

Psychopathology and Personality Traits in Patients with Treated Wilson Disease Grouped According to Gene Mutations

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Abstract

Wilson disease (WD) is a recessively inherited copper storage disorder mainly affecting liver and brain. Genotype/phenotype correlations have been reported but as yet not regarding psychic symptoms. Our aim was to investigate if a correlation might exist between genotype and phenotype concerning psychopathology and/or personality traits in patients with treated WD. Nine homozygous and three compound heterozygous Swedish patients were retrospectively investigated, representing four different mutation settings. Psychopathological symptoms were studied using the Comprehensive Psychopathological Rating Scale (CPRS), personality traits using the Karolinska Scales of Personality (KSP) and mutations were analyzed by manifold sequencing. Psychopathological symptoms: Patients with the Trp779Stop mutation had the lowest scores on the total CPRS, due to less pronounced reported CPRS items, as compared to the other three groups of patients. Compound heterozygotes for the His1069Gln/Arg1319Stop mutation showed the highest total CPRS scores. Personality traits: Patients homozygous for the Trp779Stop and the Thr977Met mutations had high scores on Psychopathy related scales whereas patients with His1069Gln/Arg1319Stop mutations had the lowest scores on these scales. Serum ceruloplasmin levels were undetectable in all patients with the Trp779Stop and Thr977Met mutations. The results show a trend towards a genotype/phenotype correlation regarding psychopathological symptoms and personality traits in treated patients with WD. If replicable, these results might contribute to the elucidation of the possible clinical importance of functionally deleterious gene mutations in WD psychopathology and personality traits.

Introduction

Wilson disease (WD), hepatolenticular degeneration, is an autosomal, recessive disorder of copper metabolism. The prevalence, as described in various patient materials, ranges from 1 in 30,000 [1] to 1 in 100,000 [2] live births. The disease-causing gene, which is localized to chromosome 13 band q14.3, encodes a membrane bound, copper-transporting P-type ATPase, ATP7B [3–5]. Mutations in this gene result in insufficient biliary excretion of copper as well as reduced incorporation of copper into ceruloplasmin. As a result of the positive copper balance, copper accumulates first in the liver and later in the brain and other organs. Excess copper is toxic by inducing free radical formation, cell injury, inflammation and finally cell death. It is also harmful to mitochondria and inhibits a large number of enzymes.

Typical morphological abnormalities in the basal ganglia can be detected using

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Magnetic Resonance Imaging (MRI) of the brain, and have been found to correlate to clinical symptoms [6,7]. Upon examination of the brain, the distribution of neuronal damage has been found to be highly variable, including structural abnormalities in corpus striatum and a certain degree of brain atrophy [1,8].

The disease often presents in the late teens or twenties but has been described in patients as early as 3 or as late as 70 years of age [9,10]. WD may present under a variety of clinical conditions, most commonly as liver disease and/or neurological/neuropsychiatric disturbances. Four clinical subgroups: hepatic, neurological, mixed (hepatic and neurological) as well as an asymptomatic group were described by Denning and Berrios [11] and later verified by Oder *et al.* [12] Four main neurological syndromes have been described: parkinsonian, pseudosclerotic, dystonic and choreic [13].

Neurologic WD is a progressive movement disorder and the most common presenting symptoms are dysarthria, dysphagia, apraxia and tremor-rigidity syndrome. Psychopathological symptoms can include reduced performance in school or at work, depression and psychosis. The diagnosis is usually made on the basis of clinical findings and/or Kayser-Fleischer corneal rings and laboratory abnormalities, e.g. low serum ceruloplasmin levels and increased amounts of urinary and liver copper [14]. Without treatment, WD is fatal, but with proper therapy, the disease progress can be halted and symptoms often improve. The treatment is aimed at removing excess accumulated copper and preventing its reaccumulation and it has to be lifelong. Three options are available for drug therapy in WD: chelating agents (penicillamine, trientine), zinc and tetrathiomolybdate [1,15,16]. Outcome is mainly determined by the amount of damage that has occurred before the institution of treatment. In patients with irreversible severe liver damage, liver transplantation is indicated and the only option in the presence of fulminant liver failure [17].

