

## Characterization of the Human *MDR1* Gene

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### ABSTRACT

P-glycoprotein (Pgp), an ATP-dependent efflux transporter that protects the body from environmental toxins and xenobiotics, is encoded by the human *MDR1* gene. Human *MDR1* is located on chromosomal region 7q21. Although several different single nucleotide polymorphisms were shown to influence Pgp expression and activity, the reported length of the *MDR1* gene in Genbank and other databases continues to evolve and varies between 6.3 kilobases (kb) and 210 kb. With DNA derived from human cell lines and tissues, we have characterized the *MDR1* genomic sequence to be 209 kb.

**KEYWORDS:** *MDR1* gene, P-glycoprotein, gene arrangements.

### INTRODUCTION

P-glycoprotein (Pgp), a member of the ATP binding cassette family of membrane transporters, is encoded by the human multidrug-resistance (*MDR1*, *ABCB1*) gene.<sup>1,2</sup> This integral membrane protein functions as an energy-dependent drug efflux pump and reduces the intracellular concentrations of a wide range of drugs and xenobiotics. Comprehensive reviews were published recently on this topic.<sup>3,4</sup> Pgp exhibits wide substrate specificity for structurally different drugs and, consequently, mediates drug resistance to a variety of drugs, including Vinca alkaloids, anthracyclines, epipodophyllotoxins, taxols, actinomycin D, cardiac glycosides, immunosuppressive agents, glucocorticoids, and anti-HIV protease inhibitors.<sup>5-7</sup> Since many drugs are substrates of Pgp, its degree of expression and functionality directly affects the therapeutic effectiveness of these agents. In particular, resistance to chemotherapy has become a major obstacle in anti-cancer treatment, but the exact regulatory mechanism of the protein expression remains elusive. One of the possible regulatory sequences was identified in the promoter region.<sup>8</sup> Other studies found random chromosomal rearrangements causing hybrid *MDR1* messages and consequent activation of Pgp expression.<sup>9</sup>

The *MDR1* gene is located on the long arm of chromosome 7 and consists of a core promoter region and 29 exons. The

total gene length was established and reported in 1987 to be at least 70 kilobases (kb),<sup>10</sup> and later in 1990, using multidrug-resistant KB-V1 cells, the *MDR1* gene size was estimated to reach more than 100 kb.<sup>11</sup> Although widely investigated, the exact genomic size has remained uncertain for the past 16 years, with several differing entries in the Genbank database. The online databases list *MDR1* gene length to be between 120 kb (<http://www.infobiogen.fr>) and 210 kb ([www.ncbi.nlm.nih.gov/IEB/Research/Acembly](http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly)).<sup>12,13</sup> The NCBI database (<http://www.ncbi.nlm.nih.gov>) outlines a 209-kb *MDR1* genome.<sup>13</sup> Another source ([www.ensembl.org](http://www.ensembl.org)) enumerates 29 exons within the gene, while Aceview ([www.ncbi.nlm.nih.gov/IEB/Research/Acembly](http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly)) computational analysis predicts 29 exons with 18 alternative exons and 32 confirmed introns, 10 of which are alternative.<sup>14</sup> In addition, alignment of the *MDR1* mRNA with the DNA sequence deposited at the Chromosome 7 Sequencing Project ([www.chr7.org](http://www.chr7.org)) reveals 29 discrete matches suggesting that there are 29 *MDR1* exons. While several changes were made in the databank during the past decade, the latest revision available online still lists the total length of *MDR1* as 209 kb ([NT007933](http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly)) and 6326 base pairs (bp) ([AH002875](http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly), accessed September 2003). The *MDR1* mRNA consists of 4872 bp ([NM000927](http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly)). The reported gene structure is mainly based on very limited cell specimens. Comparative and validation studies with different human tissues or cells are not yet available in the literature.

The aim of this study was to evaluate and validate the overall length of the human *MDR1* gene so that detailed gene structure function studies can be performed with confidence. This knowledge can then serve as a reference when investigating mechanisms of drug resistance due to variation in the *MDR1* gene.

