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Relationship between Charcot – Marie – Tooth 1A and Smith – Magenis regions. snU3 may be a candidate gene for the Smith – Magenis syndrome

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The juxtacentromeric region of the human chromosome 17 short arm (17p11.2-p12) contains genes involved in the Charcot – Marie – Tooth type 1A disease (CMT1A) and the Smith – Magenis syndrome (SMS). CMT1A is associated with a duplication of a short segment whereas SMS is linked to microdeletions, extending toward the centromere. We describe the construction and analysis of a 5 Mb YAC contig spanning the CMT1A duplicated segment and the distal part of four SMS microdeletions. We concluded that the YAC contig contains about 1Mb of genomic DNA which is deleted in the four SMS patients analysed. Moreover two YACs contain both STS deleted in SMS (U3) and STS duplicated in CMT1A (5H5), but the proximal breakpoint associated with the CMT1A duplication is not the same as the distal SMS breakpoint we studied. Finally we located five new STS in SMS deletion. Two of them, a microsatellite (D17S805(23)) and the gene coding for small nuclear RNA U3, have been localized in the contig we described. We may also note that snU3 is the first expressed sequence localized in an SMS deletion so far. The possible participation of this gene in the SMS phenotype is discussed.

INTRODUCTION

Two genetic diseases associated with chromosomal rearrangements have been mapped on the same segment of the juxtacentromeric region of chromosome 17 short arm (17p11.2-p12): Charcot – Marie – Tooth disease type 1A (1) and the Smith – Magenis syndrome (2).

Charcot – Marie – Tooth disease (CMT) is the most frequent of the motor and sensory peripheral neuropathies (3). Subtype 1A is the most common form of the disease. It is clinically characterized by distal muscle atrophy, a decrease in nerve conduction velocity and demyelination of nerve fibers (3). Inherited as an autosomal dominant trait, the disease has recently been associated with the partial duplication of the sub-band 17p11.2 (4,5), spanning at least 1.15 Mb and including the loci D17S61, D17S122, D17S125. Recently three groups have described YAC contigs spanning the CMT1A region (7–9). They proposed the region duplicated in CMT1A to be about 1.5Mb. Moreover, Pentao et al (9) described a complex repeated element that could be involved in the duplication of the CMT1A region. This could arise through unequal crossing over during meiosis.

Smith – Magenis syndrome (SMS) is a de novo disorder linked to cytogenetically visible microdeletions of the same sub-band (17p11.2). SMS patients show several aspecific clinical signs (e.g. mental retardation, facial anomalies and slow growth) associated with more specific ones such as speech delay,

prepsychotic behaviour and sleep problems (10,11). Four markers have been mapped to the critical SMS region so far (defining the loci D17S29, D17S71, D17S258 and D17S445) (12,13). These data established that the region deleted in SMS patients is more proximal than the region duplicated in CMT patients. Recently Pentao et al pointed out that the next proximal locus which is deleted in patients SMS (D17S445) maps more than 1.5 Mb proximal to D17S122. However it remains possible that the distal end of the SMS deletion and the proximal end of the CMT duplication lie close together.

No expressed sequence has been mapped to the SMS critical region up to now. On the contrary, several groups have recently proposed the gene coding for the peripheral myelin protein-22 (PMP-22) as a candidate gene for CMT (7,14–16). This assumption came from indirect, but strong, arguments: 1) The human gene is duplicated in CMT patients. 2) In *Trembler* mutant mice (17,18) mutations in the PMP-22 gene are responsible for a phenotype strongly suggesting the human CMT1A phenotype (hypomyelination of peripheral nerves, decrease in nerve conduction velocity, muscle weakness). However whereas *Tr* mutations are due to point mutations in the PMP-22 gene, the CMT1A phenotype seems to result, in human, from a dosage effect involving the duplication of a large region (19). Moreover, Guzzetta et al (13) reported recently on a SMS patient with a microdeletion of the CMT1A region, with no CMT1A clinical

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signs. Recently Valentijn et al (15) described a point mutation segregating with the disease, in a CMT1A family showing strong linkage to 17p11.2-p12 markers without duplication. The mutation is identical with that recently found in the *Trembler-J* mouse. This indicates that the gene coding for the PMP-22 protein is the major gene involved in the CMT1A phenotype. However this region probably contains several other genes, and some of them could contribute to the CMT phenotype.