In earlier studies, we found that patients with WD have pronounced psychopathology and furthermore that this persists despite the fact that the patients have received treatment for WD for several years [18,19]. It has been claimed that the psychiatric symptomatology is related to neurological symptoms [11,12], but we were unable to demonstrate that patients with predominantly neurological symptoms differ from patients with mostly hepatic symptoms regarding psychopathological symptoms, as measured by means of the Comprehensive Psychopathological Rating Scale (CPRS) [18]. Furthermore, the differences between patients with mainly neurological symptoms and patients with mostly hepatic symptoms were small regarding personality traits, as determined by means of the Karolinska Scales of Personality (KSP) [19].

We have previously reported our analyses of the coding sequences of *ATP7B* in 24 families with WD in Sweden [20]. Sixteen different mutations were found to account for 92% of the mutant genes. Genotype-phenotype correlation has not been proven with certainty, but the most common mutation in Central and Northern Europe (His1069Gln) seems to result in a fairly late age of disease onset and a preponderance for neurological symptoms [21 and ref. therein, and 22]. Mutations causing severe protein defects seem to result in an earlier onset of the disease

[23,24] and Panagiotakaki *et al.* found significantly lower median ceruloplasmin levels in patients with nonsense and frameshift mutations as compared with missense mutations [23]. The impact of yet other genes on the WD phenotype was suggested by Schiefermeier *et al.* [25] and Merle *et al.* [26].

When searching the literature, we were unable to find publications attempting to make correlations between WD genotype and phenotype concerning psychopathological symptoms and personality traits. Thus, in the present study we tried to elucidate whether such a correlation might exist in patients with treated WD. In addition, genotype-phenotype correlations regarding age and symptoms at diagnosis, including serum ceruloplasmin levels, were investigated.

Materials and methods

Patients

Twenty-six patients from 24 families, with conventionally diagnosed WD (all fulfilling a score of at least 4, before mutation analysis, as proposed by Ferenci *et al.* [14] and at least one mutation in *ATP7B* [20]), were investigated in conjunction with their regular visits to the Department of Internal Medicine at the Uppsala University Hospital, from March 1996 through September 1998. The study was approved by the Ethics Committee of the Medical Faculty at Uppsala University.

Out of the six homozygous mutations, three were selected because they were represented by three patients each. Out of the compound heterozygous mutations, the only pair that was seen in three patients was selected for comparison. All selected patients except one, (A2), who was of Latin American origin, came from the same geographic area, i.e. Sweden. Patient data including sex, age and clinical presentation at disease onset and at interview, Kayser-Fleischer rings, serum ceruloplasmin levels, exons and mutations of the altogether twelve patients are presented in Table 1 and Figure 1. One patient, (A3), was diagnosed in her presymptomatic stage and is on medication since age 7. Her sister died of hepatic failure at 10 years of age. Two patients, (D2 and D3), are siblings.

At the time of investigation, all patients were in a copper-depleted state (as defined by normal urine and serum copper levels and normal urine copper after trientine provocation) receiving pharmaceutical treatment for WD, except for one patient, (D2), who had been liver transplanted in 1987 due to poor compliance. Four patients were on D-penicillamine (A2, A3, B1 and D1), five had triethylene tetramine dihydrochloride (trientine) (A1, B3, C1, C2, C3), one was on zinc acetate (B2) and one had both trientine and zinc (D3). Patient A1 presented with hypersalivation and a gait disturbance. After initiation of trientine he rapidly deteriorated to a wheelchair dependent state with severe contractures and anarthria. His intellect is spared and he communicates through a voice computer. He was treated with L-dopa and patient B2 had fenytoin treatment but none of the twelve subjects received psychotropic drugs. Their mean age was 37.5 ± 9.8 years (range 27–56) and the mean duration of disease from onset was 19.4 ± 7.7 years. Patient A3 is not included in

