Rapid Mutation Screening for *HRPT2* and *MEN1* Mutations Associated with Familial and Sporadic Primary Hyperparathyroidism

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Familial hyperparathyroidism, a disease of the parathyroid glands, may occur in conjunction with pituitary and pancreatic tumors (multiple endocrine neoplasia type I), kidney and bone tumors (hyperparathyroidism jaw tumor syndrome), or alone (familial isolated hyperparathyroidism). This study describes the development and validation of rapid scanning for mutations in two tumor suppressor genes linked to familial hyperparathyroidism—*MEN1* and *HRPT2*. Denaturing high-performance liquid chromatography mutation scanning for *MEN1* was performed using a set of 10 amplicons covering the nine coding exons and flanking intronic regions and for *HRPT2* using a set of three amplicons for exons 1, 2, and 7 and flanking intronic regions, in which 80% of the mutations identified to date are located. All 52 *MEN1* mutations or polymorphisms, 46 known and six unknown, were successfully detected. Mutation detection in exon 9 was not confounded by the presence of the common polymorphism D418D. In addition, all 10 *HRPT2* mutations were successfully detected, and a two-step approach was able to distinguish IVS2 common polymorphisms from exon 2 mutations. The development of rapid denaturing high performance liquid chromatography mutation scanning of *MEN1* and *HRPT2* facilitates a molecular diagnosis of the associated familial syndromes for both clinically affected and at-risk family members. (*J Mol Diagn* 2006; 8:559–566; DOI: 10.2353/jmoldx.2006.060015)

Hyperparathyroidism (HPT) is a disease of the parathyroid glands characterized by calcium-insensitive hypersecretion of parathyroid hormone and increased cell proliferation. The familial autosomal dominant cancer syndromes multiple endocrine neoplasia type 1 (MEN 1) (OMIM no. 131100) and hyperparathyroidism jaw-tumor syndrome (HPT-JT) (OMIM no. 145001) have an 80 to 90% penetrance of HPT.^{1,2} Germline *MEN1* mutations have been detected in ~70% of individuals affected by MEN 1^{1,3,4} and a small number of patients presenting with familial isolated hyperparathyroidism (OMIM no. 145000).^{5–9} In addition, 10% of germline mutations are *de novo* mutations identified in sporadic parathyroid adenomas.¹⁰ To date ~60% of HPT-JT kindreds and up to 7% of familial isolated hyperparathyroidism kindreds have been found to carry germline *HRPT2* mutations.^{8,11–19} In addition, *HRPT2* mutations have been detected in 70% of patients with parathyroid carcinomas, almost 20% of which have been found in the germline.^{13,14,19,20} These mutations may be *de novo* germline mutations or inherited mutations with low penetrance.

The MEN 1 gene *MEN1* is located at 11q13 and consists of 10 exons, of which nine are coding. The Human Genome Mutation Database, Cardiff, UK, has to date recorded more than 330 *MEN1* germline mutations distributed throughout the coding region. No genotype/phenotype correlation has been identified.²¹ Menin, the gene product of *MEN1*, is thought to have a tumor suppressor role in the formation of familial MEN 1 tumors as well as sporadic parathyroid adenomas. Loss of heterozygosity (LOH) at the *MEN1* locus has been reported in 20 to 40% of sporadic parathyroid adenomas, and somatic *MEN1* mutations were found in 46 to 100% of these tumors.^{22–25} *MEN1* mutation detection is most commonly performed by direct sequencing or single-stranded conformational polymorphism analysis (SSCP).^{3,25,26} Enhanced SSCP, using fluorescent labeling in combination with heteroduplex analysis, has recently been reported for MEN 1 with 100% detection of 27 known variants.²⁷ Denaturing high-

Supported by the Australian National Health and Medical Research Council (grant 302161; and a Dora Lush Biomedical Scholarship to V.M.H.), and the Cancer Institute NSW (fellowship to D.J.M.).

Accepted for publication August 2, 2006.

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Table 1. Samples with *MEN1* Mutations Assessed by DHPLC ($n = 52$)

