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# Direct Genomic PCR Sequencing of the High Affinity Thiamine Transporter (*SLC19A2*) Gene Identifies Three Genetic Variants in Wernicke Korsakoff Syndrome (WKS)

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Wernicke Korsakoff Syndrome (WKS) (OMIM: #277730) is caused by an absolute or relative thiamine deficiency that primarily affects alcohol misusers in western countries [Kopelman, 1995; Thomson, 2000]. In the acute phase, Wernicke's encephalopathy (WE) typically presents with confusion, opthalmoplegia, and ataxia. If Wernicke's encephalopathy is not treated promptly or if treatment is inadequate it can lead to death or to the chronic phase of the condition, Korsakoff psychosis. In some cases Korsakoff psychosis has an insidious onset with severe memory impairment as the only symptom [Kopelman, 2002]. Pathological studies of routine postmortem general hospital and forensic cases show that as many as 1.7% of individuals had previously unsuspected neuropathological lesions characteristic of WKS [Harper et al., 1989, 1995]. In alcoholic patients this rate was found to be higher than expected at 12.5% [Torvik et al., 1982; Harper et al., 1995; Cook et al., 1998]. The involvement of a thiamine deficiency in the pathogenesis of this syndrome was first postulated in the early 1940's by Alexander and co-workers [Kopelman, 1995]. Thiamine pyrophosphate plays an important role in the glucose and energy metabolism pathways as a co-factor of the enzymes, alphaketoglutarate dehydrogenase, pyruvate dehydrogenase, and transketolase [Singleton and Martin, 2001]. In the literature it has been repeatedly hypothesized that there is an inherited susceptibility to WKS [Singleton and Martin, 2001]. Several enzymes and the genes that encode them have been considered as candidate susceptibility genes [Blass and Gibson, 1977; McCool et al., 1993]. The high affinity thiamine transporter protein, SLC19A2 is mutated in Rogers syndrome and causes a thiamine deficiency and megaloblastic anemia [Ganapathy et al., 2003]. SLC19A2 has been mapped to chromosome 1q23.2-q23.3 [Diaz et al., 1999].

To test the hypothesis that the *SLC19A2* gene is involved in the pathogenesis of the WKS we carried out a mutation screening study in a group of alcoholic WKS patients and in a matched group of normal controls.

Blood samples were collected from 25 alcoholic WKS patients diagnosed by a trained psychiatrist. Information relating to the patients' drinking habits was also collected using a semi-structured interview schedule. This included a "lifetime modification" of sections 1 and 3 of the Clinical Alcoholism Interview Schedule [Caetano et al., 1978], the Lifetime Drinking History [Skinner and Sheu, 1982], and the Severity of Alcohol Dependence Questionnaire (SADQ) [Stockwell et al., 1983]. The patients were all affected by the Alcohol Dependence Syndrome (ADS), with a long history of alcohol misuse (mean age: $53 \pm 8$ year; AD age of onset: $23 \pm 12$ year). Cases of WKS with a medical history of brain injuries or concomitant neurodegenerative disorders were excluded from the study. All patients and controls were selected to be of British ancestry. After complete description of the study to the subjects, written informed consent was obtained. All 32 controls were screened for an absence of mental disorders and for drinking below the alcohol limits recommended by the Royal College of Psychiatrists, [Royal College of Psychiatrists, 1986]. They were also selected for a negative history of alcoholism and a negative history of alcoholism in their first degree relatives. Five frozen brain samples of alcoholic WKS patients (4 males; 1 female) with a neuropathologically confirmed diagnosis were obtained from the New South Wales Tissue Resource Centre, University of Sydney, Australia.

Ethical permission for this research project was obtained from the University College London Medical School Clinical Investigations Panel which has been updated in 2004 with multicentre research ethics committee (MREC) approval. Information stored on computer was registered under the UK Data Protection Act 1984.

Total genomic DNA was extracted from venous blood samples using standard protocols. DNA was extracted from the cerebellum of the autopsied brains using the Puregene DNA Isolation kit (Gentra Systems, Minneapolis, MN). Fifty nanograms of genomic DNA was amplified by polymerase chain reaction (PCR) with oligonucleotide primers using standard methodology. Mutation screening was performed

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using the Li-Cor sequencing system. The method involved direct sequencing of PCR products amplified from genomic DNA. The promoter region, six exons and 100 bases within each end of all the introns were amplified with M13 tailed PCR primers. Only Exon 6 and the flanking regions, in which the WKS genetic variants had been found, were sequenced in the control group. Primer sequences are shown in Table I.

PCR products were sequenced simultaneously in both directions with the SequiTherm Excel II DNA sequencing kit (Epicentre, Madison, WI) using two M13 fluorescently labeled oligonucleotide primers. The LiCor sequencers allow simultaneous bidirectional sequencing of both strands of DNA, so that the presence of a base pair change can be checked by observing the reverse sequence run on the same gel with a different wavelength dye.