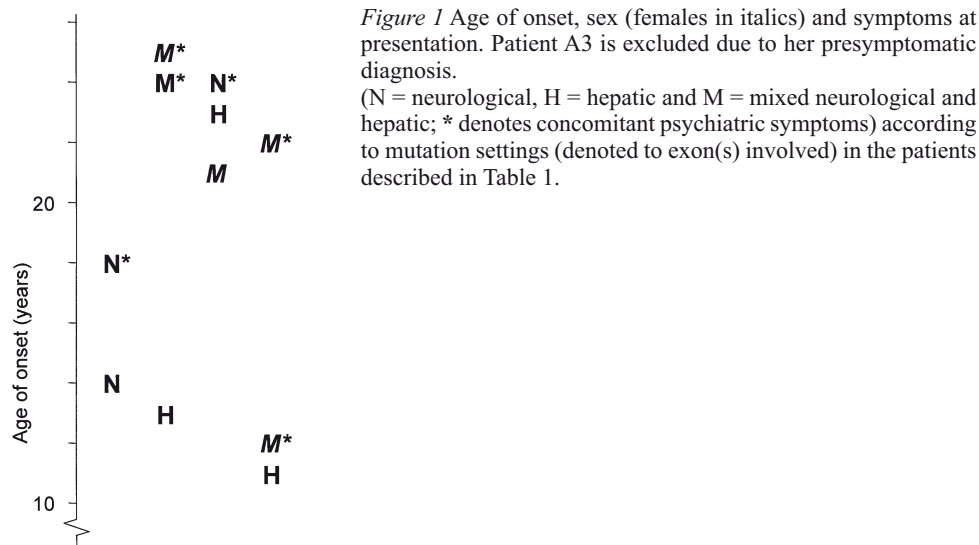


Figure 1 Age of onset, sex (females in italics) and symptoms at presentation. Patient A3 is excluded due to her presymptomatic diagnosis. (N = neurological, H = hepatic and M = mixed neurological and hepatic; * denotes concomitant psychiatric symptoms) according to mutation settings (denoted to exon(s) involved) in the patients described in Table 1.

these figures as she was diagnosed by family screening. She is also excluded from Fig. 1. Clinical data, psychopathology, neurological impairment, MRI of the brain as well as *ATP7B* gene mutations have previously been described in sub-samples of the included patients [6,18,20,27].

Manifold sequencing

Genomic DNA was isolated from whole blood collected in EDTA according to standard procedures. Each of the 21 exons (in total 4.4 kilobases) was amplified using PCR (polymerase chain reaction). Exon 2, 1234 base pairs, was amplified in four overlapping fragments. Primers were designed using the OLIGO program (Research Genetics) from adjacent intron sequence data. Sequencing was performed using a solid support, now available as AutoLoad Solid Phase Sequencing Kit (GE Healthcare, Uppsala, Sweden). For primer data and detailed procedures see Waldenström *et al.* [20]. In our total patient material, 92% of putative disease causing mutations were found. The remaining eight percent could e.g. be attributed to mutations in extraexonic sequences such as in the promoter region. However, in the present study both mutations were detected in all patients.

Ceruloplasmin analysis

Serum ceruloplasmin was analyzed at the Department of Clinical Chemistry, Uppsala University Hospital, by rate nephelometry utilizing a Beckman Array protein system (Beckman Instruments, Brea, CA, USA).

Reference values for ceruloplasmin were 0.2–0.4 g/L. Individual levels at diagnosis and at interview did not differ significantly.

Table 1 Description of the patient series concerning sex, serum ceruloplasmin levels (cpl), age at disease onset/duration of WD at interview since diagnosis (years), Kayser-Fleischer (KF) rings at onset and at interview, symptoms at onset, neurological symptoms at onset/and at interview, exons and mutations in ATP7B.

