



# Case Report: Japanese Siblings of Cystic Fibrosis With a Novel Large Heterozygous Deletion in the CFTR Gene

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Cystic fibrosis (CF) is a rare disease in the Japanese. The most common CFTR variant in Japanese CF patients is a large heterozygous deletion that can easily avoid detection by standard gene sequencing methods. We herein report a novel large heterozygous deletion in the CFTR gene in Japanese siblings with CF. A genetic analysis was performed in two patients (9-year-old boy and 5-month-old girl) who were clinically diagnosed with CF because of the positive result for the rapid fecal pancreatic elastase antigen test and the elevation of the sweat chloride concentration. In addition to conventional polymerase chain reaction (PCR) and direct sequencing, multiplex ligation-dependent probe amplification (MLPA) was performed to check for a large deletion and duplication of the CFTR gene. Based on MLPA findings, the breakpoint of heterozygous deletion was identified by real-time quantitative PCR followed by the sequence of the amplified junction fragment. In MLPA, the numbers of the fragments corresponding to exons 1, 16, 17a, and 17b and 234 nt and 747 nt upstream from the translation initiation codon of exon 1 in the CFTR gene and exon 3 in the ASZ1 gene were reduced by almost half. The c.2908+1085 3367+260del7201 variant (exon 16-17b deletion) was identified in one allele. The other allele had a large 137,567-bp deletion from g.117,361,112 (ASZ1 3' flanking region) to g.117,498,678 (CFTR intron 1) on chromosome 7. Since the deletion variant lacked the entire promoter region of CFTR, CFTR mRNA would not be transcribed from the allele, indicating it to be a novel pathogenic variant causing CF. As large mutations are frequently detected in Japanese CF patients, MPLA can be useful when searching for variants.

Keywords: cystic fibrosis, novel variant, promoter region (genetics), multiplex ligation-dependent probe amplification (MLPA), ASZ1 gene

# INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive genetic disorder that is common in Caucasian population with an estimated incidence of 1 in 3,500 newborns. CF is considered to be very rare in Japan, with an incidence of  $\sim$ 3 per 1 million individuals (1). However, this incidence of CF may be underestimated, as the sweat chloride test required for

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the definite diagnosis is not readily available, and the *CFTR* mutations identified in Japanese patients are typically rare in Caucasians (2-4). The most common variant in Japanese CF patients is a large heterozygous deletion that can easily evade detection by standard gene sequencing methods (3, 4).

We herein report Japanese siblings diagnosed with CF who had a novel large heterozygous deletion in the *CFTR* gene, in addition to the previously-known large deletion.

### **CASE PRESENTATION**

#### Case 1

A 9-year-old Japanese boy who was the first child of nonconsanguineous Japanese parents was admitted to our hospital for the treatment of acute bronchiolitis. The patient had been receiving oral and inhalant medications for the treatment of bronchial asthma since 6 years old, but the medications were discontinued at his mother's discretion 6 months before admission. He had been hospitalized for unexplained weight loss, hyponatremia and hypochloremia at 7 months of age. His maternal grandfather had lung cancer and his mother had bronchial asthma.

On admission, he had a low-grade fever  $(37.9^{\circ}C)$  and oxygen desaturation (surface oxygen saturation 89%). Rhonchi were heard on auscultation. Chest X-ray showed thickened bronchial walls around the bilateral pulmonary hilar regions, and the chest computed tomography (CT) showed hyperinflation of the lung field, thickened bronchial walls and bronchiectasis (**Figure 1A**). In addition, an atrophic pancreas with fatty infiltration was incidentally detected on CT (**Figure 1B**). He was diagnosed with sinusitis based on the gas-fluid level in the paranasal sinuses and gas bubbles within the fluid on the CT (**Figure 1C**).

CF was suspected based on these clinical findings and imaging. A low serum trypsin level [52 ng/mL, reference range (rr) 100– 550], decreased insulin secretion (insulin secretory index 0.17, rr > 1.10) and positive result for the rapid fecal pancreatic elastase antigen test indicated the pancreatic exocrine and endocrine insufficiency. The sweat chloride concentration was elevated (120



FIGURE 1 | The findings of chest (A), abdominal (B), and facial (C) computed tomography. The white arrow in (A) indicates thickened bronchial walls and bronchiectasis. The white arrow in (B) indicates an atrophic pancreas. The white arrows in (C) indicate gas-fluid level in the paranasal sinuses and gas bubbles within the fluid.

mmol/L, rr < 40). Based on these findings, he was clinically diagnosed with CF.

### Case 2

A 5 month-old girl who was a younger sister of case 1 visited our hospital because of poor feeding and weight gain. Her development was age-appropriate. The physical examination revealed almond-shaped eyes and small, unbalanced extremities. The serum levels of electrolytes were normal. The rapid fecal pancreatic elastase antigen test was positive. The sweat chloride concentration was 120 mmol/L. The patient was also clinically diagnosed with CF.

# MATERIALS AND METHODS

### **Direct Sequence of the CFTR Gene**

Genomic DNA was extracted from peripheral leukocytes. As reported in a previous study (4), Polymerase chain reaction (PCR) was performed, and all 27 exons as well as their boundaries (100–300 bp including poly T and TG repeats in intron 8) and the promoter region (up to 1,028 bp upstream of the translation initiation codon of exon 1) of the *CFTR* gene were directly sequenced.

### A Quantitative Fragment Analysis by Multiplex Ligation-Dependent Probe Amplification

MLPA, a quantitative PCR-based assay (5), was performed to check for the presence of a large deletion and duplication of the *CFTR* gene (6). The SALSA P091-C1 CFTR MLPA kit (MRC Holland, Amsterdam, Netherlands) was used. The kit contains probes for each of the 27 exons of the *CFTR* gene. Fragments were separated on CEQ-8000 capillary electrophoresis system (Beckmann Coulter, Fullerton, CA, USA) using the Beckmann D1-labeled CEQ size standard 600.

#### Detection of the Mutation of c.2908+1085\_3367+260del7201 in the CFTR Gene

We attempted to detect the mutation of c.2908+1085\_3367+260del7201 (exon 16-17b deletion) in one allele of the *CFTR* gene using the method to amplify the junction fragment (4). The primers were set on introns 15 and 17b to amplify the deleted allele.

# The Analysis of the Genomic Rearrangement of the CFTR Gene

To identify the deletion breakpoints of the novel large deletion spanning *CFTR* exon 1 and *ASZ1*, we first narrowed down the breakpoint site by repeating real-time Quantitative PCR (7), which was designed to distinguish the site of heterozygous deletion by comparing cycle threshold (Ct) values defined as the number of cycles required for the fluorescent signal to cross the threshold. Concentrations of DNA extracted from blood samples collected from Case 1 and a healthy adult (control subject) were

adjusted at  $\sim 20$  ng/µL. The real-time PCR was carried out using these samples and the Ct-values of the assay between Case 1 and the control subject were compared. The  $\Delta$ Ct-value that was determined by subtracting the Ct-value of the control subject from that of Case 1 was calculated for the comparison of the two samples. The first round of real-time PCR was carried out at  $\sim$ 4kb intervals within *CFTR* intron 1 (3<sup>'</sup>-side) and downstream of ASZ1 exon 3 (5<sup>'</sup>-side). The second and third rounds of realtime PCR were carried out at  $\sim$ 200 bp. The list of primers is shown in Table 1. Primers were added at a final concentration of 0.4 µM with TB Green Premix Ex Tag II (Takara Bio, Otsu, Japan). The assay was run in a M  $\times$  3005P Real-time QPCR system (Stratagene's An Agilent Technologies Division, La Jolla, CA, USA). The exact delineation of the deletion was carried out by PCR amplification of the junction fragment harboring the breakpoint (primers are noted in bold in Table 1) and sequence of the PCR products. The PCR products were purified and cloned using the Mighty TA-cloning Kit (Takara Bio) and sequenced directly.

# RESULTS

Direct sequencing of the CFTR gene detected no previouslyrecognized mutations related to the development of CF in either patients. Next, we performed a quantitative fragment analysis by MLPA. In the samples from cases 1 and 2, the numbers of fragments corresponding to exons 1, 16, 17a, and 17b and 234 nt and 747 nt upstream from the translation initiation codon of exon 1 in the CFTR gene and exon 3 in the ASZ1 gene were reduced by almost half, compared with that from the control (Figure 2). In the sample from their mother, 234 nt and 747 nt upstream from the translation initiation codon of exon 1 in the CFTR gene and exon 3 in the ASZ1 gene were reduced by almost half (Figure 2). These data indicated that there were large heterozygous deletions in both alleles of the CFTR gene in cases 1 and 2. One was probably the deletion of exon 16-17b inherited from their father, and the other was a novel large deletion spanning CFTR exon 1 and ASZ1 inherited from their mother. PCR amplification of the junction fragment identified the deletion of exon 16-17b (4) in one allele of cases 1 and 2 (data not shown). We could not obtain the samples from patients' father because of his disapproval.

