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Molecular Testing for Chromosomes 1p and 19q Influences Clinical Decision in Glioma Treatment

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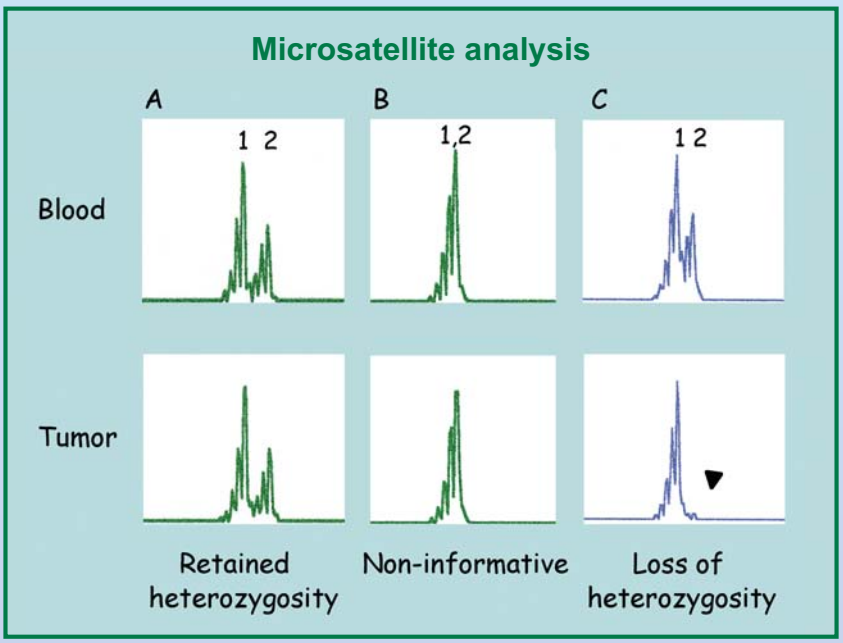
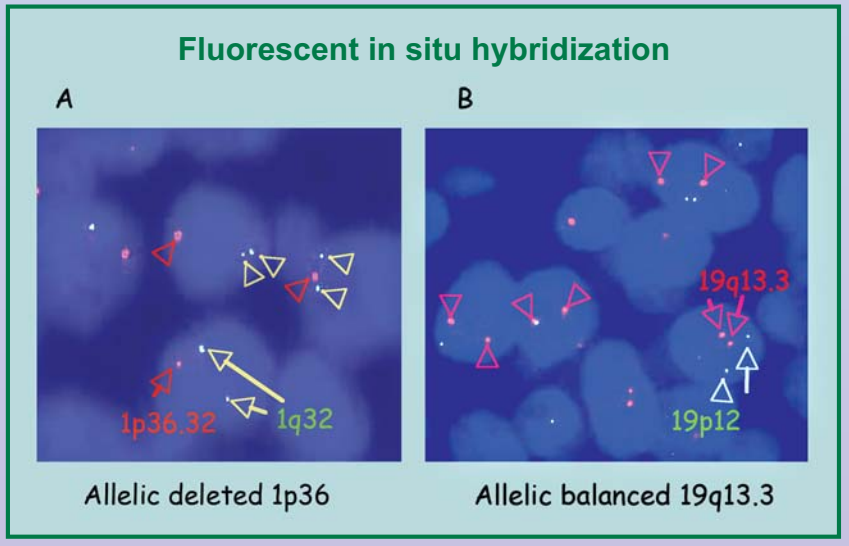
Molecular diagnostics is becoming an important ancillary tool for clinical management of human cancers. One notable example for such clinical application in neuro-oncology is the testing of combined allelic deletions of the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q) for prognosis and chemotherapeutic response in oligodendrogliomas.

