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Constitutional Mismatch Repair-Deficiency Syndrome Is a Rare Cause of Cancer Even in a Highly Consanguineous Population*

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ABSTRACT

Biallelic germline mutations in the mismatch repair genes, including *MLH*1, *MSH*2, *MSH*6 or *PMS*2, lead to a recessive constitutional mismatch repair-deficiency (CMMR-D) syndrome characterized by early onset malignancies in children and young adults. Because consanguinity unmasks autosomal recessive disorders, we hypothesized that the frequency of CMMR-D is inflated in the highly consanguineous population of Saudi Arabia. In this study, 371 pediatric and young adult patient samples from Saudi Arabia that cover the tumor spectrum of CMMR-D syndrome were analyzed for biallelic germline mutations in the *MLH*1, *MSH*2, *MSH*6 and *PMS*2 with the use of direct genomic sequencing. However, none of the 371 patients involved in the study was found to have biallelic pathological mutations of *MLH*1, *MSH*2, *MSH*6 or *PMS*2. This result indicates that CMMR-D is exceptionally rare among pediatric cancer patients and adult early onset cancer patients, even in the highly consanguineous Saudi population. Our findings suggest that larger cohorts will be needed, particularly in outbred populations, to determine the frequency of CMMR-D and that routine screening for this syndrome among cancer patients is not warranted.

Keywords: Constitutional Mismatch Repair-Deficiency (CMMR-D); Lynch Syndrome (LS); *MLH*1; *MSH*2; *MSH*6 and PMS2; Biallelic Germline Mutations

1. Introduction

The mismatch repair (MMR) system contributes to genome integrity and DNA replication fidelity; and the *MLH*1, *MSH*2, *MSH*6 and *PMS*2 genes play a major role in this process [1]. MMR primarily remedies base-base mismatches and small insertion deletion loops (IDLs) that may occur during DNA replication [2]. In order to achieve this goal this mismatch repair machinery has to distinguish and to be directed to the newly synthesized strand from the template that carries the erroneous genetics information [3].

Heterozygous germline mutations in *MLH*1, *MSH*2, *MSH*6 and *PMS*2 cause Lynch syndrome (LS), an autosomal dominant cancer syndrome. It is the most common cause of hereditary colon cancer and is characterized by an increased risk for colorectal and endometrial cancers, with recent estimates of lifetime risks ranging from 22% to 66% and 14% to 39%, respectively [4-7]. Additional LS-associated cancers may occur most notably ovarian, gastric and renal pelvis cancers although lifetime risk is typically less than 10% for these tumors [8].

While LS patients harbor heterozygous mutant MMR alleles, there are 78 patients from 46 families diagnosed as CMMR-D, a recently recognized autosomal recessively inherited cancer syndrome caused by biallelic germline mutations in one of the MMR genes [9]. The tumour spectrum in CMMR-D syndrome includes child-

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hood malignancies (mainly hematological malignancies and/or brain tumors) and very early onset LS-associated tumors [9-14]. 74% of cases show signs of neurofibromatosis type 1 (NF1), mainly café-au-lait spots (CLS) [14]. In addition, some individual cases have also been reported to be diagnosed with other cancers, including neuroblastoma, Wilms tumors, ovarian neuroectodermal tumor, myofibromatosis and sarcoma [15-18].

Middle Eastern populations in general and Saudi Arabia in particular have high rates of consanguinity, with an average prevalence of first cousin marriages (the most common form of consanguinity) of 57.7% [19,20]. Such high consanguinity rates are known to result in unmasking of autosomal recessive disorders including those that are exceedingly rare. Thus, we reasoned that CMMR-D will be relatively more common in our Saudi population. Since no data exists on the frequency of CMMR-D among patients with the various forms of cancer that are known to be associated with this disorder, we set out to systematically examine a large series of patients with these malignancies for evidence of CMMR-D. Our data suggest that CMMR-D syndrome is exceptionally rare among pediatric and young adult cancer patients even in the highly consanguineous Saudi population.