Real-time quantitative PCR demonstrated that the  $\Delta$ Ct was ~1 (the copy number of DNA in case 1 was two-fold lower than that in control) in reactions with primers of Nos. 5-24 (**Table 1**). Thus, the deletion breakpoints of the novel large deletion spanning *CFTR* exon 1 and *ASZ1* were expected to be within g.117,498.301 to g.117,499,022 (3'-side) and g.117,360,678 to g.117,361,338 (5'-side) (Figure 3A). PCR of the junction fragment amplified the 1,132-bp product from case 1 and 2 and their mother (Figure 3B). Sequence of the PCR products revealed a large 137,567-bp deletion from g.117,361,112 (*ASZ1* 3' flanking region) to g.117,498,678 (*CFTR* intron 1) on chromosome 7 (Figure 3C). Exon 1 and the promoter region of the *CFTR* gene as well as all of the *ASZ1* gene were included in this region. Since the deletion

#### TABLE 1 | Primers for real-time quantitative polymerase chain reaction.

Image: Note of the image of the im	No.	Location	g number of 5'end <sup>a</sup>		Sequence	Amplicon length (bp)	Ct <sup>b</sup> (case 1)	Ct <sup>b</sup> (control)	∆Ct <sup>c</sup>
MV72         Proof 17,216,33         Proof 2         Constrained 2         Proof 2	1	WNT2 exon2	117,315,131	F	5'-TTCCTTTCCTTTGCATCCAC	213	26.90	26.57	0.33
2         WVZ lettors 1         17.20.073         F         6 -GAGCTETECARGAGTEGACAA         249         28.81         28.80         -0.05           WVZ lettors 1         117.20.733         F         5 -AAAGAGAAGGGGCTAACCAC         218         27.10         26.83         0.27           WVZ lettors 1         117.202.739         F         5 -CTCCCTCTGCTTGCACCAC         218         27.10         26.83         0.27           AS27 5 flamking region         17.260.498         F         5 -CTCCCTCTGCCTCTGCACACACACACAGTGGA         239         2.30         1.02           AS27 5 flamking region         17.280.788         F         5 -CTCCACACACACACACACAGTGGACACACACACAGTGGA         168         24.92         2.300         1.02           AS27 5 flamking region         117.280.788         F         5 -GCACATGACTGACACACACACACAGTGGACACACACACAGTGGACACACAC		WNT2 exon2	117,315,343	R	5 <sup>′</sup> -CGGGAATCTGCCTTTGTTTA				
MM2 inten 1         117.30.763         R         5'-GETARTEGCARARTEGC           MM2 inten 1         117.322.795         R         5'-CICCCTCIGCICTGACCTG         218         27.10         28.83         0.27           MA21 of Stanking region         117.322.095         R         5'-CICCCTCIGCICTGACCTG         218         2.14         28.84         2.84         0.00           A227 of Stanking region         117.381.078         R         5'-AGETCAGACAARTEGAACTGAC         288         2.84         2.84         0.26           A227 of Stanking region         117.381.378         R         5'-AGETCAGTCAGACAARTEGACT         188         2.428         2.848         2.836         0.83           A227 of Stanking region         117.382.378         R         5'-AGECATTGCTTAGCAGCAARTEGACT         188         2.489         2.849         0.89         .827           A227 inton 12         117.382.376         R         5'-GACAARTEGACGACAART         188         2.490         2.900         0.90           A227 inton 12         117.387.979         R         5'-GACAARTEGACGACAART         188         2.400         2.900         2.900         2.900         2.900         2.900         2.900         2.900         2.900         2.900         2.900         2.900 <td< td=""><td>2</td><td>WNT2 intron 1</td><td>117,320,515</td><td>F</td><td>5<sup>′</sup>-GAGCTGTGCATGAGTGGAGA</td><td>249</td><td>26.81</td><td>26.86</td><td>-0.05</td></td<>	2	WNT2 intron 1	117,320,515	F	5 <sup>′</sup> -GAGCTGTGCATGAGTGGAGA	249	26.81	26.86	-0.05
S         MVT2 intron 1         117.322.395         F         5'-AAAAAAAAAAAGAGGGCTAACCAT         218         27.10         28.83         0.27           MAT2 intron 1         117.390.400         F         5'-TGCATGAGTGCTGGAAAGAG         239         23.64         23.64         0.00           ASZ 7' 5' fanking region         117.390.478         F         5'-CGCATGAGTGCGGAAAAAGAG         188         24.89         23.96         0.00           ASZ 7' 5' fanking region         117.392.771         F         5'-TGCATGAGTAGGGGAGAAAGAGGGAAAAGA         188         24.89         23.90         0.90           ASZ 1' 5' fanking region         117.392.771         F         5'-TGCATGAGTAGGGGAAAAGTAGGGAAAAGTAGGGAAAAGTAGGGAAAAGTAGGGAAAAGTAGGGAAAAGTAGGGAAAAGTAGGGGAAAAGTAGGGGAAAAGTAGGGGAAAAGTAGGGGGAAAGTAGGGAAAGTAGGGGAAAGTAGGGAAAGTAGGGGAAAGTAGGAGG		WNT2 intron 1	117,320,763	R	5'-GGTGATGTGCGATAATGTGC				
WN2 mcn 1         117.329.666         F         5 -CCCCCTCCCCTCRCACACGAGAGAGA         239         2384         2364         0.00           AS2 7 5 fanking region         117.300.678         R         5 -AGGTCACGAGAGAGAGAGA         158         24.92         23.94         0.01           AS2 7 5 fanking region         117.301.3181         F         5 -CAGGTCACGAGAGAGAGAGAA         158         24.92         23.96         0.33           AS2 7 5 fanking region         117.302.358         R         5 -TICOAGGAGAGAGGAGAAA         168         24.98         23.96         0.33           AS2 7 10 fanking region         117.303.358         R         5 -GAGATATTTTGGAGGAGGAGAAA         168         24.90         23.90         0.90           AS2 7 10 fanking region         117.363.346         R         5 -GAGATATTTTGGAGGAGGAAAA         168         24.00         23.90         0.90           AS2 7 10 fan 12         117.367.329         R         5 -GAGGGAAAATGAGGGAAAA         163         24.00         24.00         25.10         1.20           AS2 7 10 fan 13         117.381.924         F         5 -TACCATTGCTGAGGAAAATGAGGGAAAA         163         24.00         2.10         1.20           AS2 7 10 fan 13         117.381.931         R         5 -TACCACATGGGGAGACATTGGAA	3	WNT2 intron 1	117,322,739	F	5 <sup>′</sup> -AAAGAGAAGGGGCTCACCAT	218	27.10	26.83	0.27
4         AZZ / Stanking region         117.380.478         F         5'-TOCATGATTCCTGGAAAGA         239         23.64         23.64         20.01           AZZ / Stanking region         117.301.181         F         5'-AGCGTAGGACAGACATGGAC         158         24.02         23.00         1.02           AZZ / Stanking region         117.301.781         F         5'-TOCAGGACATGGGACAAA         168         24.02         23.00         1.02           AZZ / Stanking region         117.302.371         F         5'-GGAATGGACAGACATGGCACAAA         168         24.02         23.60         0.93           AZZ / Stanking region         117.302.378         F         5'-GGAATGGACAGACATGGCACAAA         168         24.02         24.00         0.93           AZZ / Inten 12         117.302.378         F         5'-GGAATGGACAGACATGGCACAA         163         24.02         28.00         0.90           AZZ / Inten 12         117.303.798         F         5'-GGAATGGCAGACAGAAATGGGCA         174         27.00         25.80         1.20           AZZ / Inten 8         117.319.02         F         5'-AAGCCCAGGAAATGGCAGCA         174         27.00         25.80         1.20           AZZ / Inten 8         117.319.30         R         5'-TOCACGTGCGCAAACGTGAAAATGGCAGCA         1		WNT2 exon 1	117,322,956	R	5'-CTCCCTCTGCTCTTGACCTG				
AS2T of Inarking region         117.360.075         F         5         6.4GGTCAGGAGAAGAGATGGGTCAGGAGAA         188         24.92         23.90         1.02           AS2T of Inarking region         117.361.381         F         5         5'TTGGAGGACATGTGGTCG         188         24.92         23.90         1.02           AS2T of Inarking region         117.363.365         F         5'TGGACATTGGCATTGACATTGACGCAAG         188         24.92         25.64         0.85           AS2T of Inarking region         117.363.367         F         5'GGAATGGTTGGCAAGGGAAAG         121         26.49         25.64         0.85           AS2T intron 12         117.387.967         F         5'GGAAAAATGGGCAAAG         120         26.40         25.10         1.30           AS2T intron 12         117.387.967         F         5'GGAAAAATGGGCAA         120         26.40         25.10         1.30           AS2T intron 10         117.389.070         F         5'TTGCCCTGGAAAATTGGAA         120         25.00         2.00         1.00           AS2T intron 8         117.381.927         F         5'AAACACCGGGCTGGCAAAATTGGAA         130         2.500         2.600         2.600         2.600         2.600         2.600         2.600         2.600         2.600         <	4	ASZ1 3 <sup>′</sup> flanking region	117,360,440	F	5'-TGCATGAGTGCTGGAAAGAG	239	23.64	23.64	0.00