Oligodendrogliomas are primary brain tumors, comprising up to 25% of adult gliomas. It has long been recognized that oligodendrogliomas behave differently from astrocytomas of similar grade, in that they are less aggressive with slower progression, and are responsive to chemotherapy consisting of procarbozine, lomustine (CCNU) and vincristine (PCV). Patients with oligodendrogliomas appear to survive longer than those with astrocytomas.[1] These observations have raised an important concern that stratification of patients with oligodendrogliomas from those with other glial subtypes is needed. Distinguishing glial subtypes based on nuclear and cellular morphology alone is, however, difficult and is subject to significant inter-observer variability. Moreover, there are no specific immunohistochemical markers available for differentiating oligodendrogliomas from astrocytomas. Genetic studies have demonstrated that ~70% of oligodendrogliomas carry 1p/19q codeletions. The oligodendroglial phenotype is highly associated with 1p/19q codeletions ($P<0.0001$), implicating that 1p/19q genotyping could serve as an adjunct diagnostic test for oligodendrogliomas [2]. More importantly, in 1999, Cairncross and colleagues were the first to demonstrate a strong correlation between 1p/19q codeletions and better overall patient survival ($P<0.001$) or sensitivity to PCV therapy ($P<0.001$) in patients with anaplastic oligodendrogliomas. [3] These observations have been confirmed by many major studies. Smith et al. reported that combined loss of 1p and 19q is a significant predictor of prolonged survival in patients with oligodendrogliomas, independent of tumor grade, and such correlation is not observed in patients with astrocytomas. [2] Fallon et al. further revealed that 1p/19q codeletions remain a significant predictor of survival in recurrent as well as primary oligodendrogliomas. [4] In addition, the 1p/19q codeletions are significantly associated with responses to other forms of therapy, including radiation and less toxic chemotherapeutic agents such as temozolomide.[5,6] Thus 1p/19q codeletions seem to define a 'genetically favorable' subset of oligodendrogliomas. Given these therapeutic and prognostic implications, 1p/19 genotyping has become a routine diagnostic test for oligodendrogliomas as well as tumors with oligodendroglial features.

The 1p/19q genotype can be evaluated by different molecular methods, with microsatellite and fluorescent in situ hybridization (FISH) analyses being the commonest ones employed by diagnostic laboratories worldwide. Both techniques are available in the PWH Molecular Diagnostics Laboratory. Microsatellite analysis is currently the preferred method used in 1p/19q genotyping because of its sensitivity and specificity in detecting deletions at multiple chromosomal loci in a single test and its fast turn-around time. Briefly, several polymorphic loci on chromosomes 1p36 and 19q13, regions with which clinical associations have been demonstrated, are interrogated for loss of heterozygosity (LOH) in microdissected tumor cells, with patient's peripheral blood served as constitutional control.



■ Microsatellite analysis detects allelic imbalances at polymorphic loci. In 1p/19q genotyping, at least 7 polymorphic loci on chromosomes 1p36 and 19q13.3 are evaluated for allelic loss in paired blood and tumor samples. A, a polymorphic locus shows allelic heterozygosity (i.e. alleles 1 and 2 bearing different number of tandem repeats) in DNA of constitutional blood sample, and retained heterozygosity is observed in tumor DNA. B, constitutional control displaying allelic homozygosity (i.e. both alleles carry identical number of tandem repeats) is regarded as noninformative. C, loss of heterozygosity refers to a polymorphic locus at which a deletion has converted the locus from heterozygosity to homozygosity. Arrowhead indicates a deleted allele in tumor DNA.



■ Dual-color interphase fluorescent in situ hybridization (FISH) detects DNA copy number change at specific chromosomal loci. Red signal indicates target locus (e.g. 1p36.32 or 19q13.3) and green signal represents reference locus (e.g. 1q32 or 19p12). A, one red (target) and two green (reference) signals are seen in tumor nuclei, indicating allelic loss of 1p36.3. B, two red and two green signals are detected, indicating allelic balanced 19q13.3.

