotype except a small increase in the severity of clinical findings in GSTM1 null patients that almost reached statistical significance. It is possible that a correlation might be found if a more sensitive scale for clinical or neurophysiological severity was used.

A possible reason that we did not find any significant differences is the low number of patients. In a Chinese study of 22 cases of polyneuropathy working in a printing factory and 163 controls, an association was found with CYP2E1 Dra, but not GSTM1 and GSTT1, indicating that either a more pronounced exposure to a toxic agent is necessary or that other genes may have greater importance for the development of polyneuropathy (Zhang et al. 2006). Our study sample consisted of patients diagnosed with cryptogenic polyneuropathy at departments of neurology. It is likely that general practitioners properly diagnosed persons working in an industrial setting with high exposure to toxic agents or that they were diagnosed as toxic neuropathies by a neurologist resulting in an underestimation of the risk of exposure in our study.

The solution to these problems would be to do a Genome-Wide Association Study (GWAS), which has been a successful way to find new candidate genes in, for instance, Parkinson's disease, Alzheimer's disease (Gandhi and Wood 2010), and sporadic amyotrophic lateral sclerosis (Shatunov et al. 2010). This would, however, require a very large number of patients recruited from several countries.

In conclusion, no significant correlation was found between GSTM1, GSTT1, and EPHX1 polymorphisms in patients with cryptogenic polyneuropathy compared with controls. A strong tendency, however, was seen for the GSTT1 null phenotype and smoking in these patients compared to controls (OR 3.7). The GSTT1 null polymorphism may be related to an impaired metabolism of toxic substances and reactive oxygen that could lead to nerve damage in the peripheral nervous system. This could contribute to, or directly result in an axonal or combined axonal-demyelinating neuropathy.

## Acknowledgments

This study was supported by grants from FORSS (Medical Research Council of Southeast Sweden) and Futurum—the academy for healthcare, Jönköping County council.

### References

Abu-Amero, K. K., B. Milcarek, and T. M. Bosley. 2009. GSTM1 and GSTT1 deletion genotypes in various spontaneous optic neuropathies in Arabs. Br. J. Ophthalmol. 93:1101–1104.

Arand, M., R. Muhlbauer, J. Hengstler, E. Jager, J. Fuchs, L. Winkler, and F. Oesch. 1996. A multiplex polymerase chain reaction protocol for the simultaneous analysis of the glutathione S-transferase GSTM1 and GSTT1 polymorphisms. Anal. Biochem. 236:184–186.

Barahmani, N., S. Carpentieri, X. N. Li, T. Wang, Y. Cao, L. Howe, L. Kilburn, M. Chintagumpala, C. Lau, and M. F. Okcu. 2009. Glutathione S-transferase M1 and T1 polymorphisms may predict adverse effects after therapy in children with medulloblastoma. Neuro. Oncol. 11:292–300.

Beghi, E., and M. L. Monticelli, (IGPSG) IGPSG. 1995. Chronic symmetric symptomatic polyneuropathy in the elderly: a field screening investigation in two Italian regions. I. Prevalence and general characteristics of the sample. Neurology 45:1832–1836.

Benhamou, S., M. Reinikainen, C. Bouchardy, P. Dayer, and A. Hirvonen. 1998. Association between lung cancer and microsomal epoxide hydrolase genotypes. Cancer Res. 58:5291–5293.

Chang, C. M., C. W. Yu, K. Y. Fong, S. Y. Leung, T. W. Tsin, Y. L. Yu, T. F. Cheung, and S. Y. Chan. 1993. N-hexane neuropathy in offset printers. J. Neurol. Neurosurg. Psychiatry 56:538–542.

De Palma, G., F. D. Dick, S. Calzetti, N. W. Scott, G. J. Prescott, A. Osborne, N. Haites, P. Mozzoni, A. Negrotti, A. Scaglioni, *et al*. 2010. A case-control study of Parkinson's disease and tobacco use: gene-tobacco interactions. Mov. Disord. 25:912–919.

Dick, F. D., G. De Palma, A. Ahmadi, A. Osborne, N. W. Scott, G. J. Prescott, J. Bennett, S. Semple, S. Dick, P. Mozzoni, *et al*. 2007. Gene-environment interactions in parkinsonism and Parkinson's disease: the Geoparkinson study. Occup. Environ. Med. 64:673–680.

Feldman, R. G. 1999. *n-Hexane and Methyl n-Butyl Keton. In: occupational and environmental neurotoxicology*. Lippincott-Raven, Philadelphia, PA. Pp. 290–317.

Gandhi, S., and N. W. Wood. 2010. Genome-wide association studies: the key to unlocking neurodegeneration? Nat. Neurosci. 13:789–794.

Garte, S., L. Gaspari, A. K. Alexandrie, C. Ambrosone, H. Autrup, J. L. Autrup, H. Baranova, L. Bathum, S. Benhamou, P. Boffetta, *et al*. 2001. Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol. Biomarkers Prev. 10:1239–1248.

Ginsberg, G., S. Smolenski, D. Hattis, K. Z. Guyton, D. O. Johns, and B. Sonawane. 2009. Genetic polymorphism in glutathione

 © 2011 The Authors. Published by Wiley Periodicals, Inc.

transferases (GST): population distribution of GSTM1, T1, and P1 conjugating activity. J.Toxicol. Environ. Health B: Crit. Rev. 12:389–439.