5.         AS27 5 flawking region         117,361,181         F         5 - CTATAGACTTGGTGGGCGAAAA         158         24.92         23.90         1.02           AS27 5 flawking region         117,362,77         F         5 - TGGCACTAGTCAGCCAAGA         168         24.99         25.64         0.38           AS27 5 flawking region         117,362,77         F         5 - GGCACTAGTCAGTCAGCCAAGA         168         24.90         25.64         0.85           AS27 kmont         117,363,746         R         5 - GCACTAGTCGTGGTGGTCACCAC         13         24.80         25.0         0.90           AS27 kmont         117,363,746         R         5 - GCACTAGTCGTGGTGGTCACCAC         13         24.80         25.10         1.30           AS27 kmont         117,373,827         R         5 - GCACATAAGTGGGGAAAATGTGGCTC         17         27.00         25.80         1.20           AS27 kmont         117,381,020         F         5 - TTGCCCGTGGCTGAGCAGAAA         13         28.00         27.00         1.00           AS27 kmont         117,381,502         F         5 - CACCACTGGCTGGCTGAGCAGAA         13         28.00         2.00         1.00           AS27 kmont         117,381,502         F         5 - CACCACTGGCTGGCAGACAAT         21.81         2.81 <td></td> <td>ASZ1 3' flanking region</td> <td>117,360,678</td> <td>R</td> <td>5<sup>′</sup>-AGGCTCAGGACAGAGATGGA</td> <td></td> <td></td> <td></td> <td></td>		ASZ1 3' flanking region	117,360,678	R	5 <sup>′</sup> -AGGCTCAGGACAGAGATGGA				
ASZ 7 stanking region         117,381,338         F         5 -TTGCAGGACATGTGGTCTC           6         ASZ 1 stanking region         117,382,771         F         5 -TGGCAGGAAGGAA         188         24.89         23.96         0.93           ASZ 1 stanking region         117,382,781         F         5 -CGCATGAAGTAAGGCATTGACATT         21         24.89         23.96         0.93           ASZ 1 stanking region         117,385,786         F         5 -CGATGAATGAAGGGCATTGACT         183         24.80         23.90         0.90           ASZ 1 trion 12         117,397,393         R         5 -CGATGAAAAGTGGGGAAAAG         24.80         25.10         1.30           ASZ 1 trion 10         117,393,924         R         5 -CGATGAAAAGTGGGGAAAAGTGGG         204         26.40         25.10         1.20           ASZ 1 trion 10         117,381,920         F         5 -TGCCOCTGAGGAAAGTGGGAAA         136         28.90         27.00         1.20           ASZ 1 trion 8         117,381,502         F         5 -CATACCTGGGAAAGTGGACAA         183         28.00         27.00         1.00           ASZ 1 trion 8         117,384,773         R         5 -TGCCACTGACAAGTGGAGAAA         183         28.00         27.00         1.00           ASZ 1 trion 8	5	ASZ1 3' flanking region	117,361,181	F	5 <sup>′</sup> -CTATAGGTTGGTGGGCCAAA	158	24.92	23.90	1.02
6         AS2T 3* familing region         117,382,271         F         6* 3* Concentration of the second of th		ASZ1 3' flanking region	117,361,338	R	5'-TTTGCAGGACATGTGGTCTC				
AS2! of larking region         117.382.838         R         6 'AAASTEWATGOCATTCACACAT           AS2! sort 13         117.383.736         F         5 'GCAAAGTACACGOGGAAAAT         211         26.49         26.49         0.85           AS2! riston 12         117.387.167         F         6 'GCAAAGTACACGOGGAAAAT         183         24.80         23.90         0.90           AS2! riston 10         117.387.297         F         6 'GCAAAGTACAGOGGAAAAT         204         26.40         25.10         1.30           AS2! riston 10         117.389.024         R         6 'GCAGAAAATAGAAGGGAAAAT         214         27.00         25.80         1.20           AS2! riston 8         117.381.020         F         6 'TICACTOTCOCTARGAAAATGGAA         174         27.00         25.80         24.70         1.20           AS2! riston 8         117.381.502         F         6 'AAACACCCACACGACACACACTCCTCA         350         25.80         27.00         1.00           AS2! riston 6         117.384.78         R         6 'TCACCACTGOCTGACACAT         193         28.00         27.00         1.00           AS2! riston 4         117.385.755         F         6 'AACAACCGGACACAT         178         21.81         2.8.1         2.8.1         2.8.1         2.8.1	6	ASZ1 3' flanking region	117,362,771	F	5' - TGGCACTAAGTCAGGCAAGA	168	24.89	23.96	0.93
7         AS21 s <sup>2</sup> UTR         117,383,536         F         5 "GCAATGATTITTGGATGGTC         211         26.49         25.64         0.85           AS27 intron 12         117,387,176         F         5 "GCAATGGACAATGGCGAAAATG         163         24.80         23.90         0.90           AS27 intron 12         117,387,129         F         5 "GCAATGGCTAATGGCGAAAATGTGG         204         26.40         25.10         1.30           AS27 intron 10         117,393,120         F         5 "TTACCTGGGTGGTGA         174         27.00         25.80         1.20           AS27 intron 8         117,381,183         R         5 "TGGCCCTGTGGAGAAATTGGGGTGGAGAAA         173         25.90         25.90         25.00         25.00         25.00         25.00         25.00         25.00         25.00         25.00         26.00         27.00         1.00           AS27 intron 8         117,381,50         F         5 "AGCAACGGGGGGGGGGGGGGGGGGGGAGAAA         193         28.00         27.00         1.00           AS27 intron 6         117,384,763         R         5 "GCCAATGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG		ASZ1 3' flanking region	117,362,938	R	5 <sup>′</sup> -AAAGTGAATGGCATTTGACATAT				
AS21 exen 13         117,383,746         R         5'-GCAAAATGAACGGCAAAATG           8         AS27 intron 12         117,387,167         F         5'-ACGTTAAATGACGCAAC         163         24.00         23.00         0.90           AS27 intron 10         117,387,167         F         5'-ACGGAAAATGACGGCAAA         26.40         26.10         1.30           AS27 intron 10         117,380,024         R         5'-CACGAAAAATGCAAGGGAAAT         27.00         25.80         1.20           AS27 intron 8         117,381,193         R         5'-TTACCTCCTCATGGTAC         17         27.00         25.80         1.20           AS27 intron 8         117,381,193         R         5'-TTACCTCCTCATGGTGTGACAAATTGGAAGAAT         36.0         25.90         27.00         1.00           AS27 intron 8         117,381,515         R         5'-TGCCACTGCTAGAGAAAT         193         28.00         27.00         1.00           AS27 intron 4         117,387,575         F         5'-AGACGACGAGGAGAAAT         193         28.00         27.00         1.00           AS27 intron 4         117,387,575         F         5'-AGACGACGAGGAGAAT         173         23.8         23.10         0.73           CFTR intron 1         117,480,707         F         5'-CAG	7	ASZ1 3' UTR	117,363,536	F	5 <sup>'</sup> -GCAATGATTTTTGGATGGTTC	211	26.49	25.64	0.85
8         AS21 Intron 12         117,367,167         F         S         GGGATTTGCTTAATGGGCAAC         163         24.80         23.90         0.90           AS27 Intron 10         117,337,329         R         S         GGGGAAAATGAGGGAAAA         24.80         26.10         1.30           AS27 Intron 10         117,339,024         R         S         GGGAGAAATGTGGG C0         26.00         25.10         1.30           AS27 Intron 8         117,381,020         F         S         TCACTCTCCTCATGAGAATTGGGACA         714         27.00         25.80         1.20           AS27 Intron 8         117,381,502         F         S         AAACCCCACACACGTGGGGGAAA         193         25.00         1.00           AS27 Intron 6         117,384,571         R         S         TGCCAGGGGGGAAA         193         25.00         1.00           AS27 Intron 6         117,384,571         R         S         TGCCAGGGGGAAA         193         25.00         0.69           AS27 Intron 6         117,384,571         R         S         TGCCCAGGGGGGAAA         193         23.00         0.73           AS27 Intron 4         117,489,473         R         S         TGCCCAGGGGGACTT         178         21.81         20.78 <t< td=""><td></td><td>ASZ1 exon 13</td><td>117,363,746</td><td>R</td><td>5'-GCAAAATGAACGGGAAAATG</td><td></td><td></td><td></td><td></td></t<>		ASZ1 exon 13	117,363,746	R	5'-GCAAAATGAACGGGAAAATG				
ASZ7 intron 12       117,367,329       R       5'ASGGGAAAAATGAAGGGAAA         9       ASZ7 intron 10       117,379,821       F       5'TACCCTGGGAAAAATGTGG       204       26.40       25.10       1.30         ASZ7 intron 10       117,379,821       F       5'TACCCTGGGAAAAATGTGGCTGCT       77       27.00       25.80       1.20         ASZ7 intron 8       117,381,020       F       5'TACCCCCCCAAAAATTGGAA       76       25.00       25.00       26.70       1.20         ASZ7 intron 8       117,381,521       R       5'TGCCCCCCCAAACGTTGGATGGAGAGGTGAGAAT       350       25.00       24.70       1.20         ASZ7 intron 8       117,381,751       F       5'AAACACCACCACACAGTGGATGAGATGAGAT       216       26.19       25.50       0.69         ASZ7 intron 4       117,385,755       F       5'AACAACCTGGGGTGAACCAT       216       26.19       25.50       0.69         ASZ7 intron 4       117,482,697       R       5'CGCAATGGAAGGTTGGAACCAT       218       20.78       1.00         ASZ7 intron 4       117,482,697       R       5'CAGGGAAGGTTGGAACCAT       178       21.81       20.78       1.02         ASZ7 intron 1       117,480,642       F       5'AGGAGGCACCCTCTT       178       21.81       20.7	8	ASZ1 intron 12	117,367,167	F	5 <sup>′</sup> -GCATTTGCTTAATGGCCAAC	163	24.80	23.90	0.90