	Sex	Cpl	Age at onset/ duration of WD	KF rings at onset/ at interview	Symp- toms at onset	Neurologic symptoms at onset/at interview	Exons	Mutations
A1	M	<0.03	18/15	+/-	N P	Dystonia, hypersalivation/ severe dystonia, anarthria, contractures	8	Trp779Stop
A2	M	<0.03	14/12	+/not done	N	Slight dysarthria, impaired fine motor function/ slight dysarthria, impaired fine motor function	8	Trp779Stop
A3	F	<0.03	Presymptomatic	-/-	Family screening	-/-	8	Trp779Stop
B1	F	<0.03	25/32	+/-	H N P	Severe dystonia, dysarthria/dysarthria	13	Thr977Met
B2	M	<0.03	24/7	+/-	H N P	Epilepsia, dystonia/slight tremor	13	Thr977Met
B3	M	<0.03	13/17	-/-	H	-/-	13	Thr977Met
C1	F	0.19	21/27	+/-	H N	Tremor, dysarthria/impaired fine motor function	14	His1069Gln
C2	M	0.04	24/18	+(+)	N P	Dystonia/slight dysarthria	14	His1069Gln
C3	M	0.17	23/15	-/-	H	-/-	14	His1069Gln
D1	F	0.10	22/30	+/-	H N P	Slight tremor/-	14/19	His1069Gln/Arg1319Stop
D2	F	0.12	12/23	+/-	H P	-/-	14/19	His1069Gln/Arg1319Stop
D3	M	0.06	11/21	+/-	H	-/-	14/19	His1069Gln/Arg1319Stop

M denotes male, F female, N neurologic, P psychiatric, and H hepatic

Clinical assessment

The Comprehensive Psychopathological Rating Scale (CPRS) expert rating

The Comprehensive Psychopathological Rating Scale (CPRS), comprises 65 items (40 reported items and 25 observed items) of psychopathological symptoms [28]. One item is a global measurement of illness and one item takes into account the assumed validity of the rating performed, graded 1–3, where 1 means poor and 3 represents good validity. All 65 items of psychopathological symptoms are colloquially formulated, and staged from 0 to 3 by half point steps, where 0 indicates absence of the particular symptom. A rating of 1 is a description that could apply to a pathological deviation from the individuals own norm, but might equally well be considered a normal variation in a group of people. A rating of 2 clearly indicates pathological symptoms and 3 indicates the most severe degree. Many sub-scales have been derived from the CPRS [28]. Three such sub-scales have been used in the present study; a 10-item Brief Anxiety Scale (BSA) [29], a 10-item depression rating scale – the Montgomery-Åsberg Depression Rating Scale (MADRS) [30], and an 8-items Obsessive Compulsive Symptoms Scale (OCD) [31].

The Karolinska Scales of Personality (KSP)

The Karolinska Scales of Personality (KSP) is a self-reported inventory comprising 135 questions grouped into 15 scales [32]. According to Schalling the 15 scales can be grouped in the following way: 1. *Impulsivity, sensation seeking and social withdrawal scales* [33]: Impulsiveness, i.e. acting on the spur of the moment, non-planning; Monotony Avoidance, i.e. avoiding routine, need for change and action; Detachment, i.e. avoiding involvement with others, withdrawn. 2. *Psychopathy versus conformity scales*: Socialization, i.e. positive childhood experiences, good school and family adjustment; Social Desirability, i.e. socially conforming, friendly and helpful. 3. *Anxiety-related scales*, a) Nervous tension and distress: Somatic Anxiety, i.e. autonomic disturbances, restlessness, panicky; Muscular Tension, i.e. tense and stiff, b) Cognitive-social anxiety: Psychic Anxiety, i.e. worrying, anticipating, lacking self-confidence, sensitive; Psychasthenia, i.e. easily fatigued, feeling uneasy when urged to speed up and when facing new tasks; Inhibition of aggression, i.e. non-assertive, sad rather than angry when scolded, cannot speak up. 4. *Hostility-related scales*: Suspicion, i.e. suspicious, distrusting people's motives; Guilt, i.e. remorseful, ashamed of bad thoughts. 5. *Aggressivity-related scales*: Indirect Aggression, i.e. sulking, slamming doors when angry; Verbal Aggression, i.e. getting into arguments, telling people off when annoyed; Irritability, i.e. irritable, lacking patience. The scales have been demonstrated to have long-term stability [34].

The raw scores of KSP were transformed into T-score (Mean = 50 and SD = 10) on basis of a random sample of normal controls (200 men and 200 women). Thus, 50 means a normal value while 60 is one SD above the mean and 40 is one SD below the mean.