2. Material and Methods

2.1. Patients Selection

371 Saudi pediatric and young adult archived cancer samples from the tumors spectrum of CMMR-D that have been reported in the literature were selected in this study. Cancer samples were selected based on the presence of any of the following: early onset cancer and patients with multiple tumor types and patients with affected siblings (**Table 1**). All archived samples included in this study were approved by Institutional Review Board (IRB) at King Faisal Specialist Hospital and Research Centre for the following Research Advisory Council (RAC) projects RAC# 2120017, RAC# 2040004 and RAC# 2090012.

2.2. Detection of Mutation and DNA Sequencing Analysis

To search for germline mutations, DNA (obtained from blood or normal tissue) was amplified using PCR primers. Primer pairs that were designed with "Primer 3" software are listed in **Supplementary Table 1**. All exons of the *MLH*1, *MSH*2, *MSH*6 and *PMS*2 genes, including splice junctions, were amplified by polymerase chain reaction (PCR). PCR was performed in 25 µl reactions volume containing 0.2 mM dNTPs, 0.2 units of Hot Star DNA polymerase (Qiagen), 0.2 µl forward and reverses primer respectively, 2.3 mM $MgCl_2$ and 20 ng of DNA. The PCR conditions was as follows: 95˚C for 10 min followed by 35 cycles of 95˚C for 45 s, 60˚C for 45 s and 72˚C for 1 min, and ending with an elongation step at 72˚C for 10 min. The PCR products were purified with AMPure (Agencourt, Danvers, MA) Kit and then subjected to automated cycle sequencing with the ABI Prism Big Dye Terminator Cycle v3.1 sequencing kit (Applied Biosystems, Foster City, CA). Sequence analysis was performed using the Mutation Surveyor v3.24 (Soft-genetics LLC, State College, PA) software. Reference sequence of all genes was downloaded from GenBank.

Table 1. Patient selection criteria and tumor type.

Sr no.	Systems	Total cases	Age	Median (25^{th}% - 75^{th}% tile)
I	**Hematological malignancies**	**215**		
i	ALL	84	<18 yrs	4.4 (2.9 - 7.0)
ii	AML	3	<18 yrs	
iii	DLBCL	28	<18 yrs	15.0 (9.5 - 16.0)
iv	Pediatric Hodgkins	91	<18 yrs	8.0 (5.0 - 11.0)
v	T-NHL	9	<18 yrs	11.0 (6.0 - 13.0)
II	**Brain**	**57**	<18 yrs	6.0 (4.0 - 10.7)
i	Glioblastoma	3		
ii	Astrocytoma	32		
iii	Medulloblastoma	22		
III	**Others**	**99**		
	Rhabdomyosarcoma	49	<18	3.0 (5.0 - 11.5)
i	Embryonal	26		
	Alveolar	4		
	Plemorphic	0		
	Not Otherwise Specified (NOS)	19		
ii	Thyroid Ca	30	<18	13.0 (11.7 - 16.4)
iii	Wilms tumor	20	<18	2.5 (2.0 - 5.8)
	Total	**371**		

3. Results

Previous studies suggest that the clinical features tend to differ in patients carrying biallelic germline mutation in different MMR genes. *MLH*1 and *MSH*2 mutation appear to present more frequently than *MSH*6 and *PMS*2 mutation in patients with hematologic malignancies. In contrast, the *MSH*6 and *PMS*2 biallelic germline mutation is more commonly detected in patient with brain tumor. Furthermore, the *MLH*1 and *MSH*2 mutation carriers have tendency to develop tumor earlier than those with *MSH*6 and *PMS*2 mutations. Despite the clear predominance of different type of MMR genes in different tumor types, to fully screen for CMMR-D in our study

we sequenced all the 4 MMR genes that are known to be involved in the CMMR-D tumor spectrums. However, in spite of this comprehensive approach none of the 371 patients analyzed were found to have biallelic pathological mutations of *MLH*1, *MSH*2, *MSH*6 or *PMS*2.

4. Discussion

Careful search for CMMR-D Syndrome among pediatric cancer patients is warranted by the accumulation of reports describing more patients suffering from this syndrome [11]. The recognition of the actual incidence of this syndrome allows to: 1) understand the role of impaired MMR in the pathogenesis of sporadic cancers such as hematological malignances, brain tumors, sarcomas, which deserves further investigation; 2) develop specific therapeutic considerations for the patient with CMMR-D syndrome due to the MMR-defect caused resistance to certain chemotherapeutic agents [21-24]; 3) establish surveillance/guidelines for CMMR-D syndrome families e.g. parents are obligate carriers; and finally 4) prevent this new syndrome and misdiagnosis of this new syndrome and NF1. Given the significant clinical implications for diagnosis, prognosis, therapy and family counseling and the scarcity of data regarding CMMR-D among general population [9], the prevalence of CMMR-D among young cancer patients requires further characterization.