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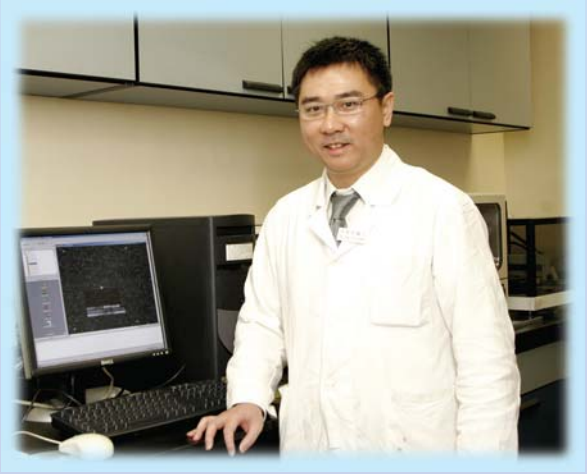
DNA-based diagnosis of Wilson Disease

Dr Ching-Wan Lam

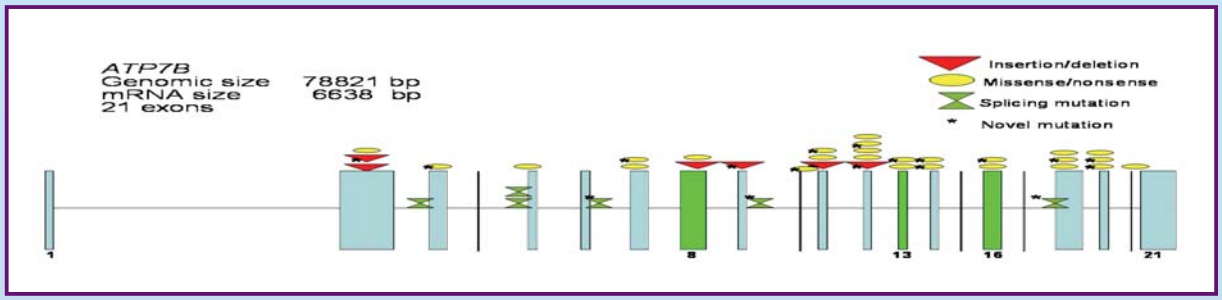


Wilson disease (WD) (MIM # 277900) is an autosomal recessive disorder of copper transport. Clinical manifestations of WD vary widely. The age of onset ranges from three to more than 50 years of age. The initial onset of symptoms can be hepatic, neurological, psychiatric or as an acute hemolytic crisis. The prevalence of WD has been estimated to be approximately 1 in 30,000 in the Caucasian population. Although the prevalence of WD in the Hong Kong Chinese has not been investigated, based on our local experiences, WD is common and is the most common inherited liver disease in Hong Kong. In addition, investigators in Japan have suggested that the prevalence of WD in Asians might be higher than that reported in the U.S. and Europe.

In 1993, the gene responsible for WD was identified, and the gene product was predicted to be a copper binding P-type adenosine triphosphatase. The *ATP7B* gene consists of 21 exons which span a genomic region of about 80 kb and encode a protein of 1465 amino acids. ATP7B is expressed primarily in the liver and kidney. The protein plays a dual function role in the hepatocyte. One role is biosynthetic, delivering copper to apoceruloplasmin within the Golgi network. The other role of ATP7B is to transport excess copper out of the cell and into the bile canaliculus for subsequent excretion from the body via the bile. ATP7B is localized in the trans-Golgi network of hepatocytes under low copper conditions, redistributes to cytoplasmic vesicles when cells are exposed to elevated copper levels, and then recycles back to the trans-Golgi network when copper is removed. Therefore, an *ATP7B* mutant will result in a reduction in the rate of incorporation of copper into apoceruloplasmin or a reduction in biliary excretion of copper, or both. For example, a WD mutant protein, Arg778Leu, has recently been shown to be extensively mislocalized, presumably to the endoplasmic reticulum. Defective biliary excretion leads to accumulation of copper in the liver with progressive liver damage and subsequent overflow to the brain, causing loss of coordination and involuntary movements. Deposition in the cornea produces Kayser-Fleischer rings, and accumulation in other sites causes renal tubular damage, osteoporosis, arthropathy, cardiomyopathy, and hypoparathyroidism.