Griffin, J. W., and D. F. Watson. 1988. Axonal transport in neurological disease. Ann. Neurol. 23:3–13.

Guengerich, F. P. 1982. Epoxide hydrolase: properties and metabolic roles. Rev. Biochem. Toxicol. 4:4–30.

Hassett, C., L. Aicher, J. S. Sidhu, and C. J. Omiecinski. 1994. Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants.[erratum appears in Hum Mol Genet 1994 Jul;3(7):1214]. Hum. Mol. Genet. 3:421–428.

Hayes, J. D., and R. C. Strange. 2000. Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology 61:154–166.

Ishikawa, K., T. Funayama, H. Ohde, Y. Inagaki, and Y. Mashima. 2005. Genetic variants of TP53 and EPHX1 in Leber's hereditary optic neuropathy and their relationship to age at onset. Jpn. J. Ophthalmol. 49:121–126.

Lancaster, J. M., H. A. Brownlee, D. A. Bell, P. A. Futreal, J. R. Marks, A. Berchuck, R. W. Wiseman, and J. A. Taylor. 1996. Microsomal epoxide hydrolase polymorphism as a risk factor for ovarian cancer. Mol. Carcinog. 17:160–162.

Lecomte, T., B. Landi, P. Beaune, P. Laurent-Puig, and M.-A. Loriot. 2006. Glutathione S-transferase P1 polymorphism (Ile105Val) predicts cumulative neuropathy in patients receiving oxaliplatin-based chemotherapy. Clin. Cancer Res. 12:3050–3056.

Lindh, J., M. Tondel, A. Österberg, and M. Vrethem. 2005. Cryptogenic polyneuropathy: clinical and neurophysiological findings. J. Peripher. Nerv. Syst. 10:31–37.

Mannervik, B. 1985. The isoenzymes of glutathione transferase. Adv. Enzymol. Relat. Areas Mol. Biol. 57:357–417.

Martyn, C. N., and R. A. Hughes. 1997. Epidemiology of peripheral neuropathy. J. Neurol. Neurosurg. Psychiatry 62:310–318.

Oesch, F. 1973. Mammalian epoxide hydrases: inducible enzymes catalysing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds. Xenobiotica 3:305–340.

Omiecinski, C. J., L. Aicher, R. Holubkov, and H. Checkoway. 1993. Human peripheral lymphocytes as indicators of microsomal epoxide hydrolase activity in liver and lung. Pharmacogenetics 3:150–158.

Rebbeck, T. R.. 1997. Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol. Biomarkers Prev. 6:733–743.

Shatunov, A., K. Mok, S. Newhouse, M. E. Weale, B. Smith, C. Vance, L. Johnson, J. H. Veldink, M. A. van Es, L. H. van den Berg, *et al*. 2010. Chromosome 9p21 in sporadic amyotrophic lateral sclerosis in the UK and seven other countries: a genome-wide association study. Lancet Neurol. 9:986–994.

Smith, C. A. D., and D. J. Harrison. 1997. Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. Lancet 350:630–633.

Söderkvist, P., A. Ahmadi, A. Akerback, O. Axelson, U. Flodin. 1996. Glutathione S-transferase M1 null genotype as a risk modifier for solvent-induced chronic toxic encephalopathy. Scandinavian Journal of Work, Environment & Health 22:360–363.

Spencer, P. S., and H. H. Schaumburg. 2000. *Experimental and clinical neurotoxicology*. Oxford Univ. Press, Oxford.

Spencer, P. S., M. I. Sabri, H. H. Schaumburg, and C. L. Moore. 1979. Does a defect of energy metabolism in the nerve fiber underlie axonal degeneration in polyneuropathies? Ann. Neurol. 5:501–507.

Tesfaye, S., N. Chaturvedi, S. E. Eaton, J. D. Ward, C. Manes, C. Ionescu-Tirgoviste, D. R. Witte, J. H. Fuller. 2005. Vascular risk factors and diabetic neuropathy. N. Engl. J. Med. 352:341–350.

Teunissen, L. L., H. Franssen, J. H. Wokke, Y. van der Graaf, W. H. Linssen, J. D. Banga, D. M. Laman, and N. C. Notermans. 2002. Is cardiovascular disease a risk factor in the development of axonal polyneuropathy? J. Neurol. Neurosurg. Psychiatry 72:590–595.

Timbrell, J. 2000. Factors affecting toxic responses: metabolism. Pp. 65–110 *in* T Francis, ed. *Principles of biochemical toxicology*. Taylor & Francis Ltd., London.

Tondel, M., J. Lindh, P. Jonsson, M. Vrethem, and B. Persson. 2006. Occupational determinants of cryptogenic polyneuropathy. Neuroepidemiology 26:187–194.

Xu, S., Y. Wang, B. Roe, and W. R. Pearson. 1998. Characterization of the human class Mu glutathione S-transferase gene cluster and the GSTM1 deletion. J. Biol. Chem. 273:3517–3527.

Zhang, Y., Q. Liu, H. Duan, J. Cheng, S. Jiang, X. Huang, S. Leng, F. He, and Y. Zheng. 2006. Association between metabolic gene polymorphisms and susceptibility to peripheral nerve damage in workers exposed to n-hexane: a preliminary study. Biomarkers 11:61–69.

© 2011 The Authors. Published by Wiley Periodicals, Inc. 