### MATERIALS AND METHODS

Human liver samples harvested from brain-dead donors that had been rejected for transplantation, mostly because of a high degree of fatty infiltration, were obtained from the University of Washington, Seattle, WA, Solid Organ Transplant Program. The human lymphocytes were isolated from blood obtained from the Blood Bank at the University of Washington. Human cell lines (Messa Dox [ovarian carcinoma], CaCo2 [colon carcinoma], and UW 228 [primary human medulloblastoma cells]) were purchased from American Type Culture Collection (ATCC, Manassas, VA). Genomic DNA was extracted and purified from the samples

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Table 1. Experimental Design of PCR and Southern Blot Analysis, and the Expected Products According to the 2 Reference Sequences of the *MDR1* Gene\*

Analytical Method		Expected Product and Fragment According to the Respective Size of <i>MDR1</i>	
PCR Analysis		6.3 kb <i>MDR1</i> Length (AH_002875)	209 kb <i>MDR1</i> Length (NT_007933)
Primers	Primer Sequence 5' to 3'		
F19	CCCATCATTGCAATAGCAGG	190 bp	10 573 bp
R19	GTTCAAACCTTCTGCTCCTGA		
F27	TCCAGTTTCCTTTTGGAGGA	214 bp	2835 bp
R27	TGTCGTTTTGTTTCAGGATCA		
F28	TGGTTTCGATGATGGAGTCA	285 bp	1112 bp
R28	AGCTTCTGTCTTGGGCTTG		
Southern Blot Analysis			
Restriction Enzyme Pairs	Radiolabeled Probe	Target Regions	
<i>Bam</i> HI/ <i>Pac</i> I	P1	Introns 8 and 9-14	6 kb
<i>Bam</i> HI/ <i>Kpn</i> I	P2	Introns 15-19 and 20-21	5 kb
<i>Bam</i> HI/ <i>Pac</i> I	P3	Introns 21 and 15-20	6 kb

\*PCR indicates polymerase chain reaction; kb, kilobase; and bp, base pair.

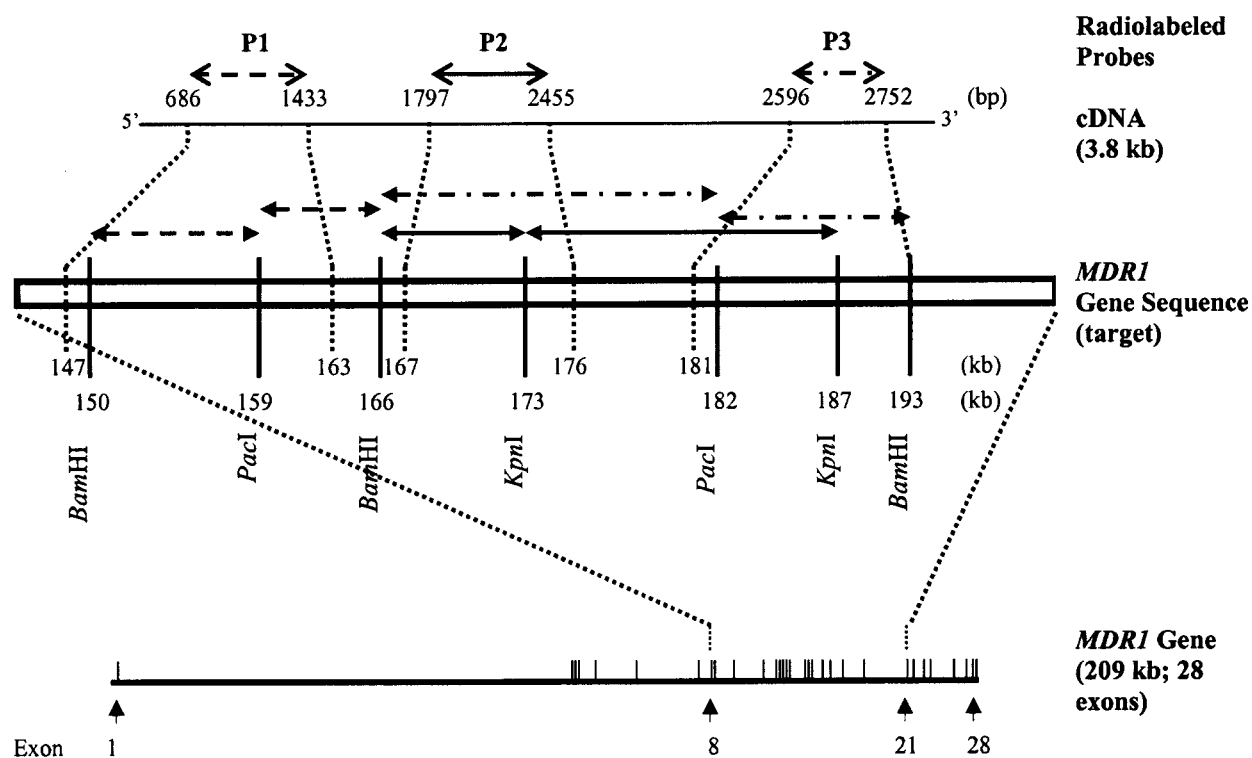
using the Puregene DNA Isolation Kit (Gentra Systems Inc, Minneapolis, MN). Primers were designed with an online primer design software program (Primer 3) and synthesized by Invitrogen Co (Carlsbad, CA). The list of primers and their target *MDR1* products are listed in Table 1. In the case of primer sets F27-R27 and F28-R28, the reaction mixtures (50 µL total volume) contained 100 ng genomic DNA, 200 µM each dNTP, 3.0 mmol MgCl<sub>2</sub>, 4.0 units of Taq polymerase (Promega, Madison, WI), 10 mmol of primers, 5.0 µL Thermo DNA Poly 10X reaction buffer (Promega, Madison, WI), and nuclease-free H<sub>2</sub>O. Thermocycling was conducted in a PerkinElmer DNA Thermocycler (model 2400, Foster City, CA) with the following profile: initial denaturation at 94°C for 3 minutes immediately followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute. In the case of the F19-R19 primer set, designed to produce over 10 kb of product, the same amount of genomic DNA was amplified in 2 units of rTth DNA polymerase (PerkinElmer), containing 10 mmol of primers, 200 µmol each dNTP, 15 µL 3.3X XL buffer, 2.4 µL Mg(OAc)<sub>2</sub> solution, and nuclease-free H<sub>2</sub>O. The long-range polymerase chain reaction (PCR) was conducted as follows: initial denaturation at 94°C for 1 minute followed by 16 cycles of 94°C for 15 seconds, 58°C for 30 seconds, and 68°C for 10 minutes. This was immediately followed by 12 cycles of 94°C for 15 seconds, 58°C for 30 seconds, and 68°C for 10 minutes with an incremental increase of 15 seconds per cycle. Products were visualized in a 2% agarose gel stained with ethidium bromide.

