Assignment of microsatellite sequences to the region duplicated in CMT1A (17p12): a useful tool for diagnosis

C Cudrey, C Chevillard, D Le Paslier, A Vignal, E Passage, M Fontes

Abstract
Charcot-Marie-Tooth disease type 1A (CMT1A), the most prevalent form of the peripheral hereditary neuropathies, has been associated with a duplication of a genomic segment of 1-5 Mb, located in 17p11.2. Recently, the same segment has been found to be deleted in patients with another peripheral neuropathy, hereditary neuropathy with liability to pressure palsies (HNPP). Highly polymorphic markers are rare in this area, rendering the diagnosis highly dependent either on invasive examinations (like nerve biopsy) or not totally reliable (like gene dosage). Thus, we used a contig of YACs, including the whole region duplicated in CMT1A, to map highly polymorphic microsatellite loci, designed in Genethon. We showed that four of these loci are located in the duplicated region, allowing us to propose them as diagnostic markers for CMT1A and HNPP.

Materials and methods
PCR AMPLIFICATION
Each reaction contained 200 ng of YAC DNA. The concentration of reagent was: primers 0-5 mmol/l, Tris-HCl 10 mmol/l, pH 8-8, KCl 50 mmol/l, MgCl2 1-5 mmol/l, non-ionic detergent 0-1%, and 0-3 U of Taq DNA polymerase. The PCR reaction was performed on a Perkin Elmer Cetus DNA thermal cycler with the following conditions: 96°C for three minutes followed by 30 cycles (denaturation 94°C for one minute, annealing 55°C for 30 seconds, extension 72°C for 30 seconds). Amplification products were analysed on 2% agarose gels and stained with ethidium bromide. The sequence of individual primer pairs can be obtained from Chance et al.8 Chevillard et al.9 or GDB databases (for Genethon microsatellites).

YAC RESTRICTION ANALYSIS
Yeast DNA, embedded in agarose block, was digested overnight with NoI (New England Biolabs). The resulting products were analysed by PFGE on a Biorad slab DR II. The DNA

Table 1 Description of the Genethon microsatellites used in this study. N0. all=n=number of alleles. Sequences and additional information have been deposited in GDB.

<table>
<thead>
<tr>
<th>D number</th>
<th>Name</th>
<th>Het</th>
<th>PIC</th>
<th>N0 all</th>
<th>Size</th>
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</thead>
<tbody>
<tr>
<td>D17S793</td>
<td>165sd4</td>
<td>0.7</td>
<td>0.765</td>
<td>7</td>
<td>99</td>
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<tr>
<td>D17S839</td>
<td>200y12</td>
<td>0.5548</td>
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<td>D17S955</td>
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<td>D17S921</td>
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<td>0.7352</td>
<td>0.9063</td>
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<td>D17S922</td>
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<td>0.5082</td>
<td>0.5352</td>
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<td>184</td>
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</table>
Table 2  Presence of previously described STSs and new Genethon microsatellites in the different YACs of the contig. This has been determined using a PCR assay (as described in Materials and methods). The status of the pre-existing STS versus CMT1A duplication is indicated, according to Chance et al.8

<table>
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<tr>
<th>YACs</th>
<th>D17S548</th>
<th>D17S455</th>
<th>D17S12</th>
<th>D17S125</th>
<th>D17S61</th>
<th>D17S549</th>
<th>D17S547</th>
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</table>

was fractionated in 1% agarose gels in 0.5×TBE at 200 V, with pulse time from 45 to 120 seconds at 12°C for 24 hours. The DNA was transferred to Pall Biodyne B membrane and hybridised to end probes, in order to localise the NotI sites in the YACs.

Results and discussion

We analysed YACs described in a previous paper8 to determine if these clones contained microsatellites which had been localised, by Genethon, in the pericentromeric region of the short arm of chromosome 17.1011 Using primers designed by Genethon (table 1), we determined the PCR conditions under which the reaction produces a unique amplification product (without extra bands) on agarose gels. The results of these experiments are summarised in table 2, describing the presence of the various STSs in YACs. From data already published,8 it was obvious that the microsatellite locus D17S805 was proximal to the region duplicated in CMT1A, as it was present in YACs which do not encompass it. The four loci D17S793, D17S839, D17S921, and D17S955 are located in YACs covering the CMT1A region. Two loci, D17S839 and D17S955, were found to be present in the YAC 884G12, totally included in the CMT1A duplicated region (and in the 1.1 Mb NotI fragment). Locus D17S839 is probably proximal to D17S955, as the latter is not present in YAC 804F11, a YAC showing chimerism at its distal end. However, locus D17S921 was not present on YACs 884G12 and 804F11 which cover the central part of the CMT1A region. Locus D17S922 was found to be distal to the others. Thus we deduced that the order of the Genethon microsatellite sequences is: cen-D17S805-D17S793-D17S839-D17S921-D17S922. This physical order is totally consistent with the results of the genetic map.

A genomic map of this region has recently been constructed by Penta et al.7 Of particular interest is the localisation of the NotI sites, which have been positioned with respect to the breakpoints associated with the CMT1A duplication. We mapped the NotI sites in the different YACs (chimeric YACs, like 856A6 and 955E12, were not taken into account in this work), allowing us to propose the map presented in the figure. From the presence of the microsatellite loci in the various YACs, we deduced intervals where these clones should be localised on the genomic DNA. We concluded that the three microsatellite loci D17S793, D17S839, and D17S955 are located in CMT1A duplicated region of CMT1A, as they are all present in at least one of the three YACs, 124C3, 884G12, and 804F11, entirely included in this region. D17S921 is present in YAC 887H5 and not in 884G12 and thus may be located at the distal end of the CMT1A duplicated region. The present YAC mapping does not give sufficient resolution to determine this and the issue may be resolved by genotyping of affected subjects. Interestingly, three of the newly isolated markers are located in the distal half of the CMT1A region, allowing its duplicated status to be determined (as partial duplications, involving only the proximal part of the region, as has been recently described).

These microsatellites are very informative (table 1), and this enhancement in the number of polymorphic markers in the region will increase the chances that parent and child are informative for one marker. These molecular resources will thus constitute a very powerful tool in the diagnosis of CMT1A. Testing this on a large number of CMT1A patients is in progress.

Localisation of five microsatellites on a NotI genomic map obtained from YAC restriction analysis (see Materials and methods). The extent of the CMT1A duplicated region is indicated from the work of other groups.712 Vertical bars represent the boundaries of the internals we defined from the ends of the different YACs of the contig. Coligated YACs (like 804F11), or clones providing inconsistent mapping data (like 856A6 and 955E12), are not indicated in the figure.
We are very grateful to Dr J Weissenbach for his constant help. This work has been supported by AFM (Association Française contre les Myopathies) and GREG (Groupement de Recherche et d’Etude sur les Genomes) fellowships. C Cudrey is a recipient of an AFM fellowship.


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