The results of the current study demonstrate that the prevalence of CMMR-D among young cancer patients in Saudi population is exceedingly low. There are no available data to compare our result with, since our knowledge of the CMMR-D syndrome originates primarily from case reports. To our knowledge, this is the largest study ever done to delineate the prevalence of CMMR-D among pediatric and young adult cancer patients. An additional strength of our study is the inclusion of samples from the tumor spectrum of CMMR-D that have been reported in the literature. These include four groups of tumors: 1) hematological malignancies [10]; 2) brain tumors [25,26]; and others [17]. We also chose to sequence all four genes known to cause CMMR-D instead of resorting to easier screening methods like microsatellite instability (MSI) and or immunohistochemistry (IHC). Typically, confirmation of the diagnosis of CMMR-D involves the analysis of MSI and/or IHC followed by mutational analysis [10,27] but since MSI analysis can be unreliable in CMMR-D-related brain tumors and IHC can show normal results in CMMR-D cases with underlying missense mutation [9,11,26,28], we decide to go directly to confirmatory mutation analysis of all MMR genes known to be involved in CMMR-D including *MLH*1, *MSH*2, *MSH*6, *PMS*2 despite the markedly higher cost associated with this approach.

Although a size of 371 samples that represent the spectrum of CMMR-D-related cancers and of the right age appears large, we concede that our sample size calculation was complicated by the lack of any published case series that systemically investigate the frequency of CMMR-D. So while it is clear that a larger cohort is required to identify CMMR-D, the minimal size required is difficult to predict. Nevertheless, our data show that the frequency is <0.0027 (<1/371) and since this estimate is based on a consanguineous population in which autosomal recessive disorders are usually higher in frequency, we expect that the frequency will be even lower in outbred populations. Another implication from our study is that CMMR-D is not an important causal factor in the unexplained trend of high frequency of CRC in our population and the younger age distribution [29-31]. This leaves open the possibility of other recessive loci contributing to this pattern and/or perhaps other environmental factors, an area of research that we are currently pursuing. Until then, our data suggest that routine screening for CMMR-D among Saudi pediatric cancer patients is not recommended.

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Supplementary

Table 1. Primer sequence used in MLH1, MSH2, MSH6 and PMS2 mutation analysis.