	Mutation	Location		Mutation	Location
	c.122ins5del3	Exon 2		c.1164delAAC	Exon 8
†	c.352delC	Exon 2		c.1167T>G (Y353D)	Exon 8
	c.359delGTCT	Exon 2	†	c.1193insTAC	Exon 8
	c.366ins18	Exon 2	*	c.1197delGAG	Exon 8
	c.379delAT	Exon 2	*	c.1210T>G (V367G)	Exon 8
†	c.452delC	Exon 2		c.1242G>T (E378X)	Exon 8
	c.512delC	Exon 2		c.1227C>T (P373S)	Exon 8
	IVS2-3C>G	Intron 2		c.1337C>A (C409X)	Exon 9
*	c.588G>C (A160P)	Exon 3		c.1354G>C (R415L), c.1364C>T (D418D)	Exon 9
†	c.593GG>TT (V161V+V162F), R171Q	Exon 3		c.1362G>C (D418H)	Exon 9
	c.622G>A (R171Q)	Exon 3		c.1364C>T (D418D)	Exon 9
	c.700C>T (T197I)	Exon 3		c.1378G>A (W423X)	Exon 9
†	c.707C>GG	Exon 3	*	c.1390G>T (S427I)	Exon 9
	c.738delACAG	Exon 3		c.1414delG	Exon 9
	IVS3 + 1G>T	Intron 3		c.1458C>T (Q450X), c.1364C>T (D418D)	Exon 9
	c.769G>A	Exon 4		IVS9 + 1del11	Intron 9
*	c.795C>T (R229C)	Exon 4		c.1462del14	Exon 10
	c.788delATACAT	Exon 4		c.1488C>T (R460X)	Exon 10
†	c.868C>T (S253L)	Exon 4	†	c.1539delG	Exon 10
	IVS4 + 2T>A	Intron 4		c.1656delC	Exon 10
	IVS4-9G>A	Intron 4		c.1657insC	Exon 10
†	c.911insT	Exon 5	*	c.1668insT	Exon 10
	IVS5 + 1G>A	Intron 5		c.1700delA, c.1702G>C (G531A)	Exon 10
†	c.960G>C (A284P), c.934+39C>T	Exon 6		c.1798A>T (Q563V), c.1800delCT	Exon 10
†	IVS6 + 2T>A	Intron 6		c.1809delG	Exon 10
	c.1120C>A (A337D)	Exon 7	†	c.1814delC	Exon 10

Sequence information was derived from GenBank accession numbers U93236 (2.7 kb) and U93237 (9.2 kb). The cDNA numbering starts at exon 1 so that c.111 is the first base of amino acid 1.

*Analyzed as part of test set ($n = 6$).

†Tumor DNA samples with LOH.

performance liquid chromatography (DHPLC) with or without SSCP and sequencing has been used for the detection of *MEN1* mutations^{28,29}; however, DHPLC validation studies, taking into consideration the possible confounding influence of common polymorphisms, have not been reported.

The HPT-JT gene *HRPT2* is located at 1q25 and consists of 17 exons.¹¹ To date, 45 different *HRPT2* mutations and seven polymorphisms have been identified.¹¹⁻²⁰ Eight of the 17 exons have been found to harbor mutations, and 80% of the mutations are located in exons 1, 2, and 7 or their flanking introns. *HRPT2*, like *MEN1* is a putative tumor suppressor gene; however, the incidence of LOH at the *HRPT2* locus is less than 13% in tumors tested.^{14,25,30,31} Three studies that included investigation of two HPT-JT families have reported DHPLC detection of *HRPT2* variants.^{14,15,19} All other mutation reports for *HRPT2* have used direct sequencing for mutation detection^{8,11,13,16-18,20} with confirmation of one mutation by allele-specific-oligonucleotide hybridization.¹²

DHPLC is a technique for automated mutation scanning that detects heteroduplexes formed during polymerase chain reaction (PCR) amplification of heterozygous template DNA.³² After amplification, PCR fragments are injected into the flow path of a reverse phase column in a HPLC system. Under partially denatured conditions, the less stable (more denatured) heteroduplexes are eluted from the column earlier than the more stable homoduplexes and are detected by the presence of additional or aberrant peaks. Amplicons presenting with multiple or aberrant peaks are sequenced to characterize the variants.

DHPLC enables automated mutation scanning of PCR products amenable to high-throughput workloads. In a comparative study of *BRCA1*, DHPLC detected 100% of mutations assessed compared with 65% detection by SSCP.³³ Further advantages of DHPLC over SSCP, the most common method reported for *MEN1* mutation scanning, include larger fragment sizes able to be analyzed, automated sampling, and the ability to program multiple conditions per run or project.³⁴ Mutation scanning by DHPLC has been successfully applied to a number of tumor suppressor genes including *PTEN*, *VHL*, and *RET*.³⁵ A limitation of this and other mutation scanning techniques is likely interference from the detection of common polymorphisms. An anticipated problem specific to tumor suppressor genes is the possibility of false negative results attributable to LOH when testing tumor DNA. The loss of an allele will result in the loss of heteroduplexes and thus the absence of the mutant chromatographic profile.

We have evaluated the sensitivity of mutation scanning by DHPLC for the putative tumor suppressor genes *MEN1* and *HRPT2*. Germline and tumor DNA samples harboring previously identified germline or somatic mutations were analyzed. Because both genes contain known common polymorphisms, interference by common polymorphisms was also assessed. After establishment of the mutation scanning conditions, assay sensitivity was tested further by mutation scanning for unknown mutations in several *MEN1* samples.

Table 2. Samples with *HRPT2* Mutations ($n = 10$) and Presumptive Polymorphisms ($n = 4$) Assessed by DHPLC ($n = 15$)

	Variant	Location
\$	c.33C>T (Y11Y)	Exon 1
‡	c.76delA	Exon 1
‡	c.162C>G (Y54X)	Exon 2
‡	c.165C>G (Y55X), IVS2+28C>T	Exon 2
†	c.165delC	Exon 2
	c.191T>C (L64P)	Exon 2
\$	IVS2+28C>T	Intron 2
\$	IVS2+28del4	Intron 2
‡	IVS6-1delC	Intron 6
*	c.636delT	Exon 7
*	c.636delT, IVS7+33(GA) ₈	Exon/intron 7
	c.679delAG	Exon 7
‡	c.686delGAGT	Exon 7
	c.700C>T (R234X)	Exon 7
\$	IVS7+33(GA) ₈	Intron 7

*Two different samples with the same mutation, one of which also carries a polymorphism in the same amplicon.