For the Southern Blot analysis, 10 µg of genomic DNA was digested to completion with the restriction endonuclease

pairs *Bam*HI/*Pac*I and *Bam*HI/*Kpn*I obtained from New England Biolabs (Beverly, MA). The DNA was electrophoresed on a 0.8% agarose gel, transferred to a nitrocellulose membrane, and hybridized with <sup>32</sup>P-labeled probes prepared by random primed incorporation of α<sup>32</sup>P-dATP by Klenow DNA polymerase (New England Biolabs). These probes were obtained by first generating 3 different *MDR1* cDNA regions using primer sets F7 (CTGCTGTCTGGGCAAAGATAC)-R7 (ACAGGTTCTGACTCACCAC), F28-R29 (CGAGCCTGGTAGTCAATGCT), and F19-R19 (see Table 1 for sequence), designated as P1, P2, and P3, respectively (Figure 1). Probe P1 is labeled as target for introns 8 and 9 to 14; probe P2 is labeled as target for introns 15 to 19 and 20 to 21, and probe P3 is labeled as target for introns 21 and 15 to 20.

## RESULTS AND DISCUSSION

We have chosen a critical region of the *MDR1* gene (spanning exons 8-21; 43 kb), which would distinguish the 6.3- vs 209-kb version of *MDR1* gene for detailed analysis. To do so, we isolated DNA from liver samples, continuous cell lines expressing Pgp, and human lymphocytes. These DNA samples were analyzed with 3 regions of the *MDR1* gene and 3 different primer sets were used to amplify the respective gene sequences by PCR to distinguish the 2 versions of *MDR1*. The predicted lengths for 6.3-kb (AH002875) vs 209-kb (NT007933) *MDR1* gene amplicons are shown in Table 1. The sequences of the primer pairs F19-R19, F27-R27, and F28-R28 were designed to yield significant differences in the product sizes according to the 2 proposed gene lengths. The



**Figure 1.** Schematic representation of restriction map based on the estimated 209-kb length and the radiolabeled cDNA fragments used as probes. The corresponding hybridization targets (arrows) are marked. Radiolabeled probes, P1, P2, and P3 were generated with F7-R7, F28-R29, and F19-R19 primer sets (Table 1), respectively.