9         ASZT intron 10         117,379,821         F         5 -TTACCCTGGGAAAATGTGG         204         26.40         25.10         1.30           ASZT exon 10         117,381,024         R         5 -GCAGAAAATTGTGGGTGCTC         774         27.00         26.80         1.20           ASZT intron 8         117,381,020         F         5 -AAACACCCACACAGTGCTG         350         25.90         24.70         1.20           ASZT intron 8         117,381,851         R         5 -TTACCCAGTGCTAACCTTGA         350         25.90         24.70         1.20           ASZT intron 6         117,384,753         R         5 -TTACCCAGTGGGTGAACATTGAAAATTGGAAA         133         28.00         27.00         1.00           ASZT intron 4         117,384,753         R         5 -TGCCAAGGAGGTGGGTGAACCAT         218         26.19         25.50         0.81           ASZT intron 4         117,419,085         F         5 -GCATGAGGAGGTGGACCAT         218         21.81         20.78         1.03           ASZT intron 4         117,419,024         R         5 -GCAGGGTTAGGCCACAC         178         21.81         20.78         1.03           CFTR intron 1         117,488,070         F         5 -GAGATGCTGAAGGGGGGTTGGCACACT         178         23.83 <th2< td=""><td></td><td>ASZ1 intron 12</td><td>117,367,329</td><td>R</td><td>5<sup>′</sup>-AGGGGAAAAATGAAGGGAAA</td><td></td><td></td><td></td><td></td></th2<>		ASZ1 intron 12	117,367,329	R	5 <sup>′</sup> -AGGGGAAAAATGAAGGGAAA				
ASZ1 exon 10       117,380,024       R       5'-GCAGAAAATCTGGGCTGCTC       174       27.00       25.80       1.20         ASZ1 exon 9       117,381,020       F       5'-TTCATCTTCCCTCAGAGAATTGGAA       174       27.00       25.80       1.20         ASZ1 intron 8       117,381,620       F       5'-AAACACCCACACACATGGTTG       350       25.90       24.70       1.20         ASZ1 intron 8       117,381,681       R       5'-TTACCCACTGCCTAACCTTCA       180       28.00       27.00       1.20         ASZ1 exon 6       117,384,571       F       5'-AAACACCCACAGTGGAGATGAAA       193       28.00       27.00       1.00         ASZ1 exon 5       117,385,755       F       5'-AACAACCTGGGCTGGACCAT       216       26.19       25.50       0.69         ASZ1 intron 4       117,419,068       F       5'-GCAAGGAAGGTTGGACTTTT       178       21.81       20.78       1.03         ASZ1 intron 4       117,419,068       F       5'-CAAGGACGTGAGCCCAGAC       171       23.83       23.10       0.73         CFTR intron 1       117,480,012       R       5'-CATGCCAAGGCTCTGGCCCTTGT       178       21.81       20.71       22.89       0.82         CFTR intron 1       117,480,029       F       5'-CATGC	9	ASZ1 intron 10	117,379,821	F	5'-TTACCCTGGGAAAATGTGG	204	26.40	25.10	1.30
10       ASZ/ exon 9       117,881,920       F       5'-TTGATCTTCCCTCATGGTCA       174       27.00       25.80       1.20         ASZ/ intron 8       117,381,930       R       5'-TGTCCCCTCTAGAAAATTGGAA       174       27.00       25.80       1.20         ASZ/ intron 8       117,381,931       R       5'-TGCCCACTGACCACCACCACGTGCTGA       350       25.90       24.70       1.20         ASZ/ intron 8       117,381,451       R       5'-TGCCCACTGCGTGAGAGAAA       193       28.00       27.00       1.00         ASZ/ exon 6       117,384,763       R       5'-TGCCAGTGGAGAAA       193       28.00       27.00       1.00         ASZ/ exon 6       117,385,755       F       5'-ACACACCTGGGTGTGACCAT       216       26.19       25.50       0.69         ASZ/ intron 4       117,419,068       F       5'-GTGCAGGGATTGGACAT       178       21.81       20.78       1.03         ASZ/ intron 1       117,480,690       R       5'-CATACACACGCGCGGCATTGT       178       23.71       22.89       0.82         OFTR intron 1       117,480,892       F       5'-GGCACCCCACAGGTTGACCAT       165       24.68       23.94       0.74         OFTR intron 1       117,480,895       R       5'-GCACCCCCACAGGTTGA		ASZ1 exon 10	117,380,024	R	5' -GCAGAAAATTCTGGCTGCTC				
ASZ1 intron 8         117,381,193         R         5'-TGTCCCCTCTAGAMANTTGGAA           ASZ1 intron 8         117,381,922         F         5'-AAACACCACACAGTGGTTG         350         25.90         24.70         1.20           ASZ1 intron 8         117,381,951         R         5'-TTACCACGGACGAGAA         193         26.00         27.00         1.00           ASZ1 intron 6         117,384,763         R         5'-TGCCAAGTGAGATTGCAACAA         216         26.19         25.00         0.69           ASZ1 intron 4         117,385,755         F         5'-AACAACCTGGAGGTGACACAT         216         21.81         20.78         1.03           ASZ1 intron 4         117,419,068         F         5'-GGGGAGGTTGGACATT         17.8         21.81         20.78         1.03           ASZ1 intron 4         117,419,068         F         5'-AGGAGGAGGTTGGACCTT         17.8         23.83         23.10         0.73           CFTR intron 1         117,480,042         F         5'-GAGAGGTTGAGCCTCTTT         17.8         23.81         23.71         22.89         0.82           CFTR intron 1         117,480,707         F         5'-GAGACCCCACACAGGTGACT         15         24.68         23.94         0.74           CFTR intron 1         117,480,707 </td <td>10</td> <td>ASZ1 exon 9</td> <td>117,381,020</td> <td>F</td> <td>5<sup>′</sup>-TTCATCTTCCCTCATGGTCA</td> <td>174</td> <td>27.00</td> <td>25.80</td> <td>1.20</td>	10	ASZ1 exon 9	117,381,020	F	5 <sup>′</sup> -TTCATCTTCCCTCATGGTCA	174	27.00	25.80	1.20
11       ASZ7 intron 8       117,381,502       F       5'-AAACACCCCACACAGTGCTTG       350       25.90       24.70       1.20         ASZ7 intron 8       117,381,851       R       5'-TTACCCACTGCTAACCTTCA       193       28.00       27.00       1.00         ASZ7 intron 6       117,384,571       F       5'-CGCAACGTGGAGGAAA       193       28.00       27.00       1.00         ASZ7 intron 6       117,385,755       F       5'-AGCAACGTGGGTGAACCAT       216       26.19       25.50       0.69         ASZ7 intron 4       117,495,755       F       5'-GGTGTCAACAATGGGACAT       178       21.81       20.78       1.00         ASZ7 intron 4       117,419,068       F       5'-GAGGGGTATTGGCACATG       178       21.81       20.78       1.03         ASZ7 intron 4       117,419,245       R       5'-CAGGGGTATTGGCACAGGCACTGT       178       21.81       20.78       1.03         ASZ7 intron 1       117,480,042       F       5'-GAGGGTATGGACACGCCCACGAGAC       171       23.83       23.10       0.73         CFTP intron 1       117,480,042       F       5'-GAGCACTCAGGAGAGTCAC       26       24.55       23.73       0.83         CFTP intron 1       117,489,848       R       5'-GCCCACTCAGGAG		ASZ1 intron 8	117,381,193	R	5'-TGTCCCCTCTAGAAAATTGGAA				
ASZ1 intron 8         117,381,851         R         S <sup>-</sup> TTACCCACTGCCTGACGGAAA         193         28.00         27.00         1.00           ASZ1 exon 6         117,384,763         R         S <sup>-</sup> TGCCAAGTGAGATGAAAAA         193         28.00         27.00         1.00           ASZ1 exon 5         117,385,755         F         S <sup>-</sup> AACAACCTGGGTGGAGCAAT         16         26.09         25.50         0.69           ASZ1 intron 4         117,495,755         R         S <sup>-</sup> CGAGGGAGTTGGACCAT         178         21.81         20.78         1.03           ASZ1 intron 4         117,490,025         R         S <sup>-</sup> CGAGGGGTTTGACCCATGGCACT         178         21.81         20.78         1.03           ASZ1 intron 1         117,480,022         R         S <sup>-</sup> CATACACACGGGGGCTTTGACCCATGT         178         21.81         2.8.9         0.82           OFTR Intron 1         117,480,707         F         S <sup>-</sup> AAGACGTGAGGGCCTTGACCCATGT         153         23.71         22.89         0.82           OFTR Intron 1         117,480,707         F         S <sup>-</sup> CATCCCACAGGTGAGCCATGT         153         23.71         22.89         0.82           OFTR Intron 1         117,480,707         F         S <sup>-</sup> CATCCCACAGGTGAGCTAC         26         24.55         23.73         <	11	ASZ1 intron 8	117,381,502	F	5'-AAACACCCACACAGTGCTTG	350	25.90	24.70	1.20
12         ASZ1 intron 6         117,384,751         F         5'-CATTACCTGGCTGGAGGAAA         193         28.00         27.00         1.00           ASZ1 exon 6         117,384,763         R         5'-TGCCAAGTGAGATTGCAAAA         117.385,755         F         6'-ACAGAGGAGGTTGGACCAT         216         26.19         25.50         0.69           ASZ1 intron 4         117,385,755         F         5'-AGCAGGAGGTTGGACCAT         178         21.81         20.78         1.03           ASZ1 intron 4         117,419,058         F         5'-CGTGCAGGGAGTTTGGCACT         178         21.81         20.78         1.03           ASZ1 intron 4         117,490,042         F         5'-TGAGAGGTTGACCCAGGCACTCT         178         21.81         20.78         1.03           CFTR intron 1         117,480,070         F         5'-AGATCGTAAGGGGGCTTCTT         153         23.71         22.89         0.82           OFTR intron 1         117,488,707         F         5'-GGCCACCTCTTCAGCCACAAGCT         165         24.68         23.94         0.74           OFTR intron 1         117,489,790         R         5'-GGCCACCTCTTCAGCCACAAG         286         24.55         23.73         0.83           OFTR intron 1         117,490,714         R         5'-GGCCAG		ASZ1 intron 8	117,381,851	R	5'-TTACCCACTGCCTAACCTTCA				