†Tumor DNA sample with LOH.

‡Tumor DNA sample without LOH.

\$_n = 4.

Materials and Methods

DNA Samples

Deidentified germline and parathyroid tumor DNA with previously identified heterozygous *MEN1* or *HRPT2*^{14,15} variants were obtained according to the ethical guidelines of

the human research ethics committees of participating centers and are listed in Tables 1 and 2. All variants detected were confirmed by commercial automated sequencing (Supamac; Sydney University, Sydney, Australia). Tumor DNA samples previously identified with LOH at the *MEN1* locus ($n = 11$) and the *HRPT2* locus ($n = 1$) were tested both unmixed and mixed with an equal amount of known wild-type DNA (Tables 1 and 2). After optimization of the *MEN1* amplicons for DHPLC using a training set of known samples ($n = 46$), a further six samples known to harbor a mutation in one of the *MEN1* exons were used as a test set to validate the optimized conditions (Table 1). A normal DNA panel ($n = 20$) was also used as part of this validation.

MEN1 and *HRPT2* Amplicons

The primers and amplicon sizes for *MEN1* amplicons for exons 2 through 10, and *HRPT2* amplicons for exons 1, 2, and 7 are detailed in Table 3. PCR amplifications were performed in 50 μ L containing 200 μ mol/L dNTP (Roche Molecular Systems Inc., Branchburg, NJ), 1.5 mmol/L MgCl₂, except for *MEN1* exon 3, which contained both 4 mmol/L MgCl₂ and 1.3 mol/L Betaine (Sigma Aldrich Co., St. Louis, MO), 1 $\times$ Buffer II (Applied Biosystems, Foster City, CA), 1.5 U of AmpliTaq gold DNA polymerase (Applied Biosystems), 0.4 μ mol/L of each primer (Pacific Oligos, Lismore, Australia), and 200 ng or 50 ng of DNA for *MEN1* or *HRPT2*, respectively. All PCRs were performed in a DNA Engine Dyad thermal cycler (MJ Research, Incline Village,

Table 3. Primers, Amplicon Sizes, and DHPLC Injection Temperatures for *MEN1* and *HRPT2*

Exon	Primers	Size (bp)	T ⁱ (°C) (range)	T ^p (°C)
A: <i>MEN1</i>				
2 (c)*	F: 5'-GGCTTCGTGGAGCATTCT-3' R: 5'-TTGAAGAAGTGGGTCATGG-3'	369	62 (62–64)	61.8
2 (b)*	F: 5'-GGGTGGAACCTTAGCGGACCCTGG-3' R: 5'-CTCGAGGATAGAGGGACAGG-3'	428	65 (64–67)	64.9
3	F: 5'-GCACAGAGGACCCTCTTTCATTAC-3' R: 5'-TGGGTGGCTTGGGCTACTACAG-3'	306*	64	62.3
4	F: 5'-GGGCCATCATGAGACATAATG-3' R: 5'-GGTCCCACAGCAAGTCAAGT-3'	228	63	61.4
5 + 6*	F: 5'-AAGGACCCGTTCTCCTCCCTGTTCC-3' R: 5'-GGCCCCTGCCTCAGCCACTGTTAG-3'	324	62	62
7	F: 5'-GGCTGCCTCCCTGAGGATC-3' R: 5'-CTGGACGAGGGTGGTTGG-3'	334	63	62.5
8	F: 5'-GTGAGACCCCTTCAGACCCTAC-3' R: 5'-TGGGAGGCTGGACACAGG-3'	218	63 (61–63)	61.6
9	F: 5'-GGGTGAGTAAGAGACTGATCTGTGC-3' R: 5'-TGAGTGCCAGACCTCTGTG-3'	246	62	63.6
10 (e)*	F: 5'-CCAAGAAGCCAGCACTGGAC-3' R: 5'-GGTCCCCACAAGCGGTCCGAAGTC-3'	381	62 (62–63)	62.4
10 (c)*	F: 5'-ACCTTGCTCTCACCTTGCTCT-3' R: 5'-CACTCTGGAAAGTGAGCACT-3'	351	67 (66–67)	66
B: <i>HRPT2</i>				
1	F: 5'-CGAGGCGACAAGAGAAGAAG-3' R: 5'-ACAGCCATGCCGACTTA-3'	226	62 (60–65)	61.2
2	F: 5'-TGAATCCAGCCTGAAGAGTTG-3' R: 5'-AGGCCAGACCCTGTCTCTTA-3'	247	53 + 57	54.4
7	F: 5'-GGAATGCCTGCTGTGAAAAT-3' R: 5'-TGAACCTCCACCTAAAAGCAA-3'	367	56 + 57	55.8

*Sets of two overlapping amplicons were used for exons 2 (c and b) and 10 (c and e), with exons 5 and 6 amplified in a single amplicon. Primers from Refs. 3, 40, and this study.

T^p is the Navigator software predicted optimal temperature for DHPLC, and Tⁱ is the experimentally determined optimal temperature for DHPLC. Where two temperatures were required for detection of all mutations, these are noted by +, and the temperatures in Parentheses indicate the temperature range over which all mutations in that amplicon were detected.