Gene	Exon	Forward Primer	Reverse Primer
MLH1	Exon1	GTAAAACGACGGCCAGTACGTTTCCTTGGCTCTTCTG	CAGGAAACAGCTATGACCCATGCGCTGTACATGCCTCT
	Exon2	GTAAAACGACGGCCAGTTTGTTATCATTGCTTGGCTCA	CAGGAAACAGCTATGACCATGAAGCGCACAAACATCCT
	Exon3	GTAAAACGACGGCCAGTTGGGAATTCAAAGAGATTTGG	CAGGAAACAGCTATGACCTGACAGACAATGTCATCACAGG
	Exon4	GTAAAACGACGGCCAGTGTGAGGTGACAGTGGGTGAC	CAGGAAACAGCTATGACCCACTGGTGTTGAGACAGGATT
	Exon5	GTAAAACGACGGCCAGTTCTCTTTTCCCCTTGGGATT	CAGGAAACAGCTATGACCTCCAATATTTATACAAACAAAGCTTCA
	Exon6	GTAAAACGACGGCCAGTGCCAGGACATCTTGGGTTT	CAGGAAACAGCTATGACCTGTTCAATGTATGAGCACTAGAACAC
	Exon7	GTAAAACGACGGCCAGTAAAAGGGGGCTCTGACATCT	CAGGAAACAGCTATGACCCTCATGGCTGAGACTGAAACA
	Exon8	GTAAAACGACGGCCAGTTCAGCCATGAGACAATAAATCC	CAGGAAACAGCTATGACCTGTGATGGAATGATAAACCAAGA
	Exon9	GTAAAACGACGGCCAGTTGGGAAGGAACCTTGTGTTT	CAGGAAACAGCTATGACCGTGGATTTCCCATGTGGTTC
	Exon10	GTAAAACGACGGCCAGTTCACCCCTCAGGACAGTTTT	CAGGAAACAGCTATGACCTGTTCCTTGTGAGTCTTGGTTG
	Exon11	GTAAAACGACGGCCAGTCCATATGTGGGCTTTTTCTCC	CAGGAAACAGCTATGACCTCAAAGGCCCCAGAGAAGTA
	Exon12A	GTAAAACGACGGCCAGTCTCCATTTGGGGACCTGTAT	CAGGAAACAGCTATGACCACTGGACAGGGGTTTGCTC
	Exon12B	GTAAAACGACGGCCAGTGGAACAGAAGCTTGATGCATTT	CAGGAAACAGCTATGACCACTTTTCCCAAAAGGCCATA
	Exon13	GTAAAACGACGGCCAGTTGCTCCTCCAAAATGCAAC	CAGGAAACAGCTATGACCAGTTGAGGCCCTATGCATCC
	Exon14	GTAAAACGACGGCCAGTATGAAGTGGGGTTGGTAGGA	CAGGAAACAGCTATGACCTAGCTTTTGTGCCTGTGCTC
	Exon15	GTAAAACGACGGCCAGTTTCAGGGATTACTTCTCCCATT	CAGGAAACAGCTATGACCGGAGAGCTACTATTTTCAGAAACG
	Exon16	GTAAAACGACGGCCAGTGATGCTCCGTTAAAGCTTGC	CAGGAAACAGCTATGACCACCCGGCTGGAAATTTTATT
	Exon17	GTAAAACGACGGCCAGTGGAAAGCACTGGAGAAATGG	CAGGAAACAGCTATGACCCATGCATGTACCGAAATGCT
	Exon18	GTAAAACGACGGCCAGTTGATCTCCGTTTAGAATGAGAATG	CAGGAAACAGCTATGACCCTAGTCCTGGGGTGCCAGT
	Exon19	GTAAAACGACGGCCAGTGATGCAAACAGGGAGGCTTA	CAGGAAACAGCTATGACCTCGGAATACAGAGAAAGAAGAACA
MSH2	Exon1A	GTAAAACGACGGCCAGTGAAACAGCTTAGTGGGTGTGG	CAGGAAACAGCTATGACCACCCCCTGGGTCTTGAAC
	Exon1B	GTAAAACGACGGCCAGTTCGTGCGCTTCTTTCAGG	CAGGAAACAGCTATGACCTCTCTGAGGCGGGAAAGG
	Exon2A	GTAAAACGACGGCCAGTGCAGCATGAAGTCCAGCTAA	CAGGAAACAGCTATGACCTGCCAAATACCAATCATTCTC
	Exon2B	GTAAAACGACGGCCAGTTTTTGTAAAAGATCTTCTTCTGGTTC	CAGGAAACAGCTATGACCTGCTATTAAAGTGTCTCAAACCATTC
	Exon3A	GTAAAACGACGGCCAGTTTGGATTTTTCCTTTTTGCTT	CAGGAAACAGCTATGACCGGGAATTCACACAGTCCTAGTTTC