Results

Genotype-phenotype correlation in patients homozygous or compound heterozygous for WD gene mutations

Three groups of patients homozygous for three different mutations: group A in exon 8 (Trp779Stop), group B in exon 13 (Thr977Met) and group C in exon 14 (His1069Gln), and in addition, group D, with patients compound heterozygous for His1069Gln and Arg1319Stop, were characterized according to age and symptoms at disease onset and at interview, respectively, and serum ceruloplasmin levels, (Table 1 and Figure 1). The four groups, A–D were compared with respect to their psychopathological symptoms determined by means of CPRS (Table 2 and Figure 2) and regarding their personality traits by means of KSP (Table 3 and Figure 3). The assumed validity of the CPRS test was at the highest level in the majority of the patients (3 points). In one patient, A1, the psychiatric rating was difficult due to anarthria, severe dystonia and contractures.

All patients with the His1069Gln mutation were older than 20 years of age at disease onset, 2/3 with neurological symptoms in combination with hepatic symptoms (C1), psychiatric symptoms (C2) and 1/3 with hepatic symptoms only (C3). Patients with mutations Trp779Stop and Thr977Met all had undetectable ceruloplasmin levels (less than 0.03 g/L which is the lower detection limit of the currently used method) whereas the His1069Gln homozygous or heterozygous patients all had measurable levels. It is noticeable that the patients with psychiatric symptoms at presentation were older than the others, implying that psychiatric symptoms are a late phenotypic manifestation (Table 1 and Figure 1).

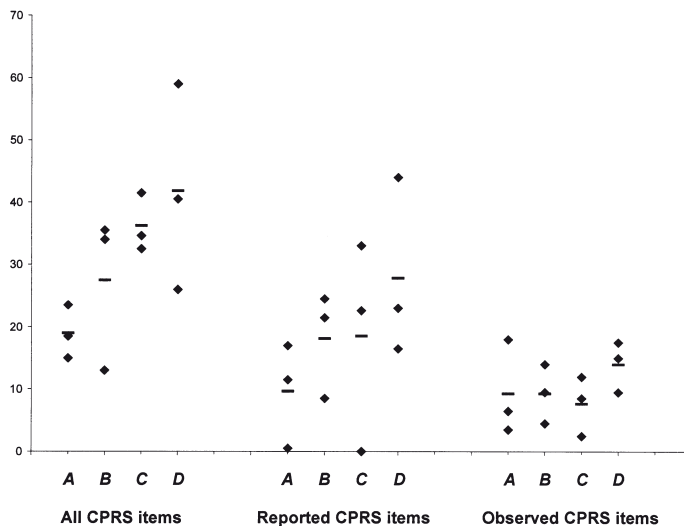


Figure 2 Symptomatology as estimated by means of the Comprehensive Psychopathological Rating Scale (CPRS) in patients with treated WD with four separate mutations (N = 12) A = Trp779Stop, B = Thr977Met, C = His1069Gln, D = His1069Gln/Arg1319Stop. — = Mean.

Table 2 Background data concerning Figure 2. CPRS data for individual patients with four separate mutations in the WD gene

	Mutations	Total CPRS	Reported items	Observed items	MADRS	BSA	OCD	NS	PS
A1	Trp779Stop	18.5	0.5	18.0	0.5	0.5	0	2.0	5.5
A2	Trp779Stop	23.5	17.0	6.5	4.0	6.5	5.0	1.5	4.0
A3	Trp779Stop	15.0	11.5	3.5	3.5	5.5	3.0	1.0	2.0
B1	Thr977Met	35.5	21.5	14.0	8.5	6.0	7.5	7.0	1.0
B2	Thr977Met	34.0	24.5	9.5	10.0	8.5	8.5	3.0	3.0
B3	Thr977Met	13.0	8.5	4.5	2.0	6.0	1.0	1.5	2.0
C1	His1069Gln	34.6	22.6	12.0	6.0	10.5	4.5	1.0	4.0
C2	His1069Gln	41.5	33.0	8.5	13.0	9.0	10.5	1.5	7.0
C3	His1069Gln	2.5	0	2.5	0	0	0	1.5	0
D1	His1069Gln/Arg1319Stop	26.0	16.5	9.5	4.5	4.0	2.5	3.5	3.0
D2	His1069Gln/Arg1319Stop	59.0	44.0	15.0	14.5	15.5	14.0	3.5	12.5
D3	His1069Gln/Arg1319Stop	40.5	23.0	17.5	9.0	7.0	4.5	5.0	6.0