NV). A touchdown cycling protocol with 30-second steps and cycles consisting of denaturation at 95°C, followed by annealing at various temperatures, and extension at 72°C, was used for *MEN1* PCR. An initial denaturation at 95°C for 10 minutes was performed followed by five cycles with the annealing temperature starting at 65°C and decreasing 1°C per cycle, five cycles with the annealing temperature at 60°C, five cycles with the annealing temperature starting at 59°C and decreasing 1°C per cycle, and then 30 cycles with the annealing temperature at 55°C and a final extension for 5 minutes. The thermal cycling for the *HRPT2* amplicons consisted of an initial denaturation at 95°C for 10 minutes followed by 35 cycles of 30 second steps of denaturation at 95°C, annealing at 60°C (except for exon 1 for which the annealing temperature was 55°C), and extension at 72°C, followed by a final extension at 72°C for 5 minutes. Before DHPLC, heteroduplex enhancement was performed for all PCR products, as previously described.¹⁴

DHPLC

DHPLC was performed on a WAVE System for Mutation Detection 2100 (Transgenomic Inc., Omaha, NE) as previously described.³⁵ In brief, a 5-μl aliquot of heteroduplex enhanced PCR product was injected into the mobile phase in the direction of the flow path of the DNasep cartridge (Transgenomic Inc.). The conditions for each amplicon are listed in Table 3. The results were analyzed using Navigator software (Transgenomic Inc.). For each amplicon, the chromatograms were normalized against the wild type, and resulting peaks were assessed both visually and by the software using the scattergraph function.

Results

MEN1 Mutation Detection

DHPLC mutation scanning of *MEN1* was developed to span the nine coding exons and flanking intronic regions. The final set of 10 different amplicons resulted in the successful detection of all 46 samples that made up the training set (Table 1, Figure 1) as well as six unknown samples with different *MEN1* sequence variants (Table 1, Figure 2). There was decreased sensitivity of mutation detection for amplicons greater than 550 bp. Only five of seven mutations in exons 2 and 4 of 10 mutations in exon 10 were detected within large amplicons (data not shown). The somatic mutations in eight of the 11 tumor samples (all of which had previously identified LOH) were detected without mixing with wild-type DNA (data not shown). These tumor samples therefore contained greater than 0% but less than 50% nontumor cell DNA.

Concurrent Detection of Common Polymorphisms and Mutations in *MEN1* Exon 9

The presence of common polymorphisms can reduce the efficiency of DHPLC mutation scanning. The exonic *MEN1* polymorphism c.1364C>T (D418D) has a reported

Figure 1. DHPLC results for *MEN1* exon 9 amplicon at 62°C. **A:** Discriminant analysis using the scattergraph function of Navigator software of exon 9 wild type, D418D (c.1364C>T) heterozygous, and unknown samples at 62°C. Scattergraph grouped the chromatograms into three different clusters, labeled 1 to 3 (**B**), and matched the three different genotypes determined by subsequent sequencing. The clusters are shown in three-dimensional space with clear separation between the clusters. **C:** Chromatograms for the remaining seven *MEN1* mutations assessed for exon 9. All were distinguishable from the polymorphism (**A**), and the mutation c.1458C>T was discernible in the presence of the polymorphism.

A Amplicon 3 @ 64°C

B Amplicon 4 @ 63°C

C Amplicon 8 @ 61°C

D Amplicon 10C @ 67°C

Figure 2. *MEN1* test set analysis. Six samples known to harbor a *MEN1* mutation were analyzed in a blinded manner, along with 19 to 20 control DNA samples from a panel of healthy volunteers. Scattergraph plots identified mutations (circled) in exons 3 (A), 4 (B), 8 (C), and 10 (D). The results for the exon 9 unknown sample are shown in Figure 1.

heterozygous frequency greater than 29%.³⁶ Comparative analysis of wild-type known c.1364C>T heterozygous and unknown samples using the scattergraph function of the Navigator software was important for accurate assessment of unknown *MEN1* exon 9 samples (Figure 1, A and B). The polymorphism presented as a subtle broadening peak and did not interfere with mutation detection within exon 9. The pattern for this polymorphism was different to the polymorphism in combination with a nearby missense mutation (c.1354G>C + c.1364C>T) as well as to another base substitution on the same codon (c.1362G>C, D418H) (Figure 1C).

HRPT2 Mutation Detection

Mutation scanning of the HPT-JT gene *HRPT2* was developed to screen three exons (1, 2, and 7) that together harbor 80% of mutations detected in this gene to date. In

total, 15 genomic or tumor DNA samples harboring *HRPT2* mutations or putative polymorphisms were successfully assessed: one mutation and one polymorphism in exon 1 (Figure 3A), five mutations and one polymorphism (as well as one sample heterozygous for both a mutation and polymorphism) in exon 7 (Figure 3B), and four mutations and two polymorphisms in exon 2 (Figure 4). The tumor DNA sample c.165delC, which had previously been shown to have LOH, was successfully detected without mixing with wild-type DNA (Figure 4).