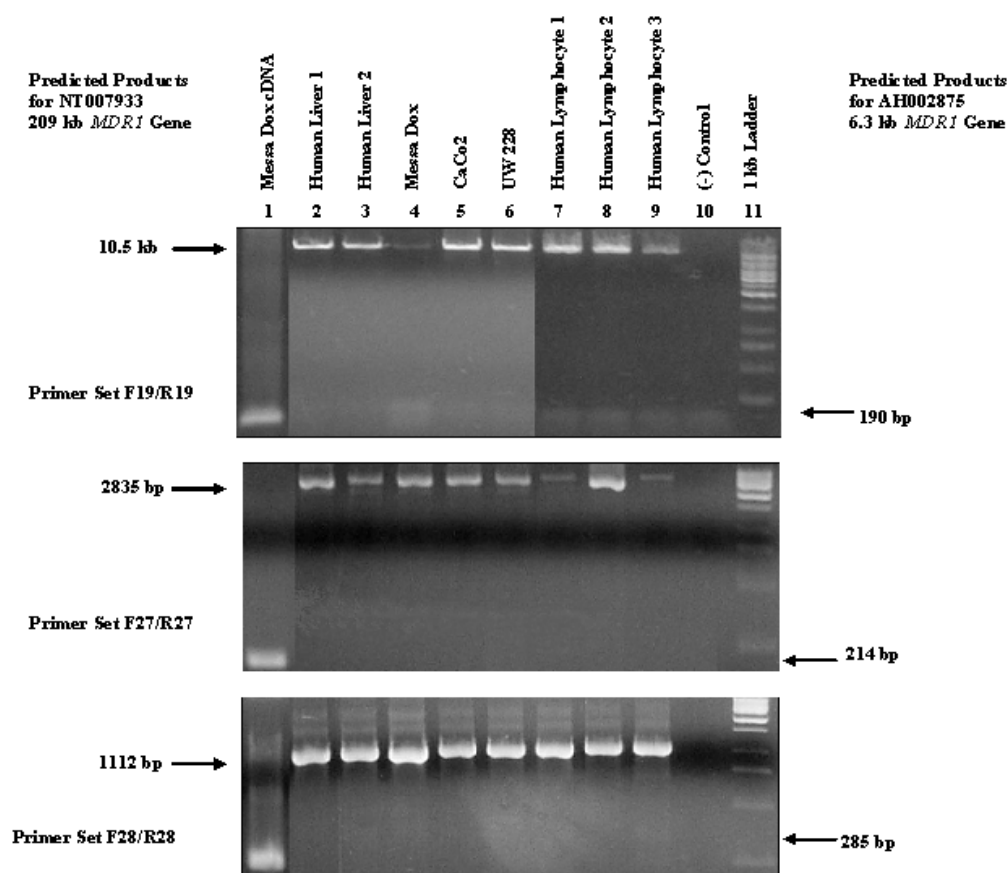
3 primer sets are designed to probe the sequences spanning introns 15, 17, and 21. Table 1 also lists the expected results of Southern Blot analysis for the 2 candidate sequences for *MDR1*. The schematics of restoration map of the 209-kb *MDR1* gene sequence (accession number [NT007933](#)) is presented in Figure 1.

With DNA isolated from 4 human livers, 3 human lymphocytes, and established cell lines—Messa Dox, CaCo2, and UW 228—we performed PCR with the 3 sets of primers listed in Table 1, and the results are shown in Figure 2. We were not able to detect any product in the reactions corresponding to the 6.3-kb *MDR1* candidate sequence. Instead, we found for all the liver tissues (2), lymphocytes (3), and cell lines (3), the PCR products corresponded to the length predicted with a 209-kb *MDR1* sequence ([NT007933](#)). As shown in Figure 2, the products of F19/R19, F27/R27, and F28/R28 were 10.5, 2.84, and 1.1 kb, respectively. The inability to detect products of short fragments was not due to PCR conditions, as the control *MDR1* cDNA derived from Messa Dox resulted in positives for all the reactions with the 3 sets of primers (Figure 2).