ASZ1 exon 6         117,384,763         R         5'-TGCCAAGTGAGATTGCAAAA           13         ASZ1 exon 5         117,385,755         F         5'-AACAACCTGGGTGTGACCAT         216         26.19         25.50         0.69           ASZ1 intron 4         117,395,750         R         5'-TGCAGGAAGTTGGATTTT         778         21.81         20.78         1.03           ASZ1 intron 4         117,419,245         R         5'-CAGAGGGTATTTGGCAATGT         778         23.31         0.73           15         CFTR intron 1         117,480,042         F         5'-CATACACACGCCCTCCTCT         78         23.71         22.89         0.82           16         CFTR intron 1         117,480,707         F         5'-CAGCCACTCTTCAGCCCACACT         165         24.68         23.94         0.74           17         CFTR intron 1         117,489,382         F         5'-GCCCACTCATCAGCCACAGGAGGTCA         286         24.55         23.73         0.83           CFTR intron 1         117,499,744         R         5'-CAGGAAACCCAGGAGAGTCA         286         24.55         23.73         0.83           CFTR intron 1         117,499,744         R         5'-CACCCAGGAAGTCATAT         122         23.61         22.62         0.90           CFTR i	12	ASZ1 intron 6	117,384,571	F	5 <sup>′</sup> -CATTACCTGGCTGGAGGAAA	193	28.00	27.00	1.00
13       ASZ1 exon 5       117,385,755       F       5'-AACAACCTGGGTGTGACCAT       216       26,19       25,50       0.69         ASZ1 intron 4       117,385,970       R       5'-TGCAGGAAGGTTGGACTTTT       178       21.81       20.78       1.03         ASZ1 intron 4       117,419,088       F       5'-GGTTGGACCAT       178       21.81       20.78       1.03         14       ASZ1 intron 4       117,419,088       F       5'-CAGGGGTATTGGCACATGT       171       23.83       23.10       0.73         15       CFTR intron 1       117,480,042       F       5'-CAAGGGGCTTGAGCCCAGAC       171       23.83       23.10       0.73         16       CFTR intron 1       117,480,042       F       5'-GGACCCACAGGGCGCTTGT       153       23.71       22.89       0.82         CFTR intron 1       117,480,042       F       5'-GGACCCACAGGGTGACTC       165       24.68       23.94       0.83         CFTR intron 1       117,489,146       R       5'-GCATCCCACAGGGGCTAT       286       24.55       23.73       0.83         CFTR intron 1       117,490,714       R       5'-CACGGCAGTGTTAGCCAGTAGT       122       23.61       22.62       0.99         CFTR intron 1       117,493,080		ASZ1 exon 6	117,384,763	R	5'-TGCCAAGTGAGATTGCAAAA				
ASZ1 Intron 4         117,385,970         R         5 <sup>-</sup> -TGCAGGAAGGTTGGATTTTT           14         ASZ1 Intron 4         117,419,068         F         5 <sup>-</sup> -GGTTTCTCAACCATGGCACT         178         21.81         20.78         1.03           ASZ1 Intron 4         117,419,245         R         5 <sup>-</sup> -CATGGAGGCTTGAGCCCAGAC         171         23.83         23.10         0.73           CFTR Intron 1         117,480,212         R         5 <sup>-</sup> -CATACACACGCGCCCCCTT         153         23.71         22.89         0.82           CFTR Intron 1         117,486,707         F         5 <sup>-</sup> AGATCGTAAGGGGGGCTTGACCCATC         165         24.68         23.94         0.74           CFTR Intron 1         117,486,707         F         5 <sup>-</sup> GGCACCCCATCGTCACCC         165         24.68         23.94         0.74           CFTR Intron 1         117,486,799         R         5 <sup>-</sup> GGCCACCCAGGCAGCTC         165         24.68         23.94         0.74           CFTR Intron 1         117,490,749         F         5 <sup>-</sup> CAGGCAACCCAGGAGAGTCA         286         24.55         23.73         0.83           CFTR Intron 1         117,490,749         F         5 <sup>-</sup> CATCCCAGGAAGCCAGGAGAGTAT         122         23.64         22.72         0.96           CFTR Intron 1	13	ASZ1 exon 5	117,385,755	F	5 <sup>′</sup> -AACAACCTGGGTGTGACCAT	216	26.19	25.50	0.69
14       ASZ1 intron 4       117,419,068       F       5'-GGTTTCTCAACCATGGCACT       178       21.81       20.78       1.03         ASZ1 intron 4       117,419,045       R       5'-CAGGGGTATTTGGCAATGT       171       23.83       23.10       0.73         15 <i>CFTR</i> intron 1       117,480,042       F       5'-CATACACACGCCCCAGTC       171       23.83       23.10       0.73         16 <i>CFTR</i> intron 1       117,486,707       F       5'-GATCGTAAGGGGGGGCTTGT       153       23.71       22.89       0.82 <i>CFTR</i> intron 1       117,486,707       F       5'-GATCCCACCCCTCTTCT       165       24.68       23.94       0.74 <i>CFTR</i> intron 1       117,489,892       F       5'-GGTCCCACCAGGGTTAGCCCACC       165       24.68       23.94       0.74         17 <i>CFTR</i> intron 1       117,490,714       R       5'-GGCCTATGTCTGGCAATT       122       23.61       22.62       0.99         19 <i>CFTR</i> intron 1       117,492,959       F       5'-ATCCCACCAGTCTGAAT       122       23.68       22.72       0.96 <i>CFTR</i> intron 1       117,493,080       R       5'-ATCCCACCAGTCTGAAT       122       23.68       22.72       0.96 <i>CFTR</i> intron		ASZ1 intron 4	117,385,970	R	5 <sup>′</sup> -TGCAGGAAGGTTGGATTTTT				
ASZ1 intron 4       117,419,245       R       5'-CAGGGGTATTTGGCAATGT         15       CFTR intron 1       117,480,042       F       5'-TTAGGAGCTTGAGCCCAGAC       171       23.83       23.10       0.73         16       CFTR intron 1       117,480,212       R       5'-CATACACACGCCCTCCTCTT       153       23.71       22.89       0.82         17       CFTR intron 1       117,486,859       R       5'-GGCCACTCTTAGCTCAGCTCATC       155       24.68       23.94       0.74         17       CFTR intron 1       117,489,828       F       5'-GGCCACTCATTAGCTGAGCTAGCTGACCT       165       24.68       23.94       0.74         18       CFTR intron 1       117,499,146       R       5'-CGCCCACAGGGAGTCA       286       24.55       23.73       0.83         19       CFTR intron 1       117,490,714       R       5'-CACGCCAGTGTGCGGAGTTAT       122       23.61       22.62       0.99         17       OFTR intron 1       117,492,959       F       5'-ACGCTGGCAGATTAT       122       23.61       22.62       0.99         17       OFTR intron 1       117,493,980       R       5'-ACGCTGGCAGATTCT       122       23.61       22.62       0.97         10       CFTR intron 1 <td< td=""><td>14</td><td>ASZ1 intron 4</td><td>117,419,068</td><td>F</td><td>5'-GGTTTCTCAACCATGGCACT</td><td>178</td><td>21.81</td><td>20.78</td><td>1.03</td></td<>	14	ASZ1 intron 4	117,419,068	F	5'-GGTTTCTCAACCATGGCACT	178	21.81	20.78	1.03
15       CFTR intron 1       117,480,042       F       5'-TTAGGAGCTTGAGCCCAGAC       171       23.83       23.10       0.73         16       CFTR intron 1       117,480,212       R       5'-CATACACACGCOCTCCTCT       153       23.71       22.89       0.82         17       CFTR intron 1       117,486,599       R       5'-GGCCACTCTTTCAGCTCATC       165       24.68       23.94       0.74         17       CFTR intron 1       117,488,982       F       5'-GGCCACCACGAGGAGTGACTC       165       24.68       23.94       0.74         18       CFTR intron 1       117,490,429       F       5'-CAGGAAACCCAGGAGAGTCA       286       24.55       23.73       0.83         0FTR intron 1       117,492,959       F       5'-CAGGCCATGTGTGCGAGATT       122       23.61       22.62       0.99         0FTR intron 1       117,492,959       F       5'-CAGGCCAGTGAGTAGAAACA       102       23.68       22.72       0.96         0FTR intron 1       117,492,959       F       5'-ACCCGCAGTGTGCCAAGGAATCTGT       122       23.68       23.62       0.97         0FTR intron 1       117,495,976       F       5'-ACCCGCCAGTGTGCCAAGAGTGGCAT       122       23.68       23.62       0.97         0FTR i		ASZ1 intron 4	117,419,245	R	5 <sup>′</sup> -CAGGGGTATTTGGCAATGT				
CFTR         Intron 1         117,480,212         R         5'-CATACACACGCCCTCCTCTT           16         CFTR         Intron 1         117,486,707         F         5'-AGATCGTAAGGGGGGCTTTGT         153         23.71         22.89         0.82           17         CFTR         Intron 1         117,486,859         R         5'-GGCACCCACAAGGTTGACCATC         165         24.68         23.94         0.74           17         CFTR         Intron 1         117,489,982         F         5'-GGCACCCACAGGGGAGTCA         286         24.55         23.73         0.83           18         CFTR         Intron 1         117,490,429         F         5'-CAGGACACCAGGAGAGTCA         286         24.55         23.73         0.83           19         CFTR intron 1         117,490,299         F         5'-CAGGCCAGTGGCAGTGGCAAT         122         23.61         22.62         0.90           19         CFTR intron 1         117,493,080         R         5'-ATCCTGGAAAGGCACTCTGA         102         23.68         22.72         0.96           17         JFR intron 1         117,495,395         R         5'-ATCCACACACACATCTGA         102         23.68         23.62         0.97           17         CFTR intron 1         117,495,708 </td <td>15</td> <td>CFTR intron 1</td> <td>117,480,042</td> <td>F</td> <td>5<sup>′</sup>-TTAGGAGCTTGAGCCCAGAC</td> <td>171</td> <td>23.83</td> <td>23.10</td> <td>0.73</td>	15	CFTR intron 1	117,480,042	F	5 <sup>′</sup> -TTAGGAGCTTGAGCCCAGAC	171	23.83	23.10	0.73