Concurrent Detection of Common Polymorphisms and Mutations in HRPT2 Exon 2 Amplicon

The common *HRPT2* polymorphisms IVS2+28C>T and IVS2+28del4 have distinct multipeak patterns that may

Figure 3. DHPLC results for *HRPT2* exon 1 and exon 7 amplicons. **A:** Chromatograms for the exon 1 wild type, polymorphism c.33C>T, and mutation c.76delA at 62°C. **B:** Chromatograms for the exon 7 samples at 56°C, and the wild type and mutation c.636delT also at 57°C, at which temperature this mutation was more readily detected.

interfere with mutation detection (Figure 4B). By taking advantage of the different melt domains in the exon 2 amplicon (Figure 4A), it was possible to determine the presence of these polymorphisms but also to melt these intronic variants and determine the presence of exonic mutations (Figure 4B).

Discussion

In this study, we have determined DHPLC to be both efficient and sensitive for the detection of mutations in the putative tumor suppressor genes *MEN1* and *HRPT2* by the successful detection of all 52 *MEN1* mutations and polymorphisms, and 10 *HRPT2* mutations. To confirm good DNasep cartridge resolution, at least one wild-type

Figure 4. DHPLC results for *HRPT2* exon 2. **A:** Navigator software generated melt curves for the exon 2 amplicon at 53°C and 57°C. The exonic region of the amplicon is shaded, and the numbers above the curves indicate the locations of the mutations as listed in **(B)**. **B:** Chromatograms for the exon 2 samples run at both 53°C and 57°C. At 53°C, the two IVS2 polymorphisms have markedly aberrant chromatograms, whereas two mutations (c.191T>C and c.165delC) show only subtle changes from the wild-type peak. At 57°C, the chromatograms for the two IVS2 polymorphisms are indistinguishable from the wild-type sample, whereas the two mutations c.191T>C and c.165delC are now easily discernible as having aberrant chromatograms. The remaining two mutations displayed aberrant chromatograms at both temperatures.

sample and samples with mutations (where available) in each amplicon should be run in conjunction with the samples under investigation. To ensure sensitive detection of mutations, careful assessment of the resulting chromatograms is required. Although the majority of mutations present with overtly aberrant peaks, some mutant patterns are subtle and require rigorous comparison with wild-type samples. When analyzing tumor DNA samples for mutations in genes where LOH is suspected, prior knowledge of the presence or extent of LOH is not necessary. We observed that by running samples with and without mixing with normal DNA, all mutations in the tumor samples ($n = 12$) were detectable.

For DHPLC scanning of the nine coding *MEN1* exons and flanking intronic regions, a 10-tube PCR protocol per patient, cycled under identical conditions, was established. This DHPLC assay enables amplicon localization of mutations, if present, in less than 24 hours. Subsequent identification of the mutation would require se-

quencing of one amplicon rather than all nine, although the presence and detection of rare polymorphisms would increase the number of amplicons requiring sequencing. DHPLC detection of *MEN1* mutations has been previously reported with the successful detection of four mutations in three exons (exons 2, 7, and 9).²⁸ However, the impact of the presence of the common D418D polymorphism in exon 9 was not addressed. The work in this study showed that the common *MEN1* polymorphism, D418D, which has a reported frequency greater than 29%,³⁶ can be identified by scattergraph analysis of multiple wild-type and D418D (c.1364C>T) heterozygous samples (Figure 1B) and did not confound mutation detection in the exon 9 amplicon (Figure 1, A and C). Concurrent accurate DHPLC detection of mutations and polymorphisms has similarly been shown recently for other genes.³⁷ It is however, conceivable that a mutation will present with the same pattern as this polymorphism. Ultimately, the approach taken for the exon 9 amplicon may be different for each unknown sample and will depend on the DHPLC findings in the other nine amplicons. For family studies of previously identified mutations, DHPLC offers an efficient alternative or adjunct to repeat sequencing.

To date, 45 *HRPT2* mutations have been reported. Hotspot regions are evident, with 80% of mutations localized to exons 1, 2, and 7. A three-tube PCR protocol was established for DHPLC mutation scanning of exons 1, 2, and 7 and flanking intronic regions and involved analysis of chromatograms of one or two different temperatures per amplicon (Table 3B). This assay demonstrated 100% sensitivity for the available 15 DNA samples with different *HRPT2* genotypes (10 mutations, four polymorphisms, and one sample harboring both a mutation and polymorphism in the same amplicon) (Figures 3 and 4).

HRPT2 mutations have been found in ~60% of HPT-JT families. It remains to be determined whether the other 40% of HPT-JT families harbor currently undetected mutations. These families may carry large deletions spanning one or more exons as have been found recently in *MEN1* and *BRCA1*.^{38–40} The two most common polymorphisms (IVS2+28C>T and IVS2+28del4), have a combined heterozygous frequency of ~60% in the general population.¹⁴ These polymorphisms are readily detected in the exon 2 amplicon, enabling confirmation of the presence of two *HRPT2* alleles in the majority of individuals and eliminating the possibility of full gene or exon 2 deletion in the germline of these individuals. However, by adjusting the DHPLC running conditions, the interference by these two polymorphisms in mutation detection was able to be minimized (Figure 4).

