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Editorial

Role of genotyping in Wilson's disease ☆

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The molecular characterization of hereditary diseases has given rise to major advancements in the understanding of pathophysiology in human disease. Among these inborn defects of metabolism, Wilson's disease (WD) serves as an excellent model to study processes such as copper metabolism, oxidative stress, neurodegeneration, psychiatric disease, acute and chronic liver failure, as well as hepatocarcinogenesis. WD results from a hepatic copper transporter defect, which is responsible for translocating copper into the bile [1,2]. It therefore represents a genetic copper storage disease, whose clinical features include acute, acute on chronic, and chronic liver disease, hemolysis, but also neuropsychiatric symptoms too [3]. Since the first description of WD patients by Kinnear Wilson in 1912 as hepatolenticular degeneration there have been many clinical reports highlighting the phenotypical heterogeneity of this disease [4,5]. The ophthalmologists Kayser and Fleischer reported already in 1902 and 1903, respectively, on corneal rings, which were subsequently linked to WD [6,7]. These so called Kayser-Fleischer rings illustrate a common characteristic finding especially in WD patients presenting with neurological disease. WD represents an orphan disease with a prevalence estimated to be about 1:30,000 to 1:100,000; it is more common in some isolated regions [3,5].

The genetic trait was demonstrated to be autosomal recessive inherited [8]. In 1985 Frydman et al. assigned the gene for WD to chromosome 13 [9]. In 1991 the groups of Tanzi and Bull identified the *ATP7B* gene as the underlying genetic cause (MIM# 277900) [10,11]. This gene encodes a transmembrane copper-transporting P-type ATPase (ATPase 7B) consisting of 1465 amino acids, with six copper-binding domains, eight transmembrane domains (Tm), a transduction domain converting the energy of ATP hydrolysis to cation transport, and a phosphorylation domain [12]. Since then, more than 400 mutations have been identified, which have been reported in part by the Diane W. Cox databank (http://www.medicalgenetics.med.ualberta.ca/wilson/index.php). Furthermore, the *ATP7B* gene provides many additional SNPs, whose clinical relevance has to be determined in the future. This mutational diversity in WD underlines the genetic heterogeneity, thus limiting the introduction of simple and rapid genetic testing for WD [13–22]. Furthermore, disease causing mutations on both alleles can only be identified in about 80% of studied patients. Interestingly, in children presenting with hepatic WD the chance of identifying disease causing mutations is up to 100%. Regional clustering of mutations has been very well established [23–29]. This makes it possible to use genetic diagnostics for regional screening programs.

Affected subjects within a family usually present with a similar phenotype, but even within families phenotypical variety occurs [30–32]. In addition to the broad spectrum of clinical manifestations, the age of onset has been observed to range from 2 y.o. up to 71 y.o. [33,34]. There are increasing reports on genotype-phenotype relationship in WD, which are very important to define the role of genotyping [35–44]. The overall impression from these reports is that the more striking the *ATP7B* mutation is affecting the gene product

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ATPase 7B, the earlier the onset of disease, the greater the likelihood of the presentation of hepatic manifestation. However, variability even within the same families carrying the same genotype exists. The substitution mutation c.3207C>A (p.H1069Q) is identified more commonly in adult patients with neuropsychiatric manifestations, but can also occur in children with acute liver failure [45]. However, very little is known with regard to children.