In this paper, we describe the construction of a 5 Mb contig spanning the CMT 1A region and the distal end of four Smith-Magenis microdeletions. This allows us to physically link the CMT1A duplications to the end of the SMS deletions on a

genomic region about 500 Kb. The possible relationship (in terms of molecular mechanism) between the SMS deletion and the CMT1A duplication is discussed. Moreover we describe for the first time the localization of an expressed sequence coding for snRNA U3 in the SMS deletion. The possible involvement of this gene in the SMS phenotype is discussed.

RESULTS

YAC library screening and contig construction

The last version of the CEPH library (20), containing YACs (21) with large inserts (1 Mb average length, Ougen et al, in preparation), was screened by PCR with four markers located in 17p11.2-p12 (the characteristics, origin and PCR conditions of the primers are given in Methods). Two of these STS were previously mapped in CMT duplications D17S122 (VAW409) and D17S125 (VAW412R3). One was located distally to CMT duplication D17S124 (VAW411R2). The last one D17S458 (5G7) lies close to the end of the proximal boundary (7,9). Using these tools, we isolated a series of YACs. The characteristics of these YACs, presented in table 1 and 2, indicate that they range from 700 Kb up to 2 Mb, with an average length of 1 Mb. As a first approach we used IRS-PCR fingerprints (22) to detect overlapping regions between YACs. This approach allowed us to detect coligations since the presence of a band in only one YAC of the contig likely suggests a chimeric YAC, an example is given in figure 1. As indicators of a potential coligation event in a YAC, we used the following criteria: 1) Inconsistent IRS-PCR fingerprints (using different combinations of primers). 2) Discrepancies in the restriction fragment lengths/STS content. These elements also indicated on which arm the coligation occurred. Finally the coligation status was checked by FISH.

In a further step these YACs were analyzed using the STS present in the region. Moreover, since some PCR results could

Table 1. Characterization of the YAC clones isolated from the CEPH YAC library

YAC	Size (Kb)	coligation
915C12	1200	YES
838E4	860	NO
938G3	2000	YES
768B12	2000	YES
761C6	1200	NO
124C3	500	YES
804F11	>1400	YES
884G12	1080	NO
887H5	960	NO
961F10	1600	NO
856A6	860	NO
955E12	1200	NO
402D12	750	NO

The size was estimated by PFGE on a Biorad CHEF DRII Figure. Coligation was estimated by 'Alu-PCR' fingerprints, by restriction analysis of YACs and FISH. Positive loci were checked by PCR and southern Blot analysis.

Table 2. Loci present (+) in individual YACs

LOCI	D17871	D178445	D178805	U3	D178458	D178455	D178793	D178261	D178122	D178125	D17861	D178459	D178457	D178460	D178124
828B9			+	+	+	+	+								
761C6				+	+	+	+								
915C12					+	+	+								
838E4					+	+	+								
938G3					+	+	+	+	+						
768B12					+	+	+	+	+						
804F11									+	+	+				
884G12									+	+	+				
887H5										+	+	+	+		
961F10										+	+	+	+	+	+
856A6														+	+
955E12															+
402D12					+	+	+	+							
124C3						+	+	+	+						
49G7						+	+	+	+						

be due to artefacts, we checked the YACs for the presence of the corresponding locus by hybridization on YAC Southern blots (figure 2).

As shown in table 2, the majority of the YACs contained at least two STS (6 in the YAC 961F10, 1.6 Mb). This allowed us to propose the YAC contig presented in figure 3. It spans at least 5 Mb; the distal end lies clearly outside the CMT region (about 1.7 Mb) because several YACs contain the loci D17S460 and D17S124, localized outside the CMT1A region (7). Using only four YACs (828B9, 49G7, 884G12 and 961F10) we were able to construct a contig spanning 4 Mb. FISH analysis of this 4 Mb contig (figure 4) showed a signal only on the short arm of the chromosome 17. This indicates that these YACs are not coligated over the majority of their length (a coligated fragment of the size of a phage insert, 20 Kb, could be easily detected by FISH). Moreover we found the distance between the centromere and the contig to be 40% of the distance between the centromere and the telomere of the short arm. This indicates that the contig is localized in the proximal part of the band p12.

