

Deletions in *STK11* in Peutz-Jeughers Syndrome detected by Multiplex Ligation-dependent Probe Amplification (MLPA)

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Introduction

Peutz-Jeghers syndrome is an autosomal dominant disorder characterized by melanocytic macules of the lips, buccal mucosa and digits; multiple gastrointestinal hamartomatous polyps; and an increased risk of various neoplasms. Defects of the *STK11* (*LKB1*) gene are the cause of PJS.

Point mutations and small deletions / insertions in the *STK11* gene can be detected by sequencing, DHPLC and other methods. Deletions and duplications of complete exons are often not detected by sequencing or DHPLC as there is usually also a normal allele of that exon present.

The gene *STK11* has 10 exons. Exon 10 is located entirely in the 3' untranslated region.

An MLPA kit was designed for *STK11* and consists of probes for all exons and promoter region, including exon 10. This kit will detect copy number aberrations of the *STK11* exons. It is not meant to detect point mutations.

Methods

Multiplex Ligation-dependent Probe Amplification (MLPA) is a sensitive, low cost, high throughput technique for the quantitative assessment of each exon of a gene. This technique, makes use of oligonucleotides designed to hybridise adjacently to a target sequence. After the ligation of the oligonucleotides, the probe is amplified and quantified. Up to 40 different probe sets can be multiplexed to assess relative copy numbers for all probes. Deletions and duplications in genes can be easily detected.

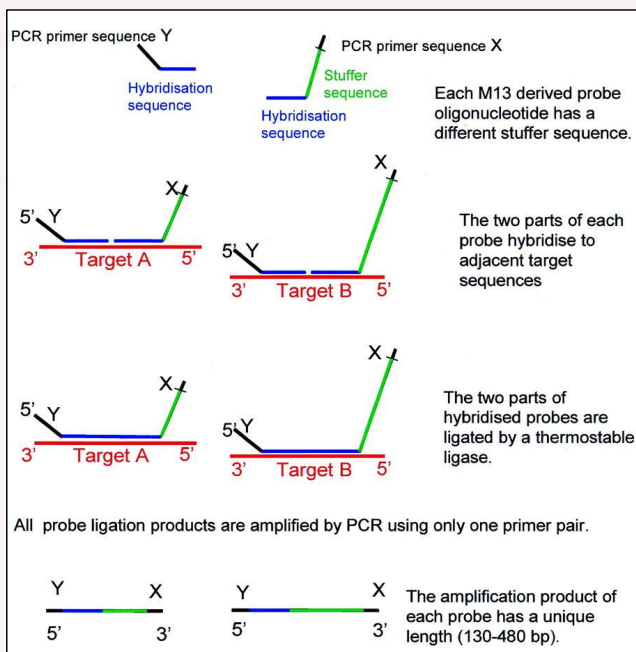


Figure 1. Each MLPA probe consists of two oligonucleotides, one synthetic and one M13-derived. Denatured DNA is hybridized with a mixture of probes. Only perfectly hybridized probes are ligated and therefore exponentially amplified. Relative amounts of probe amplification products reflect the relative copy number of target sequences.

Results

We analyzed 36 PJS families using conventional methods for mutation detection (DHPLC and DNA sequencing) and MLPA. There were 14 families (39%) with point mutations in *STK11* and 7 with genomic deletion (20%).

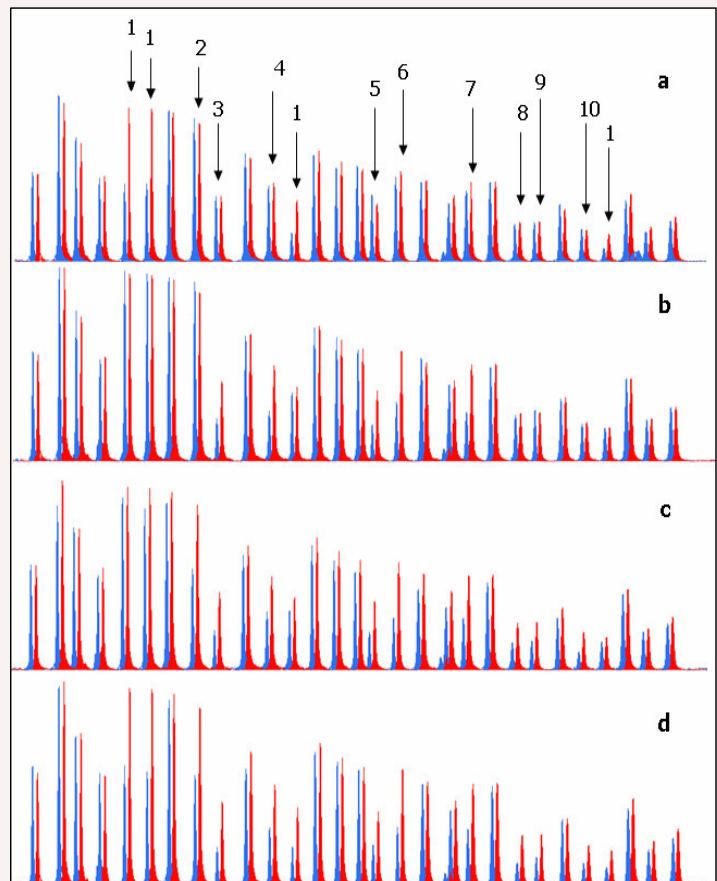


Figure 2. Numbered peaks represent probes for each *STK11* exon. Four probes are present for exon 1/promoter region. Non-numbered peaks are control probes. Blue=patient DNA; red=control DNA. One of the analyzed cases had the promoter region and exon 1 deleted (a), another had exons 3-7 deleted (b), one had exons 2-10 deleted (c) and four cases had the entire *STK11* gene deleted (d).

Conclusions

These data show that 33% of pathogenic mutations in *STK11* are genomic deletions which demonstrates that the deletion in *STK11* recently described by Le Meur et al., Eur. J. Hum. Genet. 2004, is not a rare phenomenon.

We also conclude, once again, that MLPA is a sensitive, robust and easy to perform technique and therefore an important and full of potential tool for deletion detection in genes that are not detected by direct sequencing and other screening methods.

For more information on MLPA, contact MRC Holland, Hudsonstraat 68, 1057 SN Amsterdam, The Netherlands.

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