Montgomery-Åsberg Depression Rating Scale (MADRS), Anxiety symptoms (BSA), Obsessive symptoms (OCD), Negative symptoms (NS), and Positive symptoms (PS)

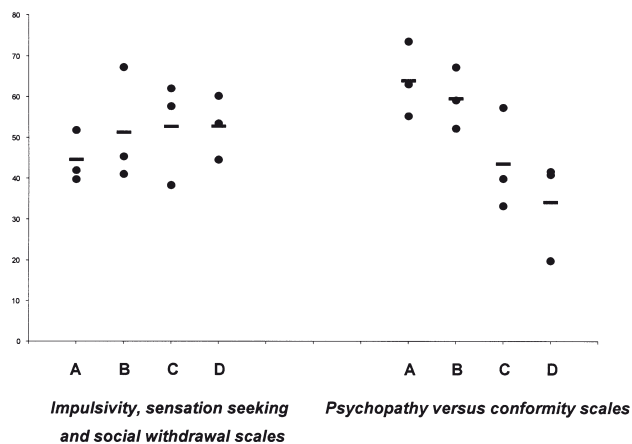


Figure 3 Personality scales according to the Karolinska Scales of Personality (KSP) in patients with treated WD with four separate mutations (N = 12) A = Trp779Stop, B = Thr977Met, C = His1069Gln, D = His1069Gln/Arg1319Stop, - = Mean.

Impulsivity, sensation seeking and social withdrawal scales are represented including: Impulsiveness, Monotony Avoidance and Detachment. *Psychopathy versus conformity scales* are represented including: Socialization and Social Desirability.

Psychopathological symptoms measured by CPRS

Concerning psychopathological symptoms, the homozygous patients with the Trp779Stop mutation had the lowest scores on the total CPRS, mainly due to less pronounced reported CPRS items and a low variability as compared to patients homozygous for the Thr977Met and His1069Gln mutations respectively, and to those compound heterozygous for His1069Gln/Arg1319Stop. The three patients with His1069Gln/Arg1319Stop mutations had the highest scores on the total CPRS as well as on reported and observed items (Table 2, Figure 2).

Personality traits measured by KSP

The patients who were homozygous for the Trp779Stop mutation, had the highest scores on the *Psychopathy vs. conformity* related scales; Social desirability and Socialization scales as well as the lowest scores on the *Impulsivity, sensation seeking and social withdrawal scales*; Impulsiveness, Monotony Avoidance and Detachment scales. The patients homozygous for the Thr977Met mutation also had relatively high scores on the *Psychopathy vs. conformity* related scales whereas patients with His1069Gln/Arg1319Stop mutations had the lowest scores on these scales, below those of healthy volunteers (Table 3, Figure 3).

Discussion

The patients in the present study were evaluated following long-term treatment for their WD, pharmaceutical as well as following liver transplantation to a copper depleted state but in the absence of treatment with psychotropic drugs. All patients had normal free serum and urinary copper at the time of evaluation. Despite this, the patients described in the present study deviated from healthy volunteers regarding several personality traits as well as psychopathological symptoms [18, 19].

Table 3 Background data concerning Figure 3. Data on separate KSP scales for individual patients with four separate mutations in the WD gene