16       CFTR intron 1       117,486,707       F       5'-AGATCGTAAGGGGGGCTTTGT       153       23.71       22.89       0.82         17       CFTR intron 1       117,486,859       R       5'-GGCCACTCTTTCAGCTCATC       165       24.68       23.94       0.74         17       CFTR intron 1       117,489,146       R       5'-GTGCTGAGCTTAGGCGACTT       165       24.68       23.94       0.74         18       CFTR intron 1       117,490,429       F       5'-CAGGAAACCCAGGAGAGTCA       286       24.55       23.73       0.83         CFTR intron 1       117,490,429       F       5'-CGCCCTATGTCTGGGCATTAT       122       23.61       22.62       0.99         CFTR intron 1       117,492,959       F       5'-ACCGTGTGCCAGATTGTCT       12       23.68       22.72       0.96         CFTR intron 1       117,495,294       F       5'-ACCCTGCACCACTCTGAA       102       23.68       22.72       0.96         CFTR intron 1       117,495,395       R       5'-ACCCGGCTACCAGCACTTGA       23       24.59       23.62       0.97         CFTR intron 1       117,495,595       R       5'-ATCCCGACCACTCTGAACAGCACTTGA       23       24.59       23.62       0.97         CFTR intron 1       117		CFTR intron 1	117,480,212	R	5'-CATACACACGCCCTCCTCTT				
CFTR intron 1         117,48,585         R         5'-GGCACTCTTTCAGCTCATC           17         CFTR intron 1         117,488,982         F         5'-GCATCCCACAAGGTTGACTC         165         24,68         23,94         0,74           17         CFTR intron 1         117,489,146         R         5'-GTGCTGAGCTTAGGCGACTT         165         24,68         23,94         0,74           18         CFTR intron 1         117,490,429         F         5'-CAGGAAACCCAGGAGAGTCA         286         24,55         23,73         0,83           CFTR intron 1         117,490,714         R         5'-CACGCCCATGTGTCGCAGATTAT         122         23,61         22,62         0,99           CFTR intron 1         117,492,959         F         5'-ACCGTGTGCCAGATTCTCT         122         23,68         22,72         0,96           CFTR intron 1         117,495,395         R         5'-ATCCTGGAAAGGCACTCTGA         102         23,68         22,72         0,96           CFTR intron 1         117,495,395         R         5'-GCGTGTGACAGGAAGGCACTCTGA         12         24,59         23,62         0,97           CFTR intron 1         117,495,798         R         5'-GGGTGGTGACAAGGCAGTGTACCT         12         24,59         23,62         0,97	16	CFTR intron 1	117,486,707	F	5' -AGATCGTAAGGGGGCTTTGT	153	23.71	22.89	0.82
17       CFTR intron 1       117,488,982       F       5'-GCATCCCACAAGGTTGACTC       165       24.68       23.94       0.74         18       CFTR intron 1       117,489,146       R       5'-GCAGGAAACCCAGGAGAGTCA       286       24.55       23.73       0.83         18       CFTR intron 1       117,490,429       F       5'-CAGGAAACCCAGGAGAGTCA       286       24.55       23.73       0.83         19       CFTR intron 1       117,490,714       R       5'-CACGCCCATGTCTGGCAATAT       122       23.61       22.62       0.99         CFTR intron 1       117,493,080       R       5'-ACCCTGGAAAGGCACTCTGA       102       23.68       22.72       0.96         CFTR intron 1       117,495,395       R       5'-ACCCACCCACTTGAAAGGCACTCTGA       102       23.68       22.72       0.96         CFTR intron 1       117,495,395       R       5'-ACCCACCCACTTGAAAGGCACTTGAA       102       23.68       24.59       23.62       0.97         CFTR intron 1       117,495,395       R       5'-GCGTGTGACAAGCGACTTCAA       233       24.59       23.62       0.97         CFTR intron 1       117,495,708       R       5'-GGCTTGGCTACAGCAAGTGAT       141       24.56       23.69       0.87 <t< td=""><td></td><td>CFTR intron 1</td><td>117,486,859</td><td>R</td><td>5'-GGCCACTCTTTCAGCTCATC</td><td></td><td></td><td></td><td></td></t<>		CFTR intron 1	117,486,859	R	5'-GGCCACTCTTTCAGCTCATC				
CFTR intron 1         117,489,146         R         5'-GTGCTGAGCTTAGGCGACTT           18         CFTR intron 1         117,490,429         F         5'-CAGGAAACCCAGGAGAGTCA         286         24.55         23.73         0.83           18         CFTR intron 1         117,490,714         R         5'-CAGGCAAACCCAGGAAGGTCA         286         24.55         23.73         0.83           19         CFTR intron 1         117,492,959         F         5'-CACGCCCAGTGACGAGATTCTCT         122         23.61         22.62         0.99           CFTR intron 1         117,493,080         R         5'-ACCCTGGAAAGGCACTCTGA         102         23.68         22.72         0.96           CFTR intron 1         117,495,395         R         5'-ATCCCGCACCATCTGAAAAGCCCATGAACA         102         23.68         24.59         23.62         0.97           20         CFTR intron 1         117,495,395         R         5'-GCCTGTGACAAGTGACAACA         233         24.59         23.62         0.97           21         CFTR intron 1         117,495,708         R         5'-GGCTGTGACAAGCAATCCT         141         24.56         23.69         0.87           24         CFTR intron 1         117,497,521         F         5'-GAGCTTTTCCAAGGCGATA         140	17	CFTR intron 1	117,488,982	F	5 <sup>′</sup> -GCATCCCACAAGGTTGACTC	165	24.68	23.94	0.74
18       CFTR intron 1       117,490,429       F       5'-CAGGAAACCCAGGAGAGTCA       286       24.55       23.73       0.83         19       CFTR intron 1       117,490,714       R       5'-CGCCCATGCCAGGAATT       122       23.61       22.62       0.99         CFTR intron 1       117,492,959       F       5'-CATGCCCAGTGATGGTAAT       122       23.61       22.62       0.99         CFTR intron 1       117,493,080       R       5'-AACGCTGTGCCAGATTCTCT       102       23.68       22.72       0.96         CFTR intron 1       117,495,294       F       5'-ATCCCACCCATCTGAAAGGCACTCTGA       102       23.68       22.72       0.96         CFTR intron 1       117,495,395       R       5'-ATCCCACCCATCTGAAAGGCACTCTGA       102       23.68       23.62       0.97         CFTR intron 1       117,495,395       R       5'-GGCTGTTAGAAGGCACTCTGAACAG       233       24.59       23.62       0.97         CFTR intron 1       117,497,712       F       5'-GGCTGTGCACAAGCAAGTGAT       141       24.56       23.69       0.87         CFTR intron 1       117,497,521       F       5'-GGACTTTGCCAACAAGCCAAGTCCA       140       25.05       23.83       1.21         CFTR intron 1       117,497,521		CFTR intron 1	117,489,146	R	5'-GTGCTGAGCTTAGGCGACTT				
CFTR intron 1         117,490,714         R         5'-CGCCCTATGTCTGGCATTAT           19         CFTR intron 1         117,492,959         F         5'-CCATGCCCAGTGATGGTAAT         122         23.61         22.62         0.99           CFTR intron 1         117,493,080         R         5'-AACGCTGTGCCAGATTCTCT         122         23.68         22.72         0.96           20         CFTR intron 1         117,495,294         F         5'-ATCCTGGAAAGGCACTCTGA         102         23.68         22.72         0.96           CFTR intron 1         117,495,395         R         5'-ATCCCACCCACTTGAAACA         102         23.68         23.62         0.97           21         CFTR intron 1         117,495,708         R         5'-GGCTGTGGCTACAGCAAGTGGAT         233         24.59         23.62         0.97           21         CFTR intron 1         117,497,712         F         5'-GGCTTTGGTGTCACAAGCAAGTGAT         24.56         23.69         0.87           23         CFTR intron 1         117,497,521         F         5'-GAGCTTTTCCAAGGCGATA         140         25.05         23.83         1.21           CFTR intron 1         117,497,660         R         5'-TACGAATCCCCAGTGACCTG         140         25.05         23.83         1.21 </td <td>18</td> <td>CFTR intron 1</td> <td>117,490,429</td> <td>F</td> <td>5<sup>′</sup>-CAGGAAACCCAGGAGAGTCA</td> <td>286</td> <td>24.55</td> <td>23.73</td> <td>0.83</td>	18	CFTR intron 1	117,490,429	F	5 <sup>′</sup> -CAGGAAACCCAGGAGAGTCA	286	24.55	23.73	0.83
19       CFTR intron 1       117,492,959       F       5'-CCATGCCCAGTGATGGTAAT       122       23.61       22.62       0.99         CFTR intron 1       117,493,080       R       5'-AACGCTGTGCCAGATTCTCT       102       23.68       22.72       0.96         20       CFTR intron 1       117,495,294       F       5'-ATCCCGCACCATCTGAA       102       23.68       22.72       0.96         CFTR intron 1       117,495,395       R       5'-ATCCCACCCATCTTGAAACA       233       24.59       23.62       0.97         21       CFTR intron 1       117,495,395       R       5'-GGCTGTTAGAAGTGGCCTTTCA       233       24.59       23.62       0.97         24       CFTR intron 1       117,495,708       R       5'-GGCTTTGGTGCACAAGTGAT       141       24.56       23.69       0.87         25       CFTR intron 1       117,497,512       F       5'-GAGCTTTTCCAAGGCGATA       140       25.05       23.83       1.21         24       CFTR intron 1       117,497,521       F       5'-AGGCTTGTCTTTAGCGAGCAT       140       25.05       23.83       1.21         24       CFTR intron 1       117,497,521       F       5'-AGGCTTGTCTTTAGCGAGCACTG       140       25.05       23.83       1.21 <td></td> <td>CFTR intron 1</td> <td>117,490,714</td> <td>R</td> <td>5<sup>′</sup>-CGCCCTATGTCTGGCATTAT</td> <td></td> <td></td> <td></td> <td></td>		CFTR intron 1	117,490,714	R	5 <sup>′</sup> -CGCCCTATGTCTGGCATTAT				