Nicastro et al. report, in this issue of the Journal, on the genotype–phenotype relation in Italian children presenting with WD [46]. They retrospectively studied 58 patients derived from 47 unrelated families. SSCP and direct sequencing using a 2-step approach (1st: exon 5, 6, 8, 10, 12-19; 2nd: remaining exons in case of lack of identified disease causing mutations on both alleles) revealed 34 different mutations accounting for 91.3% of studied WD alleles. The majority of the children presented with hepatic symptoms (86%). Kayser-Fleischer rings were detectable in 5 children. Mutations were grouped in homozygotes and compound heterozygotes for missense mutations versus homozygotes for nonsense and frameshift mutations. The latter group of patients presented with lower serum ceruloplasmin and serum copper concentration at onset of disease compared to the group of patients carrying missense mutations on both alleles. Normalization of ALT serum concentration before and under therapy (zinc, penicillamine) was used as criteria for effective treatment response, which was achieved more commonly in the group of missense mutations. The correlation between the age at diagnosis and the urinary copper excretion in this observational study highlights the problem of using this parameter in young children for diagnosing WD. Taken together, the authors succeeded to describe a large cohort of children presenting with WD in detail and related the phenotype to the genotype.

The data provided substantiates that the genotype plays a role to some extent in predicting the phenotype. However, there is also the information on two affected brothers carrying the same genotype but presenting with different phenotype (neurological versus hepatic manifestation). Homozygosity for c.3207C>A (p.H1069Q) occurs more likely in adults with neurological features, but here they were also detected in WD children. What additional factors may influence the clinical presentation of WD has still to be elucidated. Clearly, larger numbers of studied patients, ideally carrying a homozygous genotype, are required to determine modifier genes (www.eurowilson.org). This is a common problem in all association studies. Suggested modifiers just as the impact of apoE genotype or the HFE genotype have not been confirmed so far [47,48]. The role of the prion protein has also to be evaluated in larger cohorts [49,50]. The canine copper toxicosis gene COMMD1 (MURR1) seems to play no role in the clinical presentation of WD [51,52]. Genes e.g. involved in metal transport, oxidation or mitochondria metabolism serve as candidates for impacting WD. However, epigenetic regulation or non-genetic factors may also be involved in the presentation of WD.

Nicastro et al. detected mutations on both alleles in almost all children. The high mutational yield is in contrast to many reports on genetics in adults. This phenomenon may be explained, that the younger the age of onset, the more likely mutations will be discovered. However, SSCP and direct sequencing may also miss mutations. Even more interestingly, treatment response related to normalization of ALT was more likely to be observed in the analyzed group carrying missense mutations. The differentiation of missense mutations versus frameshift/nonsense mutations seems to be very valuable and may relate to residual functional activity of ATPase 7B in patients carrying missense mutations, whereas patients carrying frameshift/nonsense mutations will likely have no functional protein activity. Thus genotype may in future enable us to predict treatment efficacy; surely this needs to be confirmed.

Another important feature deriving from this study is that children present primarily with hepatic manifestations, however, neurological disease can occur. Furthermore, diagnosis for WD is limited in children, since characteristic findings such as Kayser-Fleischer ring or pronounced increase of urinary copper excretion is not usually helpful in this population. But this makes the case: (i) WD can manifest as early as 2 years of age, (ii) first manifestation can be acute liver failure and death, (iii) effective treatment is available, (iv) preliminary data imply that presymptomatic treatment prevents onset of WD. Taken together, there is a need for screening programs to minimize the disease burden of WD. Genetic testing has limitations, but seems to be the best tool to detect WD in children. Since this is a rare disease, a genetic screening program can only be justified once we develop simple, rapid, precise and non-invasive tests at a low cost. At this point, the identification of disease-causing mutations or informative haplotypes in WD would allow the screening of relatives, especially the screening of children. Thus genetic screening should be recommended, although e.g. the AASLD practice guidelines did not make clear recommendations on this topic [53]. Since effective treatment is available, newborn screening or screening within the first two years of life seems to be most appropriate. DNA chip technology may fulfil these criteria in the future. These technologies have to overcome the genetic heterogeneity in WD and mutational analysis may differ depending on the region. Current developments in genetic testing are promising for use in e.g. newborn bloodspot analysis or genomic DNA derived from buccal swaps [54,55]. Consequently, population-based genetic screening programs for Wilson's disease may become a reality in the near future.

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