	Mutations	Imp	MA	D	So	SD	SA	MT	PA	Ps	Inh Agg	Susp	Guilt	Ind Agg	Verb Agg	Irr
A1	Trp779Stop	39.7	51.8	27.9	63.3	83.8	39.2	49.9	31.3	30.3	44.2	43.1	44.5	31.6	28.8	36.5
A2	Trp779Stop	59.6	43.2	52.9	54.4	56.0	52.2	61.9	54.1	58.7	58.7	50.7	49.1	47.3	49.0	42.5
A3	Trp779Stop	36.0	58.3	41.4	53.9	72.1	47.7	47.8	57.5	38.0	55.4	49.5	50.0	51.0	43.7	44.9
B1	Thr977Met	45.0	30.9	60.1	58.1	60.1	43.2	37.9	40.0	52.1	53.8	40.8	44.4	48.4	33.6	27.1
B2	Thr977Met	44.6	37.0	41.6	60.8	73.7	49.9	52.5	63.9	80.8	62.7	37.8	36.0	37.9	25.0	32.3
B3	Thr977Met	70.1	60.3	71.3	56.9	47.6	43.6	75.2	49.8	48.3	56.1	43.1	44.7	47.6	52.8	36.4
C1	His1069Gln	63.9	61.1	61.2	36.6	43.2	67.2	58.2	59.5	55.4	56.5	54.0	44.8	50.4	57.9	49.2
C2	His1069Gln	58.7	66.6	47.6	23.4	43.1	59.9	62.1	56.7	71.5	58.1	55.0	52.6	65.9	55.0	53.4
C3	His1069Gln	30.1	41.8	43.0	64.0	50.6	41.0	42.3	40.8	29.4	43.1	33.5	39.9	42.4	43.8	32.6
D1	His1069Gln/Arg1319Stop	53.4	64.1	42.7	53.1	28.7	40.0	45.3	35.2	59.2	51.1	62.9	57.5	54.3	55.0	68.0
D2	His1069Gln/Arg1319Stop	70.0	59.4	54.2	9.06	30.5	64.4	79.1	58.9	70.3	73.1	67.2	62.7	71.3	48.1	48.7
D3	His1069Gln/Arg1319Stop	43.2	35.3	56.0	28.4	54.8	39.2	43.1	37.3	50.4	44.2	29.2	35.9	38.2	28.8	32.3

Data are presented as T-scores. For comparison healthy controls have a mean of 50 and a standard deviation of 10.

Imp = Impulsiveness, MA = Monotony Avoidance, D = Detachment, So = Socialization, SD = Social Desirability, SA = Somatic Anxiety, MT = Muscular Tension, PA = Psychic Anxiety, Ps = Psychasthenia, Inh Agg = Inhibition of Aggression, Susp = Suspicion, Ind Agg = Indirect Aggression, Verb Agg = Verbal Aggression, Irr = Irritability

Their total burden of psychopathological symptoms was found to be in the same range as that of patients with moderately severe to severe depressive disorders [35] and more pronounced than in patients with e.g. neurofibromatosis [36] or primary hyperparathyroidism [37].

In the WD literature, a number of publications can be found, where attempts have been made to find a correlation between genotype and phenotype regarding age of onset, severity and type of WD (hepatic, neurological, mixed) [21 and ref. therein, and 38, 39]. The interpretation of the results from these studies has encountered considerable difficulties since WD is rare, the patient materials are small but the number of mutations is large; more than 300 sequence variants are known at present (WD mutation database; <http://www.medicalgenetics.med.ualberta.ca/wilson/index.php>). Although the patients in the present investigation come from a cohort comprising approximately one third of the known Swedish patients with WD [2], the number of investigated subjects is low also in this study. Sixteen different mutations were described in the 24 families in Sweden [20], three of which in the homozygous setting and one in the heterozygous setting occurred in more than two patients. Beside genotype, additional factors like dietary copper intake, intestinal metallothionein inducibility as well as the capacity to overcome copper stress at the cellular level, e.g. via glutathione protein pathways, probably also influence the phenotypic variability in WD [40]. The existence of several other factors, such as e.g. *Ctrl* [41] and *Murr1* [42] and extrahepatic factors such as apolipoprotein (Apo) E [25] and prion-related protein (PrP) [26] genotypes add to the complexity of the phenotypic expression in WD. In the present study however we chose to focus on the possible relation between the *ATP7B* mutation and phenotype.