Continued

Exon3B	GTAAAACGACGGCCAGTGGTGTTGTGGGTGTTAAAATGTC	CAGGAAACAGCTATGACCGCCTGGAATCTCCTCTATCAC
Exon4A	GTAAAACGACGGCCAGTGAGGAATTCTGATCACAGAAAGAA	CAGGAAACAGCTATGACCTGACAGAAATATCCTTCTAAAAAGTCA
Exon4B	GTAAAACGACGGCCAGTTGCTTTTCTTATTCCTTTTCTCA	CAGGAAACAGCTATGACCCCTTTTTGCCTTTCAACAAC
Exon5A	GTAAAACGACGGCCAGTCCAAGGAAAATGAGGGACTTC	CAGGAAACAGCTATGACCTCTGACTGCTGCAATATCCAA
Exon5B	GTAAAACGACGGCCAGTTGCAGTTTCATCACTGTCTGC	CAGGAAACAGCTATGACCCCATTCAACATTTTTAACCCTTT
Exon6	GTAAAACGACGGCCAGTAATGAGCTTGCCATTCTTTC	CAGGAAACAGCTATGACCGGTATAATCATGTGGGTAACTGC
Exon7A	GTAAAACGACGGCCAGTTGGAATTTTGAGCTGATTTAGTTG	CAGGAAACAGCTATGACCTTTGCTGCTTGTCTTTGAAAC
Exon7B	GTAAAACGACGGCCAGTAAGATGCAGAATTGAGGCAGA	CAGGAAACAGCTATGACCGGACAGCACATTGCCAAGTA
Exon8	GTAAAACGACGGCCAGTTCAGTCAAAATTTTATGATTTGTATTC	CAGGAAACAGCTATGACCTTTCTTAAAGTGGCCTTTGCTT
Exon 9	GTAAAACGACGGCCAGTTTTGTCACTTTGTTCTGTTTGC	CAGGAAACAGCTATGACCAAGTCATCATCTTGGGGACA
Exon 10A	GTAAAACGACGGCCAGTTGAAAAATGGTAGTAGGTATTTATGGA	CAGGAAACAGCTATGACCGGTAAATTTAACACCATTCTTCTGG
Exon 10B	GTAAAACGACGGCCAGTGGACCCTGGCAAACAGATTA	CAGGAAACAGCTATGACCCACATCATGTTAGAGCATTTAGGG
Exon 11	GTAAAACGACGGCCAGTTCATAGGATACTTTGGATATGTTTCA	CAGGAAACAGCTATGACCAAAGCCAGGTGACATTCAGA
Exon 12A	GTAAAACGACGGCCAGTGGGTTTTGAATTCCCAAATG	CAGGAAACAGCTATGACCGCATGCCTGGATGCTTTTA
Exon 12B	GTAAAACGACGGCCAGTTTTGCTCACGTGTCAAATGG	CAGGAAACAGCTATGACCACAAAACGTTACCCCCACAA
Exon 13A	GTAAAACGACGGCCAGTATTCGACAAACTGGGGTGAT	CAGGAAACAGCTATGACCCTTCAAGGGACTAGGAGATGC
Exon 13B	GTAAAACGACGGCCAGTCAATCCATTTATTAGTAGCAGAAAGA	CAGGAAACAGCTATGACCTGCAGTCCACAATGGACACT
Exon 14A	GTAAAACGACGGCCAGTTGGCATATCCTTCCCAATGT	CAGGAAACAGCTATGACCTTGGCCAAGGCAGTAAGTTC
Exon 14B	GTAAAACGACGGCCAGTCGATGGATTTGGGTTAGCAT	CAGGAAACAGCTATGACCTTTCCCATTACCAAGTTCTGA
Exon 15A	GTAAAACGACGGCCAGTCAAGGTGAGAAGGATAAATTCCA	CAGGAAACAGCTATGACCTTGCTGCTGGTTCCATGATA
Exon 15B	GTAAAACGACGGCCAGTTTTGGGATTCATGTTGCAGA	CAGGAAACAGCTATGACCAAAAACCTTCATCTTAGTGTCCTGTT
Exon 16	GTAAAACGACGGCCAGTTGAAACAATTTGTCACTGTCTAACAT	CAGGAAACAGCTATGACCCCATTACTGGGATTTTTCACG
Exon 1A	GTAAAACGACGGCCAGTCAGAACGGTTGGGCCTTG	CAGGAAACAGCTATGACCGTTGAGGTTCTTCGCCTTGG
Exon 1B	GTAAAACGACGGCCAGTAGCTTCTTCCCCAAGTCTCC	CAGGAAACAGCTATGACCCTGCACTCATTCAAGCCAAC
Exon 2A	GTAAAACGACGGCCAGTGCCTTTAAGGAAACTTGACCA	CAGGAAACAGCTATGACCCACGGACTGATTTCCCTTTC
Exon 2B	GTAAAACGACGGCCAGTGGTGGCCTTGTCTGGTTTAC	CAGGAAACAGCTATGACCCAAACACACACACATGGCAGT