The prognosis for WD patients is excellent with early treatment with D-penicillamine, trientine, or zinc salts, but early detection, monitoring, and treatment of presymptomatic patients is critical to prevent irreversible liver damage requiring transplant. As described earlier, biochemical and symptomatic signs are not specific enough for effective diagnosis of all affected individuals. In addition, the clinical and laboratory parameters are not sufficient to exclude the diagnosis of WD in patients with liver disease of unknown origin. In these two groups of patients, the exclusion of a diagnosis of WD by clinical and biochemical parameters is very challenging. Direct detection of the mutations causing WD will eliminate these problems. Direct mutation detection in diagnosis of presymptomatic sibs is particularly important because of the difficulty in distinguishing presymptomatic patients from heterozygotes.

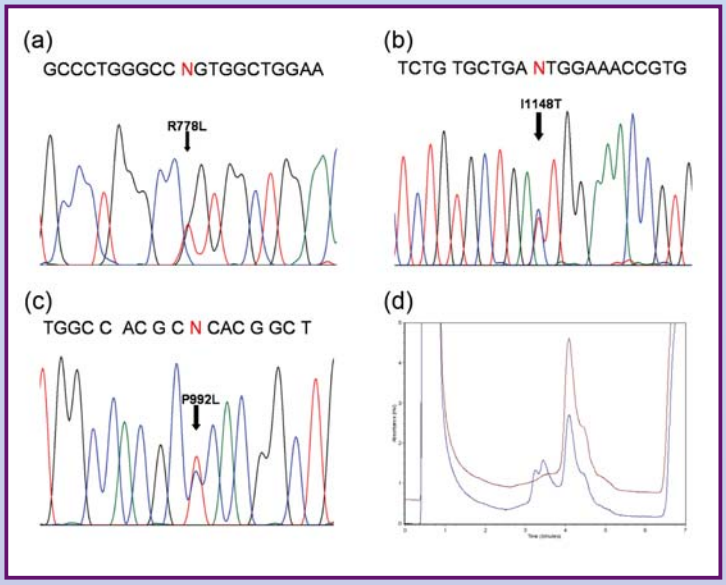
The most frequent *ATP7B* mutation in Caucasian patients is His1069Gln, which is found in 28-38% of all alleles, and the next most frequent is Gly1267Lys, which is found in 10%. No such mutations have so far been detected in Asian WD patients. This finding reveals that the mutation spectrum of the *ATP7B* gene shows a population-dependent distribution. No studies have yet been undertaken to elucidate the molecular basis of WD in Hong Kong Chinese. In one study performed in Northern Chinese using single strand conformational polymorphism analysis, however, less than 50% of WD patients had *ATP7B* mutations. [1] As a second locus for WD has not been reported, the result probably reflects the insensitivity of the screening method. In addition, the mutational spectrum of a disease-causing gene might be different between Southern and Northern Chinese, e.g. phenylketonuria. Therefore, the data cannot be used directly in our population. A study has been performed on Southern Chinese WD patients [2]. However, the



■ ***Fig. 1***
Distribution of the 38 mutations in the *ATP7B* gene.

investigators only screened 4 out of 21 exons of the *ATP7B* gene for mutations, and mutations were detected in less than 15% of WD patients. Neither of these studies has provided enough information to establish an easy and effective protocol for DNA-based diagnosis for WD in the Hong Kong Chinese population.

Toward this end, we have delineated the spectrum of mutations in the *ATP7B* gene in patients in Hong Kong. Sixty-two WD patients including 52 unrelated Chinese families together with 10 presymptomatic were recruited. The median of age at presentation was 18 years (range 4–50). We identified 38 different mutations in 52 probands (Fig. 1). Forty-six probands are compound heterozygotes and six probands were homozygotes. Interestingly, 16 mutations are novel. Over 50% of the mutations are located in 3 of the 21 exons of the *ATP7B* gene —exons 8, 13, and 16. The mutations, R778L, P992L, and I1148T are the three most common mutations in Hong Kong Chinese WD patients (Fig. 2).



■ ***Fig. 2***
Common *ATP7B* mutations. (a) R778L (b) P992L and (c) I1148T (arrows). (d) DHPLC of exon 13: *upper*: wild-type DNA; *lower*: P992L heterozygote.

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