The promoter region and all six exons including the 3′ UTR region of Exon 6 were sequenced in our sample of 30 alcoholic WKS patients (27 males, 3 females) and in 32 healthy controls.

The sample gives a power of 0.32 to detect a disease allele frequency difference of 10% in 30 cases and 30 controls with significance of $P = 0.05$ with Fishers Exact test. If there was a 30% difference in allele frequency between cases and controls then the sample would give a power of 0.91 to detect a difference at $P = 0.05$.

We detected three different polymorphisms in the 3′ UTR region of Exon 6. One patient and none of the controls, carried a base pair change (T/A) at position +3390 of the gene (c. 3390T>A). One patient and none of the controls carried a deletion at position +2657 of the gene (c. 2657delT). In three patients and in five controls a substitution (A/G) was mapped at position +2344 (c. 2344A>G). None of these variants has been described in databases or in the scientific literature before.

TABLE I. Forward (FP) and Reverse (RP) Primers' Sequences (the Sequence in Italics Corresponds to the M13 Forward and Reverse Tails)

| | Oligonucleotides' sequence |
|---|---|
| Promoter region | |
| FP | *cacgacgttgtaaaacgac*cgcttttggagtggagtt |
| RP | *ggataacaatttcacacagg*tcggcaagaaccagcatt |
| Exon 1 | |
| FP | *cacgacgttgtaaaacgac*acaagtgcctgaccctcact |
| RP | *ggataacaatttcacacagg*tttctcggtcctctcttcctc |
| Exon 2 | |
| FP | *cacgacgttgtaaaacgac*ccaagagggagtttgctgtt |
| RP | *ggataacaatttcacacagg*tcaaggttgagatctttgaggt |
| Exon 3 | |
| FP | *cacgacgttgtaaaacgac*tgggcctgtaaattgctttc |
| RP | *ggataacaatttcacacagg*caaatttgggaggggtgaat |
| Exon 4 | |
| FP | *cacgacgttgtaaaacgac*cctcccatttgcctcattta |
| RP | *ggataacaatttcacacagg*caaggccctccataatcttg |
| Exon 5 | |
| FP | *cacgacgttgtaaaacgac*tcaccctgatcaagtcacaca |
| RP | *ggataacaatttcacacagg*gttggaaaggcaattgacag |
| Exon 6 | |
| FP | *cacgacgttgtaaaacgac*tcaagtggctgctgtgaagt |
| RP | *ggataacaatttcacacagg*gctgtgatgctgctttgtgt |
| FP | *cacgacgttgtaaaacgac*agcaactgcctggatgt |
| RP | *ggataacaatttcacacagg*cacaatgggaaccaagaaca |
| FP | *cacgacgttgtaaaacgac*aaatgcaggaatcacatctatcc |
| RP | *ggataacaatttcacacagg*tcactggcaatatttgttctgg |
| FP | *cacgacgttgtaaaacgac*cctcaaacagcacttttctactt |
| RP | *ggataacaatttcacacagg*gaattgcattagtccaagg |
| FP | *cacgacgttgtaaaacgac*aagtagcaaccttcattttacattc |
| RP | *ggataacaatttcacacagg*caagttagaccaggggaag |
| FP | *cacgacgttgtaaaacgac*atcctggctaacaccgtgaa |
| RP | *ggataacaatttcacacagg*ccctggtctaacttgccta |

Although a genetic component in the pathogenesis of WKS has been postulated since the late seventies, very few genetic studies have been carried out. In this research we attempted to evaluate the potential role of genomic variants of the high affinity transporter in the pathophysiology of the WKS. All the genetic variants detected in the WKS patients and in the controls occurred in the 3′ UTR of the Exon 6 of the *SLC19A2* gene.

Several authors have postulated that the 3′ UTR is equally important as the 5′ region in terms of gene regulation [Duret and Bucher, 1997]. Recent reports have shown that the 3′ UTR regions of certain genes can regulate gene expression post-transcriptionally and therefore, mutations in this region should be sought as much as in any other part of the gene [Inoue-Murayama et al., 2002; Kim et al., 2003]. We can hypothesize a similar role for the 3′ UTR of the *SLC19A2* gene. We have found a total of three new sequence variations in the WKS sample. Two of these were not found in the control group. Inferring function and abnormal function of these variants from the position they occupy in the gene is complex and the literature encourages caution in dismissing or implicating an effect from any base pair changes in any given gene. The small sample size precludes any firm inferences about the possible functional significance of these genetic variants in WKS. The fact that a deletion and a base pair substitution were only found in cases warrants further investigation in larger samples of WKS cases.

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