In conclusion, DHPLC mutation scanning is a rapid method for the detection of both known and unknown mutations. The amplicon sets developed in this study for *MEN1* and *HRPT2* have demonstrated a sensitivity approaching 100%. These assays are an efficient first step in the detection of germline mutations in *MEN1* or HPT-JT probands as well as a suitable alternative strategy to other mutation detection methodologies for screening of family members for previously identified mutations. This is especially true for laboratories for which sequencing is

expensive and has a turn-around-time greater than 24 hours.

Acknowledgments

We thank The Humpty Dumpty Foundation, Australia, for the donation of DHPLC equipment; and C.J. Haven and H. Morreau (Leiden University Medical Center, Leiden, The Netherlands) and R.T. Zori and H.J. Stalker (University of Florida, Gainesville, FL) for providing DNA samples with *HRPT2* mutations.

References

1. Agarwal SK, Lee Burns A, Sukhodolets KE, Kennedy PA, Obungu VH, Hickman AB, Mullendore ME, Whitten I, Skarulis MC, Simonds WF, Mateo C, Crabtree JS, Scacheri PC, Ji Y, Novotny EA, Garrett-Beal L, Ward JM, Libutti SK, Richard Alexander H, Cerrato A, Parisi MJ, Santa Anna-A S, Oliver B, Chandrasekharappa SC, Collins FS, Spiegel AM, Marx SJ: Molecular pathology of the *MEN1* gene. *Ann NY Acad Sci* 2004, 1014:189–198
2. Marx SJ: Multiple endocrine neoplasia type 1. The Parathyroids, ed 2. Edited by JP Bilezikian, R Marcus, MA Levine. San Diego, Academic Press, 2002, pp 535–584
3. Lemmens I, Van de Ven WJ, Kas K, Zhang CX, Giraud S, Wautot V, Buisson N, De Witte K, Salandre J, Lenoir G, Pugeat M, Calender A, Parente F, Quincey D, Gaudray P, De Wit MJ, Lips CJ, Höppener JW, Khodaei S, Grant AL, Weber G, Kytölä S, Teh BT, Farnebo F, Phelan C, Hayward N, Larsson C, Pannett AAJ, Forbes SA, Bassett JHD, Thakker RV: Identification of the multiple endocrine neoplasia type 1 (*MEN1*) gene. The European Consortium on *MEN1*. *Hum Mol Genet* 1997, 6:1177–1183
4. Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, Debelenko LV, Zhuang Z, Lubensky IA, Liotta LA, Crabtree JS, Wang Y, Roe BA, Weisemann J, Boguski MS, Agarwal SK, Kester MB, Kim YS, Heppner C, Dong Q, Spiegel AM, Burns AL, Marx SJ: Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science* 1997, 276:404–407
5. Kassem M, Kruse TA, Wong FK, Larsson C, Teh BT: Familial isolated hyperparathyroidism as a variant of multiple endocrine neoplasia type 1 in a large Danish pedigree. *J Clin Endocrinol Metab* 2000, 85:165–167
6. Teh BT, Kytölä S, Farnebo F, Bergman L, Wong FK, Weber G, Hayward N, Larsson C, Skogseid B, Beckers A, Phelan C, Edwards M, Epstein M, Alford F, Hurley D, Grimmond S, Silins G, Walters M, Stewart C, Cardinal J, Khodaei S, Parente F, Tranebjaerg L, Jorde R, Salmela P, Menon J, Khir A, Tan TT, Chan SP, Zaini A, Khalid BAK, Sandelin K, Thompson N, Brandi M-L, Warth M, Stock J, Leisti J, Cameron D, Shepherd JJ, Öberg K, Nordenskjöld M: Mutation analysis of the *MEN1* gene in multiple endocrine neoplasia type 1, familial acromegaly and familial isolated hyperparathyroidism. *J Clin Endocrinol Metab* 1998, 83:2621–2626
7. Villablanca A, Wassif WS, Smith T, Hoog A, Vierimaa O, Kassem M, Dwight T, Forsberg L, Du Q, Learoyd D, Jones K, Stranks S, Juhlin C, Teh BT, Carling T, Robinson B, Larsson C: Involvement of the *MEN1* gene locus in familial isolated hyperparathyroidism. *Eur J Endocrinol* 2002, 147:313–322
8. Warner J, Epstein M, Sweet A, Singh D, Burgess J, Stranks S, Hill P, Perry-Keene D, Learoyd D, Robinson B, Birdsey P, Mackenzie E, Teh BT, Prins JB, Cardinal J: Genetic testing in familial isolated hyperparathyroidism: unexpected results and their implications. *J Med Genet* 2004, 41:155–160
9. Carrasco CA, Gonzalez AA, Carvajal CA, Campusano C, Oestreicher E, Arteaga E, Wohlk N, Fardella CE: Novel intronic mutation of *MEN1* gene causing familial isolated primary hyperparathyroidism. *J Clin Endocrinol Metab* 2004, 89:4124–4129
10. Chandrasekharappa SC, Teh BT: Functional studies of the *MEN1* gene. *J Intern Med* 2003, 253:606–615
11. Carpten JD, Robbins CM, Villablanca A, Forsberg L, Presciutti S,