Continued

Exon 3	GTAAAACGACGGCCAGTACCCGGCCCTTATTGTTTAT	CAGGAAACAGCTATGACCTCCCCCATCACCCTAACATA
Exon 4A	GTAAAACGACGGCCAGTGGTTTTCCAAATTTTGATTTGTTT	CAGGAAACAGCTATGACCCACTATCCCCCACTCCACTG
Exon 4B	GTAAAACGACGGCCAGTACATTGGTGGCTCTGATGTG	CAGGAAACAGCTATGACCGGGCTTGGGATTCAGAATTT
Exon 4C	GTAAAACGACGGCCAGTCAGCCACCAAACAAGCAACT	CAGGAAACAGCTATGACCATCTGCCACCACTTCCTCAT
Exon 4D	GTAAAACGACGGCCAGTCTGATCACCCCGATTTTGAT	CAGGAAACAGCTATGACCCTTCTGCACCAGGGAATCTG
Exon 4E	GTAAAACGACGGCCAGTCAGTGAACTGGGGCTGGTAT	CAGGAAACAGCTATGACCTCAGAGGGATCACCTTCCAG
Exon 4F	GTAAAACGACGGCCAGTTGGTGAGGAGGGAGATCTGT	CAGGAAACAGCTATGACCTTGTACTGGGGGATAGTGTGC
Exon 4G	GTAAAACGACGGCCAGTTTTTCAGATGATCGCCATTG	CAGGAAACAGCTATGACCAGCACCTGGGGTAACATCAC
Exon 4H	GTAAAACGACGGCCAGTATACCCGGCTCCCAGTTTT	CAGGAAACAGCTATGACCTGCTGACTGTGTCAGAATCCA
Exon 4I	GTAAAACGACGGCCAGTAAGTGAATTGGCCCTCTCTG	CAGGAAACAGCTATGACCTTAGGAGCCGCTTACCAAAA
Exon 4J	GTAAAACGACGGCCAGTTGGTGCTAGATGCAGTGACA	CAGGAAACAGCTATGACCTCAGGGGAGACCCAACATT
Exon 4K	GTAAAACGACGGCCAGTGCTTCCAGATCTTGAGAGGCTA	CAGGAAACAGCTATGACCCAGGAAAACGACCTTCAGGA
Exon 4L	GTAAAACGACGGCCAGTGGGATCATGGAAGAAGTTGC	CAGGAAACAGCTATGACCCCAATTCTGTTGCGCTGTT
Exon 4M	GTAAAACGACGGCCAGTTGACCAAGCTCTTGCTGACA	CAGGAAACAGCTATGACCCCCTCCGTTCTTCAGCATT
Exon 4N	GTAAAACGACGGCCAGTCCAAGAAGGGCTGTAAACGA	CAGGAAACAGCTATGACCGGGATAATATACAGCTGGCAAA
Exon 5A	GTAAAACGACGGCCAGTTAAAACCCCCAAACGATGAA	CAGGAAACAGCTATGACCTTTCCTGCTCCTCTTCCTCA
Exon 5B	GTAAAACGACGGCCAGTGCCATCCTTGCATTACGAAG	CAGGAAACAGCTATGACCCTGTGTTTGGAAAATGATCACC
Exon 6	GTAAAACGACGGCCAGTCGTAAGGGTTCATAAGAAAGACAAA	CAGGAAACAGCTATGACCTGAGAACTTAAGTGGGAAACAAAA
Exon 7	GTAAAACGACGGCCAGTCCGGCCAATAATTGCATAGT	CAGGAAACAGCTATGACCGTCTTCAAATGAGAAGTTTAATGTCTT
Exon 8	GTAAAACGACGGCCAGTTTCCTTTGAGTTACTTCCTTATGC	CAGGAAACAGCTATGACCAAAAACCGAATTTGTGGAAAA
Exon 9	GTAAAACGACGGCCAGTTGCTAGCACATGTATCGCTAA	CAGGAAACAGCTATGACCTGTTTCTTTGAAACTTAAGGTCAGT
Exon 10	GTAAAACGACGGCCAGTGGAAGGGATGATGCACTATG	CAGGAAACAGCTATGACCTTGTCTGAATTTACCACCTTTG
Exon 1	GTAAAACGACGGCCAGTGAGTTCAGGAGGCGGAG	CAGGAAACAGCTATGACCGTGGGTCTCAAAGAGGGC
Exon 2A	GTAAAACGACGGCCAGTTGTTGAGTCATTTCCCACAGTT	CAGGAAACAGCTATGACCACTCCTTTACCGCAGTGCTT
Exon 2B	GTAAAACGACGGCCAGTAGAACCTGCTAAGGCCATCA	CAGGAAACAGCTATGACCCAACAACATTCACAGATCATTTCTT
Exon 3-4	GTAAAACGACGGCCAGTGTGCAACGCGGTTTACAG	CAGGAAACAGCTATGACCACTTAGATTGGCAGCGAGAC
Exon 5A	GTAAAACGACGGCCAGTCCCAACATCATGGGTCTCTC	CAGGAAACAGCTATGACCAAATTCCTTATGGCGCACAG
Exon 5B	GTAAAACGACGGCCAGTGGTTGGAACTCGACTGATGTT	CAGGAAACAGCTATGACCTGCTCATGTGCATTAACCAA
Exon 6A	GTAAAACGACGGCCAGTTGGATGTTGTAACTTGAGCTGTG	CAGGAAACAGCTATGACCCTTCCACCTGTGCATACCAC