CFTR intron 1       117,493,080       R       5'-AACGCTGTGCCAGATTCTCT         20       CFTR intron 1       117,495,294       F       5'-ATCCTGGAAAGGCACTCTGA       102       23.68       22.72       0.96         CFTR intron 1       117,495,395       R       5'-ATCCCACCCATCTTGAAACA       102       23.68       22.72       0.96         21       CFTR intron 1       117,495,395       R       5'-GCTGTTAGAAGTGGCCTTTCA       233       24.59       23.62       0.97         24       CFTR intron 1       117,495,708       R       5'-GGCTGTTGGTCACAGCAAGTGAT       141       24.56       23.69       0.87         22       CFTR intron 1       117,497,521       F       5'-GAGCTTTTCCAAGGCGATA       140       25.05       23.83       1.21         23       CFTR intron 1       117,497,521       F       5'-GAGCTTTTCCAAGGCGATA       140       25.05       23.83       1.21         24       CFTR intron 1       117,497,521       F       5'-AGGCTTGTCTTTAGCGAGCAATTCAA       140       25.05       23.83       1.21         24       CFTR intron 1       117,497,660       R       5'-AGGCTTGTCTTTAGCGAGCAACTG       140       25.05       23.83       1.21         24       CFTR intron 1       117	19	CFTR intron 1	117,492,959	F	5'-CCATGCCCAGTGATGGTAAT	122	23.61	22.62	0.99
20       CFTR intron 1       117,495,294       F       5'-ATCCTGGAAAGGCACTCTGA       102       23.68       22.72       0.96         CFTR intron 1       117,495,395       R       5'-ATCCTGGAAAGGCACTCTGAAACA       102       23.68       22.72       0.96         21       CFTR intron 1       117,495,395       R       5'-GCTGTTAGAAAGTGGCCTTTCA       233       24.59       23.62       0.97         CFTR intron 1       117,495,708       R       5'-GGGTGGCTACAGCAAGTGGAT       141       24.56       23.69       0.87         22       CFTR intron 1       117,497,512       F       5'-GGCTTTGGTGTCACAAACAATTCCA       141       24.56       23.69       0.87         23       CFTR intron 1       117,497,521       F       5'-GAGCTTTTCCAAGGCGATA       140       25.05       23.83       1.21         CFTR intron 1       117,497,521       F       5'-AGGCTTGTCTTAGCAGCAGCATG       140       25.05       23.83       1.21         CFTR intron 1       117,497,521       F       5'-AGGCTTGTCTTAGCGAGCAAGTC       140       25.05       23.83       1.21         CFTR intron 1       117,498,301       F       5'-AGGCTTGTCTTTAGCGAGCACCTG       158       24.98       24.09       0.89         CFTR intron 1<		CFTR intron 1	117.493.080	R	5'-AACGCTGTGCCAGATTCTCT				
CFTR intron 1       117,495,395       R       5'-ATCCCACCCATCTTGAAACA         21       CFTR intron 1       117,495,395       F       5'-GCTGTTAGAAGTGGCCTTTCA       233       24.59       23.62       0.97         CFTR intron 1       117,495,708       R       5'-GGTGGCTACAGCAAGTGGAT       141       24.56       23.69       0.87         22       CFTR intron 1       117,497,112       F       5'-GGCTCCCACACAACAATCCT       141       24.56       23.69       0.87         23       CFTR intron 1       117,497,522       R       5'-TGATCCCCAAGGCGATA       140       25.05       23.83       1.21         CFTR intron 1       117,497,521       F       5'-GAGCTTTTCCAAGGCGATA       140       25.05       23.83       1.21         CFTR intron 1       117,497,660       R       5'-TACGAATCCCCAGTCACCTG       158       24.98       24.09       0.89         CFTR intron 1       117,498,301       F       5'-AGGCTTGTCTTTAGCGAGCA       158       24.98       24.09       0.89         CFTR intron 1       117,498,458       R       5'-CGCAGTATTGGGGTCAAGTT       158       24.98       24.09       0.89         CFTR intron 1       117,498,458       R       5'-CGCAGTATTGGGGCTCAAGTT       213       24.95	20	CFTR intron 1	117.495.294	F	5'-ATCCTGGAAAGGCACTCTGA	102	23.68	22.72	0.96
21       CFTR intron 1       117,495,476       F       5'-GCTGTTAGAAGTGGCCTTTCA       233       24.59       23.62       0.97         CFTR intron 1       117,495,708       R       5'-GGGTGGCTACAGCAAGTGAT       141       24.56       23.62       0.97         22       CFTR intron 1       117,497,112       F       5'-GGCTTTGGTGCCACAACCATCCT       141       24.56       23.69       0.87         23       CFTR intron 1       117,497,522       R       5'-TGATCCCCACAACAATTCAA		CFTR intron 1	117.495.395	R	5'-ATCCCACCCATCTTGAAACA				
CFTR intron 1       117,495,708       R       5'-GGGTGGCTACAGCAAGTGAT         22       CFTR intron 1       117,497,112       F       5'-GGCTTTGGTGTCACAATCCT       141       24.56       23.69       0.87         23       CFTR intron 1       117,497,521       F       5'-GAGCTTTTCCAAGGCAAGTCACATCCT       140       25.05       23.83       1.21         24       CFTR intron 1       117,497,660       R       5'-TACGAATCCCCAGTCACCTG       158       24.98       24.09       0.89         24       CFTR intron 1       117,498,301       F       5'-AGGCTTGTCTTTAGCGAGCA       158       24.98       24.09       0.89         CFTR intron 1       117,498,458       R       5'-CGCAGTATTGGGGTCAAGTT       158       24.98       24.09       0.89         CFTR intron 1       117,498,458       R       5'-CGCAGTATTGGGGGTCAAGTT       158       24.98       24.09       0.89         CFTR intron 1       117,498,458       R       5'-CGCAGTATTGGGGGTCAAGTT       158       24.98       24.09       0.89         CFTR intron 1       117,499,022       F       5'-TTTGGGAGAAAGTGTCATGCA       213       24.95       24.69       0.25	21	CFTR intron 1	117,495,476	F	5'-GCTGTTAGAAGTGGCCTTTCA	233	24.59	23.62	0.97
22       CFTR intron 1       117,497,112       F       5 <sup>'</sup> -GGCTTTGGTGTCACAATCCT       141       24.56       23.69       0.87         23       CFTR intron 1       117,497,521       F       5 <sup>'</sup> -GAGCTTTTCCAAGGCGATA       140       25.05       23.83       1.21         24       CFTR intron 1       117,498,301       F       5 <sup>'</sup> -AGGCTTGTCTTTAGCGAGCA       158       24.98       24.09       0.89         CFTR intron 1       117,498,458       R       5 <sup>'</sup> -CGCAGTATTGGGGGTCAAGTT       158       24.98       24.09       0.89         CFTR intron 1       117,498,458       R       5 <sup>'</sup> -CGCAGTATTGGGGGTCAAGTT       158       24.98       24.09       0.89         CFTR intron 1       117,498,458       R       5 <sup>'</sup> -CGCAGTATTGGGGGTCAAGTT       158       24.98       24.09       0.89         CFTR intron 1       117,498,458       R       5 <sup>'</sup> -CGCAGTATTGGGGGTCAAGTT       158       24.95       24.69       0.25         25       CFTR intron 1       117,499,022       F       5 <sup>'</sup> -TTTGGGAGAAAGTGTCATGCA       213       24.95       24.69       0.25		CETR intron 1	117,495,708	R	5'-GGGTGGCTACAGCAAGTGAT				
CFTR intron 1       117,497,252       R       5'-TGATCCCCACAACAATTCAA         23       CFTR intron 1       117,497,251       F       5'-GAGCTTTTTCCAAGGCGATA       140       25.05       23.83       1.21         CFTR intron 1       117,497,660       R       5'-TACGAATCCCCAGTCACCTG       140       25.05       23.83       1.21         24       CFTR intron 1       117,498,301       F       5'-AGGCTTGTCTTTAGCGAGCA       158       24.98       24.09       0.89         CFTR intron 1       117,498,458       R       5'-CGCAGTATTGGGGTCAAGTT       158       24.95       24.69       0.25         25       CFTR intron 1       117,499,022       F       5'-TTTGGGAGAAAGTGTCATGCA       213       24.95       24.69       0.25	22	CETR intron 1	117.497.112	F	5'-GGCTTTGGTGTCACAATCCT	141	24.56	23.69	0.87
23       CFTR intron 1       117,497,521       F       5'-GAGCTTTTTCCAAGGCGATA       140       25.05       23.83       1.21         24       CFTR intron 1       117,497,660       R       5'-TACGAATCCCCAGTCACCTG       158       24.98       24.09       0.89         24       CFTR intron 1       117,498,301       F       5'-CGCAGTATTGGGGTCAAGTT       158       24.98       24.09       0.89         CFTR intron 1       117,498,458       R       5'-CGCAGTATTGGGGGTCAAGTT       213       24.95       24.69       0.25         25       CFTR intron 1       117,499,022       F       5'-TTTGGGAGAAAGTGTCATGCA       213       24.95       24.69       0.25		CETR intron 1	117.497.252	R	5'-TGATCCCCACAACAATTCAA				
CFTR intron 1         117,497,660         R         5' -TACGAATCCCCAGTCACCTG         Lotor         Lotor <thlot< thr="">         Lotor</thlot<>	23	CFTR intron 1	117.497.521	F	5 <sup>′</sup> -GAGCTTTTTCCAAGGCGATA	140	25.05	23.83	1,21
24         CFTR intron 1         117,498,301         F         5'-AGGCTTGTCTTTAGCGAGCA         158         24.98         24.09         0.89           CFTR intron 1         117,498,458         R         5'-CGCAGTATTGGGGTCAAGTT         213         24.95         24.69         0.25		CFTR intron 1	117.497.660	R	5'-TACGAATCCCCAGTCACCTG			0	
CFTR intron 1         117,498,458         R         5 <sup>°</sup> -CGCAGTATTGGGGGTCAAGTT         21.00         21.00         0.00           25         CFTR intron 1         117,499,022         F         5 <sup>°</sup> -TTTGGGAGAAGTGTCATGCA         21.3         24.95         24.69         0.25	24	CFTR intron 1	117.498.301	F	5'-AGGCTTGTCTTTAGCGAGCA	158	24.98	24.09	0.89
25         CFTR intron 1         117,499,022         F         5'-TTTGGGAGAAGTGTCATGCA         213         24.95         24.69         0.25	- ·	CFTR intron 1	117.498.458	R	5 <sup>′</sup> -CGCAGTATTGGGGGTCAAGTT				2.00
	25	CFTR intron 1	117,499,022	F	5 <sup>'</sup> -TTTGGGAGAAGTGTCATGCA	213	24.95	24.69	0.25