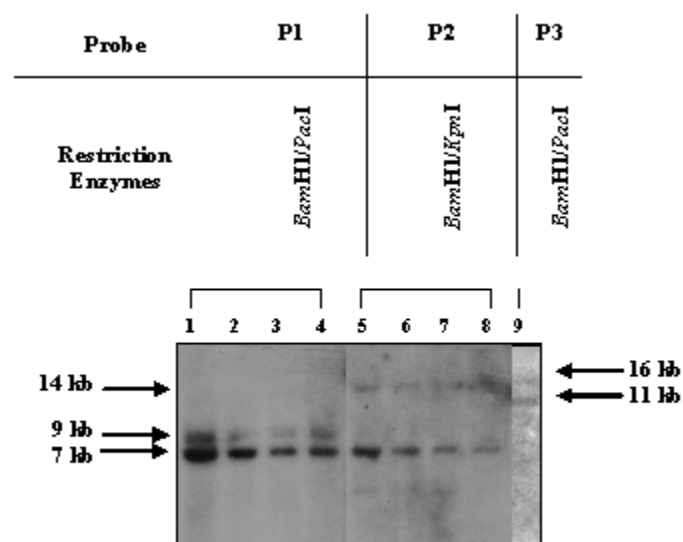
The PCR results were verified further with Southern Blot analysis to probe introns 8 to 21 (Figure 1). We found that the radiolabeled probes P1-P3 pointed to 209-kb *MDR1* sequence candidate. With *Bam*HI/*Pac*I and P1, we found 7 and 9 kb; with *Bam*HI/*Kpn*I and P2, we found 7 and 14 kb;

and finally with *Bam*HI/*Pac*I and P3, we found 11- and 16-kb fragments (Table 1 and Figures 1 and 3). The bands correspond to the estimates shown in Table 1 for the more than 200-kb gene length. Again, there were no DNA bands corresponding to the length (5 and 6 kb) of product predicted by the 6.3-kb total gene size. Collectively, the above data strongly suggest the *MDR1* gene sequence is consistent with [NT007933](#).

As the Genbank data are often based on the analysis of limited DNA specimens, we used several different human cell lines as well as lymphocyte and liver samples to investigate eventual differences between tissues and/or subjects regarding the *MDR1* gene locus. Both sets of experiments confirm the length of the *MDR1* gene being much longer than 6.3 kb, and it is most likely 209 kb as indicated in the database (accession number [NT007933](#)). The results were consistent in the cases of different human liver tissue samples, several cell lines, and lymphocytes, showing that there is very little, if any, interindividual variation with respect to the gene length (Figures 2 and 3). The recent research interest to characterize differences in gene expression related to variation in drug response among individuals has begun to provide some clues to *MDR1* gene regulatory mechanisms. Discoveries of *MDR1* sequence variation in contig sequences have provided some clues. However, detailed studies of *MDR1* gene regulation require a validated *MDR1* sequence. Toward this end, we have provided data validating the 209-kb *MDR1* sequence.



**Figure 2.** PCR analysis of the 3 *MDR1* gene sequences using F19-R19, F27-R27, and F28-R28 primer sets (Table 1), respectively. Lane 1 is cDNA of Messa Dox cells; lanes 2 and 3 are 2 different liver samples; lanes 4, 5, and 6 are Messa Dox, Caco2, and UW 228 cell lines; and lanes 7, 8, and 9 are 3 different lymphocyte samples. Lane 10 is a negative control in which no DNA was added, and lane 11 is a 1-kb ladder. There were amplifications in all the samples according to the 209-kb gene length, having sizes according to Table 1. There were no bands that showed up according to a 6.3-kb total *MDR1* gene length. Results for Messa Dox cDNA were added to show reaction sensitivity for short fragments as well; the PCR products were 167 bp, 171 bp, and 249 bp for primer sets F19-R19, F27-R27, and F28-R28, respectively.



**Figure 3.** Southern Blot analysis of *MDR1* gene with DNA isolated from human livers. Genomic human liver DNA was subjected to *BamHI/PacI* (columns 1-4), *BamHI/KpnI* (columns 5-8), and *BamHI/PacI* (column 9) restrictions and P1, P2, and P3 radiolabeled probes, accordingly.

The results of our study confirming the size of the *MDR1* gene will be of great utility when extended to analyses of mechanisms of drug-induced resistance. In other words, knowledge of both intron-exon structure and overall gene length can be used to differentiate between aberrant mRNA splicing and gene deletions in multidrug resistance. In addition, there are several references in the literature regarding “mini-Pgp,”<sup>15,16</sup> but there have been no molecular analyses to explain this phenomenon or its cause; our reference for *MDR1* gene structure will aid in these investigations.

In summary, data presented in this report have clarified and established that *MDR1* gene length is likely to be 209 kb.

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