

Commentary

Low-density oligonucleotide microarrays - A major step in Wilson's disease diagnosis

Wilson's disease (WD) is a monogenic disorder of copper accumulation caused due to mutation in the copper transporting ATPase gene *ATP7B*^{1,2}. WD follows an autosomal recessive mode of inheritance with a world prevalence of 1 in 5000 to 1 in 30,000 live births and a carrier frequency of 1 in 90³. Though a single gene disorder, mutation detection in WD has been difficult, mainly due to two reasons: (i) size of the *ATP7B* gene is large (>6.5 kb) with 21 exons, and (ii) besides the prevalent mutations, extensive presence of rare mutations throughout the entire gene. To screen the entire gene one has to undertake amplification and sequencing strategy encompassing 24-26 exonic regions including intron-exon boundaries^{4,5}. This requires substantial time and effort. The second problem is heterogeneity of mutations *i.e.* the occurrence of hundreds of rare (non-prevalent) mutations in various world populations. Moreover, there also exists a huge variability in the prevalent mutations in these populations.

Indian population vs major world populations

Among the data available for a very few major world populations, H1069Q and E1064A are the major founder mutants in Caucasians⁶, R778L and P992L among the East Asians that includes Japanese, Korean and Chinese populations⁷⁻⁹. Among patients from Brazil, c.3402delC mutation had the highest frequency (30.8%), followed by the missense change, c.2123T>C (p.L708P) (16.7%)¹⁰. The Indian population is highly heterogeneous, comprising four major linguistic groups *i.e.* Indo-European, Dravidian, Tibeto-Burman and Austro-Asiatic, and 4693 communities with several thousand endogamous groups (The Indian Genome Variation Database)¹¹. Due to high genetic heterogeneity in the Indian population, prevalent mutations for a specific sub-population might not be present in others,

even if they are subgroups of the same larger linguistic group.

The data for WD mutations from India are quite divergent reflecting the ethno-genetic diversity of this large country. None of the common mutations detected has a prevalence over 25 per cent of all *ATP7B* mutations. In patients from southern India, c.G3182A (p.G1061E) in 16 per cent and c.C813A (p.C271X) in 12 per cent accounted for major *ATP7B* mutations¹², whereas p.E122fs (10.6%) and C271X detected in western Indian patients were the prevalent ones¹³. Patients from eastern and northern India harboured five prevalent mutations. Three mutations c.813C>A (Cys271Stop), c.1708-1G>C (IVS4-G>C) (splice site mutation) and c.3182 GGG>GAG (Gly1061Glu) were found to be associated with 16, 8.5 and 8 per cent of all the mutant chromosomes^{5,14}.

To summarize, multiple studies from different zones of India suggest that detecting WD by screening an individual for one major founder mutation would not be fruitful. A comprehensive and efficient strategy to screen a panel of multiple mutations (both common and rare) in a single multiplex reaction would address the issue.

Genetic diagnosis of WD has a tremendous advantage

Disease onset varies from as early as age of 2 years to as late as the 7th decade of life¹⁵. Early or presymptomatic diagnosis can alleviate disease progression and in some cases entirely prevent onset of disease symptoms. However, there is no confirmatory biochemical or clinical tests available for early disease detection. In the last 10 years major effort has been made in genetic diagnosis of the disease. Roberts and Schilsky laid guidelines for WD diagnosis approved by the American Association for the Study

of Liver Diseases (AASLD)¹⁶. Besides, typical clinical symptoms, such as presence of Keyser Fleischer ring, low serum ceruloplasmin (<20 mg/dl) and 24 h urinary copper excretion (>40 µg), molecular testing to determine the mutation has been considered crucial for disease diagnosis¹⁶. Also, genetic testing can be carried out to determine the mutation status in neonates, much before the onset of any clinical symptoms. Further, detection of carrier status of an individual can ensure proper genetic counselling. To date, major advances have been made to identify the disease status in siblings of WD patients. Gupta *et al*^{14,17} have utilized heterozygous microsatellite markers and informative single nucleotide polymorphisms (SNPs) to identify the mutant chromosome segregating within a WD family. The advantage of this study lies in rapid identification of presymptomatic individuals. However, in such studies the specific mutation often goes undetected. In some cases, high genetic homogeneity or consanguineous marriage results in non-informativeness of the SNP or microsatellite markers. The present study by Mathur *et al*¹⁸ further improves WD diagnosis by identifying specific mutations and not relying on informativeness or heterozygosity of markers.

Introducing low-density microarray for WD detection

In this issue the study by Mathur *et al*¹⁸ on microarray based diagnosis of WD is a major development not only in the area of genetic diagnosis of WD but also for monogenic disorders as a whole. The study attempts to establish detection and analysis procedures for microarray based diagnosis of WD. Presymptomatic identification by mutation detection would be now possible in neonates.

Mathur *et al*¹⁸ describes designing and utilizing an oligonucleotide microarray for detecting WD mutations. A subset of 62 mutations that were specific to Indian population was identified in the Wilson disease database and was used to design oligonucleotide probes for a low-density WD microarray. The entire process is simple but effective and can be split into two parts. Part I involves selection of mutations from the WD database, designing oligonucleotide probes and printing them as microarrays. The second part involved designing DNA amplicons to be tested. The amplicons were DIG labelled. As a proof of principle, the test amplicons were hybridized on the microarrays containing the probes, washed and then analyzed for binding. Around 60 per cent of the mutations showed

high detectability. On including weakly hybridized but reproducible amplicons, the detection rate rose to 80 per cent. Moreover, prevalent mutations in the Indian population, *i.e.* Tyr187Stop, Cys271Stop, Gly1061Glu, c.1708-1G>C were detected in the tests against controls. Authors also included ambivalent variants, *e.g.* c.2623G>A (Gly875Arg) that exhibit dual status in the WD database. Presence of this variant is linked to disease causation (DV) in southern Indian WD patients; however, is designated as a non-disease variant (NDV) in Han-Chinese population^{19,20}. *In vitro* studies carried out in cell lines have demonstrated that copper content of the cell dictates subcellular localization of the Arg⁸⁷⁵ variant and hence affecting intracellular copper content²¹. Early detection of these variants of ambiguous status, early in life of individuals will certainly help to take proper lifestyle and nutritional measures and possibly prevent the disease.

In a nation like India with great genetic diversity, inexpensive genetic testing of common as well as rare mutations would prove to be a blessing. Efforts should be made to improve accuracy and efficiency of this WD microarray platform to make it available for the healthcare system of the Indian subcontinent. Also, similar studies should be extended towards other monogenic diseases that show high mutational heterogeneity.

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