(Continued)

#### TABLE 1 | Continued

No.	Location	g number of 5'end	a	Sequence	Amplicon length (bp)	Ct <sup>b</sup> (case 1)	Ct <sup>b</sup> (control)	∆Ct <sup>c</sup>
	CFTR intron 1	117,499,234	R	5'-TCCAAAAGACGCATCTGACA				
26	CFTR intron 1	117,499,949	F	5 <sup>′</sup> -GTGAGAGGGGAAGACAGCAG	207	23.43	23.61	-0.18
	CFTR intron 1	117,500,155	R	5 <sup>'</sup> -ACTCCAGCCACCCTTTCTTT				
27	CFTR exon 1	117,504,160	F	5'-TTCCATATGCCAGAAAAGTTGA	140	23.66	23.89	-0.23
	CFTR exon 2	117,504,299	R	5'-ATTCGAGGCGCTGTCTGTAT				

<sup>a</sup>GRCh38.p12; NC\_000007.14, F: forward, R: reverse.

<sup>b</sup>Ct-values are defined as the number of cycles required for the fluorescent signal to cross the threshold in the real-time quantitative polymerase chain reaction.

<sup>c</sup> The  $\Delta$ Ct-value is determined by subtracting the Ct-value of the control subject from that of Case 1.

Ct, Cycle threshold.



variant lacked whole of the promoter region of *CFTR*, *CFTR* mRNA would not be transcribed from the allele. This deletion (GRCh38.p12:NC\_000007.14:g.117,361,112\_117,498,678del137, 567) was thus regarded as a novel pathogenic variant causing CF.

#### DISCUSSION

We encountered Japanese siblings with CF who had a novel variant (*CFTR* promoter deletion) in addition to exon 16-17b deletion, that is the most common *CFTR* variant in Japanese CF patients (3, 4). The exon 16-17b deletion causes a deletion of 153 amino acids, p.(Gly970\_Thr1122del)-CFTR, that is located over 3 transmembrane helices of the CFTR

protein (3). p.(Gly970\_Thr1122del)-CFTR was synthesized but not transported to the membrane when expressed in CHO cells, indicating that it can be categorized as a class II mutation (3, 8). The novel variant, *CFTR* promoter deletion, is not be transcribed due to the lack of whole of the promoter region of the *CFTR* gene and thus can be categorized as a class I mutation (8).

CF is a multiorgan disease with marked phenotypic heterogeneity. The pathogenic *CFTR* variants are grouped into five classes according to the mechanism by which the variants affects the normal CFTR protein function (8). Patients who are homozygous or compound heterozygous for the severe variants belonging to classes I, II and III are underweight,



more often required pancreatic enzyme supplementation and have a higher prevalence of diabetes than patients carrying at least one class IV mutation (9). Among CF patients, pulmonary disease is the main cause of death, and the colonization of Pseudomonas aeruginosa in the respiratory tract is associated with a reduced respiratory function and increased pulmonary exacerbation (10, 11). Severe variants are also linked to a high probability of the colonization of P. aeruginosa in the respiratory tract (12). The present patients were compound heterozygous for the severe variant (class I/class II) and were immediately prescribed inhalation of recombinant human deoxyribonuclease I and replenishment of pancreatic enzyme after receiving their diagnosis. Fortunately, the respiratory function has not worsened in either of these patients, and P. aeruginosa has been never isolated from respiratory tract. The early therapeutic intervention after the diagnosis of CF helps protect the lungs from damage and improves the prognosis.

As the life expectancy of CF patients continues to increase, infertility has become a critical problem in these patients. Nearly all men with CF had infertility due to obstructive azoospermia (13), specifically congenital bilateral absence of the vas deferens, which is mainly caused by the variant in the *CFTR* gene (14). In our patients, the *ASZ1* gene

encoding ASZ1, which is only expressed in the testis and ovary and probably plays a role in germ cell development (15), was also completely deleted on the one allele. Either the deletion of Asz1 or the variant leads to male infertility in mice (16). No CF patients have shown the deletion of the ASZ1 gene. Male CF patients produce sperm, and their testicular histology is usually normal (17). Although the ASZ1gene was deleted only on the one allele in our patients, the analysis of sperm in case 1 may allow us to investigate the association between the ASZ1 gene and male infertility in humans.

As in the present sibling cases of CF, large heterozygous deletions escape detection by standard gene sequencing methods. In contrast to Caucasians, such large deletions are frequently detected in Japanese CF patients (3). MLPA can detect all deletions, not just the common one. Thus, in Japanese CF patients, MPLA can be more useful in searching for variants of *CFTR* gene.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

#### **ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by Institutional Review Boards of Kitakyushu General Hospital and Nagoya University Graduate School of Medicine. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

### **AUTHOR CONTRIBUTIONS**

MK involved the treatment of patients, carried out the initial analysis of data for work, and drafted the initial manuscript. TH and KK carried out the acquisition and analysis of data

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