MLPA-based point mutation analysis of the FBN1 gene in Marfan syndrome

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Introduction

The Multiplex Ligation-depandant Probe Amplification assay (MLPA) detects whole-exon deletions or duplications (Schouten *et al* 2002). These mutations are present at a significant level in many genes such that it is financially advantageous to pre-screen all new patients using MLPA prior to point mutation analysis (Bunyan *et al* 2004). To further improve the mutation detection rate, it would be useful if an MLPA test could also detect common point mutations. In order to facilitate this, we designed MLPA probes which are specific to the 14 most common FBN1 gene point mutations/microdeletions listed on the Marfan syndrome database (www.umd.necker.fr/Site%20Marfan/01AHOME%20PAGE.html) and these probes were directly synthesised with the second oligo of each pair being 5' phosphorylated. The 14 FBN1 mutations are listed in Table 1. We screened 55 patients using this MLPA probe mix, three of whom were known to be carriers of mutations which are contained within the kit (the mutations being previously identified by dHPLC and sequencing analysis).

Table 1. The 14 FBN1 mutations in the point mutation probe mix.

Exon	Mutation	No. of FBN1 database entries	Wessex cases
2	184 C>T	5	
4	364 C>T	3	
13	1633 C>T	4	
16	2019del4	3	1
19	2341 T>C	3	
24	3037 G>A	7	
26	3217 G>A	3	
26	3294 C>T	3	
28	3509 G>A	3	2
39	4930 C>T	3	
54	6661 T>C	4	
55	6844 C>T	3	
57	7039delAT	3	2
62	7754 T>C	6	

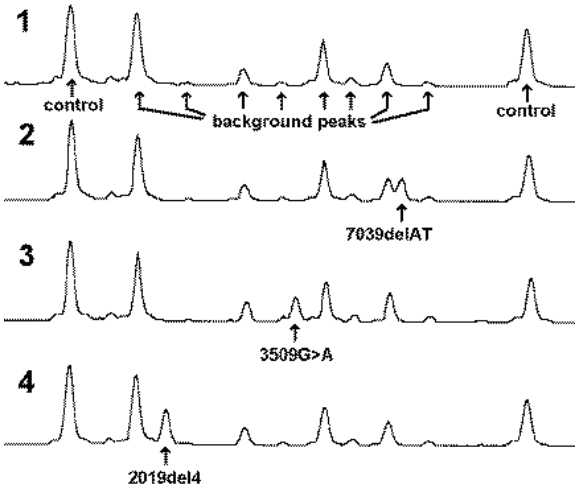
Methods

MLPA analysis was carried out using the standard MLPA protocol of MRC-Holland, the only difference being that 1µl of DNA was used per test (typically 400-600ng) rather than 100ng. If any of the FBN1 mutations were present, an additional peak would appear somewhere between 93 and 119 base pairs in size. Two control probe sets (with peaks of 90 and 124 b.p.) were included to show that the MLPA was working correctly. 1µl of the MLPA PCR product was run on an ABI3100 sequencer and the data were analysed using Genotyper (version 2.0) software. An example of the results is given in Figure 1 which shows the data from the three known carriers.

Results

As can be seen in Figure 1, several additional peaks were seen in all individuals, including normal controls. However, the mutation-specific peaks were still obvious amongst the constant pattern that was seen. Using this test we were able to confirm all three known carriers and in addition we found two additional carriers in the 52 previously-unscreened patients (1 x 3509G>A, 1 x 7039delAT).

Figure 1. Genotyper output from a normal control (track 1) and the 3 known FBN1 mutation carriers (tracks 2-4).



Discussion

Although positive results were obtained with this test, two major problems have been encountered using the FBN1 point mutation probe mix. Firstly, this probe set was originally designed in March 2004 to work in conjunction with the impending MRC-Holland FBN1 dosage kit (which was released in July 2004) such that both probe sets could be used simultaneously to perform dosage and point mutation analysis on a single DNA sample. However, the point mutation probes were designed prior to knowing the ligation sites of the dosage probes – now that the MRC-Holland dosage kit has been released we have unfortunately discovered that there is some overlap in the ligation sites of the two probe sets, so both analyses cannot be performed together.

Secondly, most of the point mutations which are contained in the kit involve a C to T substitution at the DNA level. This means that the mutation-specific probe (which ends in a T) is prone to hybridise to a normal chromosome via a G-T mismatch, hence the extra peaks seen in Figure 1.

Both of these problems could be overcome by redesigning the point mutation probes so that they hybridise to the opposite strand. Armed with this retrospective knowledge, we are exploring the possibility of designing useful point mutation probe sets for other syndromes.

References

Bunyan, D.J., Eccles, D.M., Sillibourne, J., Wilkins, E., Thomas, N.S., Shea-Simonds, S., Duncan, P.J., Curtis, C.E., Robinson, D.O., Harvey, J.F. & Cross, N.C.P. (2004) Dosage analysis of cancer predisposition genes by Multiplex Ligation-Dependent Probe Amplification. *Br. J. Cancer* in press.

Schouten, J.P., McElgunn, C.J., Waaijer, R., Zwijnenburg, D., Diepvens, F. and Pals, G. (2002) Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependant probe amplification. *Nuc. Acid Res*. **30**, e57