Physical map of the region: relationship with the diseases

In an attempt to define new markers in the SMS critical region we mapped the chromosome 17 microsatellites sequences, isolated in Genethon (23), by using a somatic cell hybrid panel

containing a SMS hybrid (24)(figure 5A). Figure 5B and 5D show that four of these sequences lie in the region deleted in the SMS hybrid (DH4A) present in the panel. Using the different YACs we were able to localize one of these microsatellites (D17S805) in YAC 828B9 (figure 5C) of our contig. The three others microsatellites therefore probably lie proximally. This work allowed us to double the number of STS localized in the SMS region.

A recent study by Mazan et al (25) localized the gene coding for U3 small nuclear RNA in sub-bands 17p11.2-p12. Using the hybrid panel described in Methods, we localized this locus in the region deleted in the SMS hybrid (figure 5F). PCR primers were used to check the YACs of the contig for the presence of this locus. We found (figure 5E) that two YACs (828B9 and 761C6) from the contig contained the U3 locus (the localization of this locus was verified on YACs and cell hybrid Southern blots, data not shown). It was interesting to note that the two YACs are at the proximal end of the contig. One YAC (828B9) contains the two loci (D17S805 and snU3). The other (761C6) contains only snU3. In a next step we screened the YAC library again, using primers corresponding to an extragenic segment of the snU3 cluster. This allows us to isolate two additional YACs (797G1 and 965G9) containing the snU3 cluster. These YACs contain also the locus D17S805 but not the locus D17S458. These two YACs have been used to extend the contig proximally.

Moreover, we demonstrated that the two YACs, 828B9 and 761C6 contained at least one locus duplicated in CMT1A (D17S455, probe 5H5) (7) and one locus deleted in SMS (D17S805 or U3). This allowed us to place the distal breakpoint

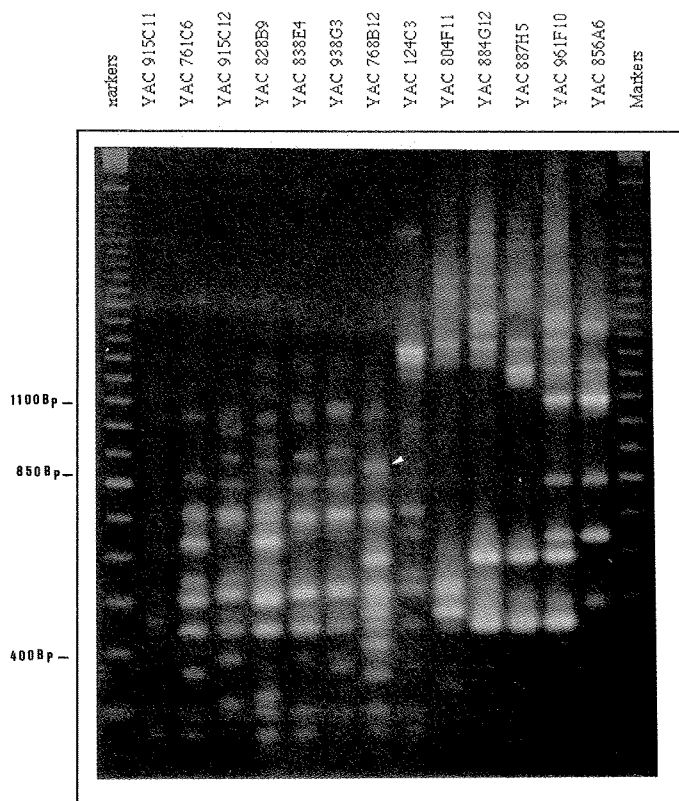


Figure 1. IRS-PCR fingerprints. Alu-PCR reactions were conducted with individual YAC DNA, using Alu 5 as primer. The reaction products were analysed on 0.8% agarose gel, and stained with ethidium bromide. The presence of common bands among different YACs confirmed the overlapping observed with PCR screening and southern blots hybridization. The presence of a band in a YAC, and not in the others, indicates a coligation in the YAC, e.g., the 850 bp band (indicated by an arrow) is present only in YAC 768B12.