Mutations in the WD gene are known to result in a deficient copper transporting P-type ATPase and subsequently to the accumulation of copper mainly in the liver and brain. Although Thr977Met seems to be a conserved amino acid substitution, it has been shown to render ATP7B completely without activity in a yeast complementation assay [43]. Data from the present study show that all patients with the Thr977Met and Trp779Stop mutations had undetectable ceruloplasmin levels. The seriousness of the Trp779Stop mutation is supported by the findings of a severe neurological state in patient A1 (the only patient in our cohort who deteriorated in conjunction with start of chelation therapy with trientine) as well as the fatal liver failure of the sister (who died at 10 years of age) of the presymptomatic patient, A3. Furthermore, a serious disease pattern was also seen in patients in the Thr977Met group (B1 and B2). The results concerning ceruloplasmin are in accordance with the previously mentioned findings by Panagiotakaki *et al.* [23]. Trp779Stop is postulated to give a prematurely terminated protein, without functional domains. This mutation is in exon 8, that has been shown to be absent in an alternative splice variant, where also exons 6, 7 and 12 are lost. This alternative splice variant has a normal reading frame and gives rise to a shorter protein. Yang *et al.* have shown that this protein is mainly present in the cytosol whereas the normal ATP7B is in the *trans*-Golgi apparatus [44]. These alternative splice variants of *ATP7B* are expressed in the brain [45] and could be an explanation for differences in central

nervous system manifestations, as tentatively shown in this study. Whether this shorter protein has any functional activity in human is unknown, so the relationship between personality traits and psychopathology and the function of a truncated WD gene product can, however, only be speculated on at present.

In our study, His1069Gln homozygosity was seen in patients who became symptomatic at a later age. We have though recently seen patients with the same mutation setting who became symptomatic already in their first decade, substantiating the findings by Vrabelova *et al.* [38] that this is a mutation that can give very diverse phenotypic expressions.

It has been reported that patients with WD express an abnormal metabolism of neurotransmitters, particularly dopamine, noradrenaline and serotonin [8, 46–49]. Neuroimaging findings in WD suggest a complex pathogenesis involving both afferent nigrostriatal dopaminergic projections [50], loss of a dopamine transporter [51] and efferent nigrostriatal dopaminergic projections (loss of D2 receptors in striatum) [27,52] as well as an alteration of presynaptic serotonergic transporters (SERT) availability [49]. Eggers *et al.* found that the degree of depression symptoms as determined by means of the Hamilton rating scale for depression correlated negatively with the density of presynaptic SERT in 23 patients with WD [53]. According to our findings, the KSP scales that mainly differ in the patients for the *APT7B* gene mutations include *Psychopathy vs. conformity* related scales as well as *Impulsivity, sensation seeking and social withdrawal scales*. Socialization, often found to be extremely low in patients with antisocial personality disorder, was linked to deficiencies in the serotonergic systems [54]. It is also of interest that Socialization could be linked to deficiencies in the glucose metabolism [55], since a reduced glucose metabolism in all brain regions except the thalamus has been demonstrated in patients with WD [50]. The withdrawal scale, i.e. Detachment has been found to correlate negatively with D2 receptor density and dopamine transporter binding in healthy humans [55,56]. Autonomic disturbances included in the Brief Anxiety Scale (BSA), have been related to disturbances in the noradrenergic systems [57]. Impulsiveness [58], Aches and Pain [59] and Hostility [60] seem to be related to disturbances in the serotonergic systems. The Negative symptoms might be related to disturbances in the dopaminergic systems [34, 61].

The results from the present study might suggest a correlation between genotype and phenotype in WD but obviously, larger patient materials are required in order to obtain statistically significant results. However, our results support the notion that patients with WD resulting from the homozygous stop codon Trp779Stop in exon 8 and the Thr977Met mutation in exon 13 respectively seem to present with a different phenotype as compared to patients with the other mutations. Patients who were homozygous for the Trp779Stop mutation but also patients with the Thr977Met mutation tended to differ the most, as concerns psychopathology and personality traits from patients with the other mutations investigated. All patients with the Trp779Stop and Thr977Met mutations also had undetectable serum ceruloplasmin levels, further supporting the functional seriousness of these two mutations. Further studies are required to support these observations but in the case of a rare disease

like WD every single contribution could eventually contribute to large meta-analyses in order to obtain reliable data on the issue of correlations between genotype and phenotype in WD.

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