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Exon 6B	GTAAAACGACGGCCAGTAGCAGGCATCCGTGTAAGTT	CAGGAAACAGCTATGACCACTGGAAGGGACAATGGAAA
Exon 7	GTAAAACGACGGCCAGTGAAAGAAAACAGATTAAGTCCACTCTG	CAGGAAACAGCTATGACCTGTAGTTCTCTTGCCAGCAATC
Exon 8	GTAAAACGACGGCCAGTGTGCCATGTGATCGTATTTC	CAGGAAACAGCTATGACCAGTTATCAATTAAAAGTCAAAGGC
Exon 9	GTAAAACGACGGCCAGTGGGCTGGGAACATTTGTCAT	CAGGAAACAGCTATGACCTTGTACTGAAATGCCAATGGA
Exon 10	GTAAAACGACGGCCAGTAGCCTAGGCGACAGAGTGAG	CAGGAAACAGCTATGACCAAGCTTTAGAAGCTGTTTGTACACTG
Exon 11A	GTAAAACGACGGCCAGTCTCCGTCCACGTTTGCTTAG	CAGGAAACAGCTATGACCGCTAGAAGACAGCATACCCCTTT
Exon 11B	GTAAAACGACGGCCAGTGAAGGAGCCCTCTAGGACAGA	CAGGAAACAGCTATGACCGGAGCTGGCCGCATACTC
Exon 11C	GTAAAACGACGGCCAGTGCAGCACTTCCGTGGATT	CAGGAAACAGCTATGACCGTGTTTGGGGTTGCGAGAT
Exon 11D	GTAAAACGACGGCCAGTAAAGCGCCTAAAACTGACGA	CAGGAAACAGCTATGACCCCAGGGGCACAACTTTCTTA
Exon 11E	GTAAAACGACGGCCAGTCAGGACATGTCAGCCTCTCA	CAGGAAACAGCTATGACCCTTATCTCTTTTCTTAGTTCATCTTCG
Exon 12A	GTAAAACGACGGCCAGTGGGACTTTATTTTGTTTTTGTATTG	CAGGAAACAGCTATGACCGCTGCAGCATCTCGAAGTTA
Exon 12B	GTAAAACGACGGCCAGTCGATGTTTGCAGAAATGGAA	CAGGAAACAGCTATGACCTCCTGCCTTGGCCTCTATTA
Exon 13	GTAAAACGACGGCCAGTTTGTTTTCATTTCATTTCTGCTG	CAGGAAACAGCTATGACCTGCTGGGATTACAGACGTGA
Exon 14A	GTAAAACGACGGCCAGTCTCCCAAAGTGCAGGGATTA	CAGGAAACAGCTATGACCGGTCCGAAGGTCCAGTTTTT
Exon 14B	GTAAAACGACGGCCAGTTCCAGTCACTGAAAGGGCTAA	CAGGAAACAGCTATGACCGAGAACGCAGACGACACAGA
Exon 15	GTAAAACGACGGCCAGTAACTACTAAAACGTTGAACC	CAGGAAACAGCTATGACCTTTTTGAGACACAGTCTTGT

Each primer includes the M13 tail in order to facilitate subsequent sequencing using universal M13 primers.