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Comparison of two PCR-based molecular methods in the diagnosis of CMT 1A and HNPP diseases in Chinese

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Abstract

Objectives: Current molecular diagnostic methods in detecting Charcot-Marie-Tooth type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsy (HNPP) diseases are either not sensitive or time-consuming and costing. The aims of this study are improving the accuracy and speeding up the diagnosis.

Patients and methods: We developed real-time quantitative PCR (QPCR) and three polymorphic short tandem repeats (STRs) methods to test 53 unrelated CMT1A patients, 12 unrelated HNPP patients and 100 normal control subjects.

Results: QPCR in detection of *pmp22* gene duplication (CMT1A) and deletion (HNPP) showed a sensitivity of 100.00% (53/53) and 100.00% (12/12), respectively. And this method also showed a specificity of 100% (100/100) in CMT1A and 100% (100/100) in HNPP, respectively. In contrast, using three polymorphic STRs method showed a sensitivity of 50/53 (94%) in CMT1A and 12/12 (100.00%) of HNPP patients, respectively. And this method showed a specificity of 97% (100/103) in CMT1A and 100% (100/100) in HNPP, respectively.

Conclusion: QPCR and three STRs methods both demonstrate a rapid and robust diagnosis with almost complete informativeness. The high sensitivity and heterozygosity of these three polymorphic markers in detecting CMT1A/HNPP subjects of Caucasian and Chinese showed the potential to become pan-ethnic screening markers in the future.

Keywords: Charcot-Marie-Tooth type 1A; Hereditary neuropathy with liability to pressure palsy; Real-time quantitative PCR; Three polymorphic short tandem repeats; Heterozygosity; Polymorphic markers

1. Introduction

Charcot-Marie-Tooth (CMT) disease is the most frequent inherited peripheral neuropathy, with an estimated prevalence of 1 in 2500 [1]. Two major forms of CMT can be identified electrophysiologically: one form shows defects in the formation or maintenance of myelin (CMT1) and the other primary axonal degeneration (CMT2) [2]. CMT1A with a duplication of 1.5 Mb containing the gene for peripheral myelin protein 22 (*PMP22*) on chromosome 17p11.2–12 is responsible for 75% of cases of the demyelinating form [3]. Hereditary neuropathy with liability to pressure palsies (HNPP), however, is characterized by deletion of the 1.5 Mb fragment containing the gene *PMP22*.

Traditional methods for the diagnosis of CMT1A and HNPP have several disadvantages. The hybridization-based techniques are time-consuming, and large amounts of high-quality DNA are needed. The more specialized fluorescence in situ hybridization (FISH) assay is available from only a

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limited number of clinical laboratories. Diagnosis with markers located inside the duplication usually is carried out using restriction fragment length polymorphism (RFLP) probes [4]. However, this diagnosis method often relies on the interpretation of differences in allele intensities, even with the most polymorphic markers that have been reported to date. Indirect methods based on microsatellite or single nucleotide polymorphic markers analysis depend on the informativeness of the markers and may lead to a wrong interpretation of analyzed results. Sensitivity of detecting the hotspot of crossover breakpoints of the CMT1a-REPs is less than the other methods [5]. Since this technique may miss small rearrangement, it is less sensitive than those techniques which can detect the number of copies of the *PMP22* gene directly [6].

Nowadays, highly sensitive and specific PCR-based quantitative strategies of detecting the CMT1A and HNPP diseases have been setup very well [7]. However, the sensitivity of these methods in detecting the CMT1A and HNPP diseases is hard to reach 100%. We develop two PCR-based methods together to detect the sensitivity and specificity of CMT1A/HNPP diseases in Taiwanese. If the results will meet our anticipation, the methods will improve the accuracy, speed up the diagnosis and have the potential to become pan-ethnic screening markers in detecting CMT1A/HNPP subjects of other ethnics.

2. Materials and methods

2.1. Patients

Patients diagnosed as CMT1A/HNPP at the Neurology Department of six medical centers were evaluated. Blood samples from 65 unrelated families with CMT1A/HNPP and 100 normal subjects as controls were analyzed. The diagnosis of CMT1A was based on the clinical presentations, abnormal nerve conduction velocities (<40 m/s), analysis of ratios obtained by Southern blotting [4]. Informed consent was obtained from each participant in accordance with protocols approved by the Chang Gung Memorial Hospital and University.

2.2. DNA extraction

Genomic DNA was extracted form leukocytes of EDTA-treated blood by using the Stratagen DNA blood kit [8].

2.3. Real-time PCR assay

A previously published method [9] was used. The final volume of each tube was reduced from 45 μl to 25 μl as compared with previously published methods. Data evaluation was carried out using the ABI Prism sequence detection system and Microsoft Excel according to a previous published article [7].

2.4. Fluorescent labeling and sizing on an automated sequencer

Three most informative STRs (4A, 9A, and 9B) were applied in this study as a previous article [10].

2.5. Statistical analysis

Software (SPSS 10.0; SPSS, Chicago, Ill) was used for statistical analysis. Categorical variables were compared using χ^2 or Fisher's exact test, wherever appropriate. The cutoff point of *pmp22* gene copy number with real-time quantitative PCR as a screening tool for CMT1A and HNPP was evaluated through receiver operating characteristic (ROC) curve. The *p*-value of significance was 0.05. All *p*-values were two-sided.

3. Results

3.1. Real-time quantitative PCR method

Detection of *pmp22* gene duplication (CMT1A) and deletion (HNPP) showed a sensitivity of 100.00% (53/53) and 100.00% (12/12), respectively. And this method showed a specificity of 100% (100/100) in CMT1A and 100% (100/100) in HNPP, respectively (Tables 1A and 1B). Through ROC curve evaluation, the *pmp22* copy number cutoff point between CMT1A and normal subjects is 1.39 (Table 2A) and the *pmp22* copy number cutoff point between HNPP and normal subjects is 0.77 (Table 2B), which cutoff points will reach the highest sensitivity (100%) and specificity (100%).

Table 1A
Diagnostic accuracy of CMT1A and HNPP detected by real-time quantitative PCR (Q-PCR)

	Positive	Negative
CMT1A subjects[a]	53	0
Normal controls[a]	0	50
HNPP subjects[b]	12	0
Normal controls[b]	0	50

[a] Sensitivity: 53/53 = 100%; specificity: 50/50 = 100%.
[b] Sensitivity: 12/12 = 100%; specificity: 50/50 = 100%.

Table 1B
Diagnostic accuracy of CMT1A and HNPP detected by three polymorphic STR (3 STRs)

	Positive	Negative
CMT1A subjects[a]	50	3
Normal controls[a]	0	100
HNPP subjects[b]	12	0
Normal controls[b]	0	100

[a] Sensitivity: 50/53 = 94%; specificity: 100/103 = 97%.
[b] Sensitivity: 12/12 = 100%; specificity: 100/100 = 100%.