- Bailey-Wilson J, Simonds WF, Gillanders EM, Kennedy AM, Chen JD, Agarwal SK, Sood R, Jones MP, Moses TY, Haven C, Petillo D, Leotlela PD, Harding B, Cameron D, Pannett AA, Hoog A, Heath III H, James-Newton LA, Robinson B, Zarbo RJ, Cavaco BM, Wassif W, Perrier ND, Rosen IB, Kristoffersson U, Turnpenny PD, Farnebo LO, Besser GM, Jackson CE, Morreau H, Trent JM, Thakker RV, Marx SJ, Teh BT, Larsson C, Hobbs MR: HRPT2, encoding parafibromin, is mutated in hyperparathyroidism-jaw tumor syndrome. *Nat Genet* 2002, 32:676–680
12. Cavaco BM, Guerra L, Bradley KJ, Carvalho D, Harding B, Oliveira A, Santos MA, Sobrinho LG, Thakker RV, Leite V: Hyperparathyroidism-jaw tumor syndrome in Roma families from Portugal is due to a founder mutation of the HRPT2 gene. *J Clin Endocrinol Metab* 2004, 89:1747–1752
13. Cetani F, Pardi E, Borsari S, Viacava P, Dipollina G, Cianferotti L, Ambrogini E, Gazzero E, Colussi G, Berti P, Miccoli P, Pinchera A, Marcocci C: Genetic analyses of the HRPT2 gene in primary hyperparathyroidism: germline and somatic mutations in familial and sporadic parathyroid tumors. *J Clin Endocrinol Metab* 2004, 89:5583–5591
14. Howell VM, Haven CJ, Kahnoski K, Khoo SK, Petillo D, Chen J, Fleuren GJ, Robinson BG, Delbridge LW, Philips J, Nelson AE, Krause U, Hammje K, Dralle H, Hoang-Vu C, Gimm O, Marsh DJ, Morreau H, Teh BT: HRPT2 mutations are associated with malignancy in sporadic parathyroid tumours. *J Med Genet* 2003, 40:657–663
15. Howell VM, Zori RT, Stalker HJ, Williams C, Jesse N, Nelson AE, Robinson BG, Marsh DJ: A molecular diagnosis of hyperparathyroidism-jaw tumor syndrome in an adolescent with recurrent kidney stones. *J Pediatr* 2004, 145:567
16. Simonds WF, Robbins CM, Agarwal SK, Hendy GN, Carpten JD, Marx SJ: Familial isolated hyperparathyroidism is rarely caused by germline mutation in HRPT2, the gene for the hyperparathyroidism-jaw tumor syndrome. *J Clin Endocrinol Metab* 2004, 89:96–102
17. Villablanca A, Calender A, Forsberg L, Hoog A, Cheng JD, Petillo D, Bauters C, Kahnoski K, Ebeling T, Salmela P, Richardson AL, Delbridge L, Meyrier A, Proye C, Carpten JD, Teh BT, Robinson BG, Larsson C: Germline and de novo mutations in the HRPT2 tumour suppressor gene in familial isolated hyperparathyroidism (FIHP). *J Med Genet* 2004, 41:e32
18. Bradley KJ, Hobbs MR, Buley ID, Carpten JD, Cavaco BM, Fares JE, Laidler P, Manek S, Robbins CM, Salti IS, Thompson NW, Jackson CE, Thakker RV: Uterine tumours are a phenotypic manifestation of the hyperparathyroidism-jaw tumour syndrome. *J Intern Med* 2005, 257:18–26
19. Moon SD, Park JH, Kim EM, Kim JH, Han JH, Yoo SJ, Yoon KH, Kang MI, Lee KW, Son HY, Kang SK, Oh SJ, Kim KM, Yoon SJ, Park JG, Kim IJ, Kang HC, Hong SW, Kim KR, Cha BY: A novel IVS2-1G>A mutation causes aberrant splicing of the HRPT2 gene in a family with hyperparathyroidism-jaw tumor syndrome. *J Clin Endocrinol Metab* 2005, 90:878–883
20. Shattuck TM, Valimaki S, Obara T, Gaz RD, Clark OH, Shoback D, Wierman ME, Tojo K, Robbins CM, Carpten JD, Farnebo LO, Larsson C, Arnold A: Somatic and germ-line mutations of the HRPT2 gene in sporadic parathyroid carcinoma. *N Engl J Med* 2003, 349:1722–1729
21. Wautot V, Vercherat C, Lespinasse J, Chambe B, Lenoir GM, Zhang CX, Porchet N, Cordier M, Beroud C, Calender A: Germline mutation profile of MEN1 in multiple endocrine neoplasia type 1: search for correlation between phenotype and the functional domains of the MEN1 protein. *Hum Mutat* 2002, 20:35–47
22. Carling T, Correa P, Hessman O, Hedberg J, Skogseid B, Lindberg D, Rastad J, Westin G, Akerstrom G: Parathyroid MEN1 gene mutations in relation to clinical characteristics of nonfamilial primary hyperparathyroidism. *J Clin Endocrinol Metab* 1998, 83:2960–2963
23. Farnebo F, Teh BT, Kytola S, Svensson A, Phelan C, Sandelin K, Thompson NW, Hoog A, Weber G, Farnebo LO, Larsson C: Alterations of the MEN1 gene in sporadic parathyroid tumors. *J Clin Endocrinol Metab* 1998, 83:2627–2630
24. Heppner C, Kester MB, Agarwal SK, Debelenko LV, Emmert-Buck MR, Guru SC, Manickam P, Olufemi SE, Skarulis MC, Doppman JL, Alexander RH, Kim YS, Saggat SK, Lubensky IA, Zhuang Z, Liotta LA, Chandrasekharappa SC, Collins FS, Spiegel AM, Burns AL, Marx SJ: Somatic mutation of the MEN1 gene in parathyroid tumours. *Nat Genet* 1997, 16:375–378
25. Dwight T, Twigg S, Delbridge L, Wong FK, Farnebo F, Richardson AL, Nelson A, Zedenius J, Philips J, Larsson C, Teh BT, Robinson B: Loss of heterozygosity in sporadic parathyroid tumours: involvement of chromosome 1 and the MEN1 gene locus in 11q13. *Clin Endocrinol (Oxf)* 2000, 53:85–92
26. Tanaka C, Yoshimoto K, Yamada S, Nishioka H, Ii S, Moritani M, Yamaoka T, Itakura M: Absence of germ-line mutations of the multiple endocrine neoplasia type 1 (MEN1) gene in familial pituitary adenoma in contrast to MEN1 in Japanese. *J Clin Endocrinol Metab* 1998, 83:960–965
27. Crepin M, Escande F, Pigny P, Buisine MP, Calender A, Porchet N, Odou MF: Efficient mutation detection in MEN1 gene using a combination of single-strand conformation polymorphism (MDGA) and heteroduplex analysis. *Electrophoresis* 2003, 24:26–33
28. Park JH, Kim IJ, Kang HC, Lee SH, Shin Y, Kim KH, Lim SB, Kang SB, Lee K, Kim SY, Lee MS, Lee MK, Moon SD, Park JG: Germline mutations of the MEN1 gene in Korean families with multiple endocrine neoplasia type 1 (MEN1) or MEN1-related disorders. *Clin Genet* 2003, 64:48–53
29. Crepin M, Pigny P, Escande F, Bauters CC, Calender A, Lefevre S, Buisine MP, Porchet N, Odou MF: Evaluation of denaturing high performance liquid chromatography for the mutational analysis of the MEN1 gene. *J Mol Endocrinol* 2006, 36:369–376
30. Farnebo F, Teh BT, Dotzenrath C, Wassif WS, Svensson A, White I, Betz R, Goretzki P, Sandelin K, Farnebo LO, Larsson C: Differential loss of heterozygosity in familial, sporadic, and uremic hyperparathyroidism. *Hum Genet* 1997, 99:342–349
31. Villablanca A, Farnebo F, Teh BT, Farnebo LO, Hoog A, Larsson C: Genetic and clinical characterization of sporadic cystic parathyroid tumours. *Clin Endocrinol (Oxf)* 2002, 56:261–269
32. Kuklin A, Munson K, Gjerde D, Haeefe R, Taylor P: Detection of single-nucleotide polymorphisms with the WAVE DNA fragment analysis system. *Genet Test* 1997, 1:201–206
33. Eng C, Brody LC, Wagner TM, Devilee P, Vijg J, Szabo C, Tavtigian SV, Nathanson KL, Ostrander E, Frank TS: Interpreting epidemiological research: blinded comparison of methods used to estimate the prevalence of inherited mutations in BRCA1. *J Med Genet* 2001, 38:824–833
34. Xiao W, Oefner PJ: Denaturing high-performance liquid chromatography: a review. *Hum Mutat* 2001, 17:439–474
35. Marsh DJ, Theodosopoulos G, Howell V, Richardson AL, Benn DE, Proos AL, Eng C, Robinson BG: Rapid mutation scanning of genes associated with familial cancer syndromes using denaturing high-performance liquid chromatography. *Neoplasia* 2001, 3:236–244
36. Correa P, Lundgren E, Rastad J, Akerstrom G, Westin G, Carling T: Multiple endocrine neoplasia type 1 polymorphism D418D is associated with sporadic primary hyperparathyroidism. *Surgery* 2002, 132:450–455
37. Colley J, Jones S, Dallosso AR, Maynard JH, Humphreys V, Dolwani S, Sampson JR, Cheadle JP: Rapid recognition of aberrant DHPLC elution profiles using the Transgenomic Navigator(TM) software. *Hum Mutat* 2005, 26:165
38. Hogervorst FB, Nederlof PM, Gille JJ, McElgunn CJ, Grippeling M, Pruntel R, Regnerus R, van Welssem T, van Spaendonck R, Menko FH, Kluijdt I, Dommering C, Verhoef S, Schouten JP, van't Veer LJ, Pals G: Large genomic deletions and duplications in the BRCA1 gene identified by a novel quantitative method. *Cancer Res* 2003, 63:1449–1453
39. Kikuchi M, Ohkura N, Yamaguchi K, Obara T, Tsukada T: Gene dose mapping delineated boundaries of a large germline deletion responsible for multiple endocrine neoplasia type 1. *Cancer Lett* 2004, 208:81–88
40. Bergman L, Teh B, Cardinal J, Palmer J, Walters M, Shepherd J, Cameron D, Hayward N: Identification of MEN1 gene mutations in families with MEN 1 and related disorders. *Br J Cancer* 2000, 83:1009–1014