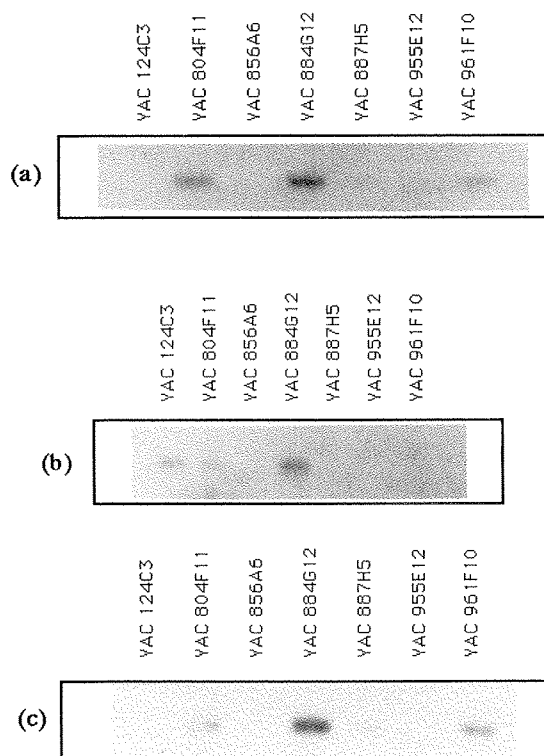


Figure 2. Mapping of various STS to a YAC panel. Southern blots from Eco RI digested DNA from different YACs located in the contig were hybridized with the following probes: pEW 401(a), VAW 409(b), pVAW 412R3(c).

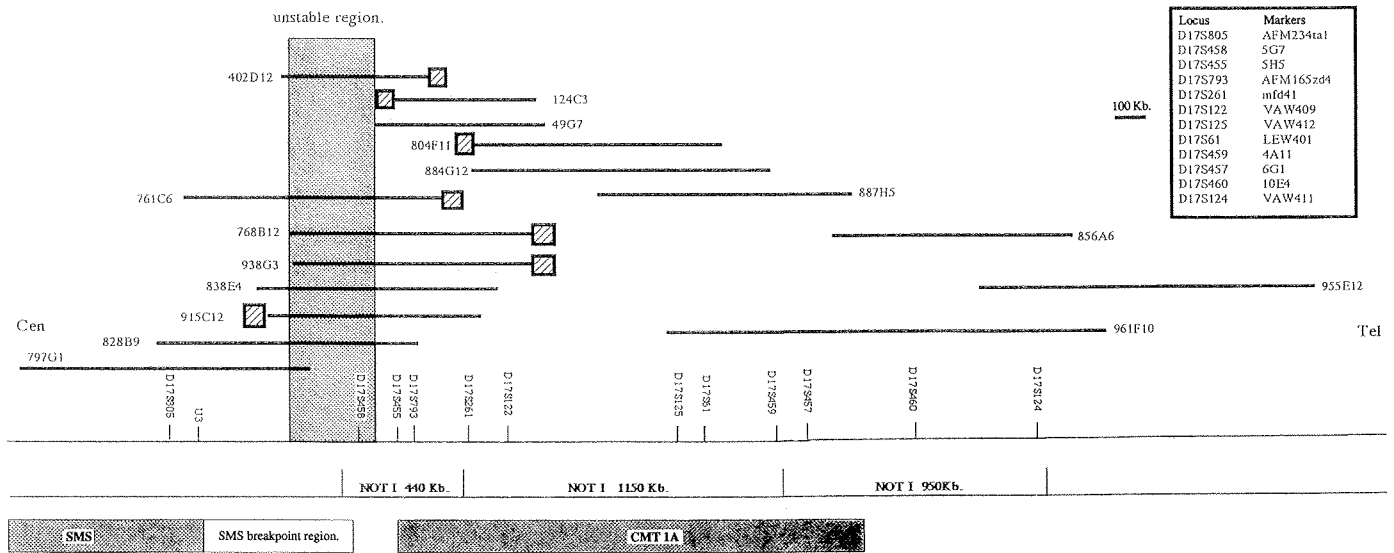


Figure 3. Schematic representation of the YAC contig spanning the CMT 1A duplication and the distal end of SMS microdeletion. YACs were ordered using PCR assay and southern blot hybridization. Distances were estimated from the lengths of the YACs and the size of Not I fragments. ▨ indicates a coligation, which were estimated by 'Alu-PCR' fingerprints and by restriction analysis of YACs. SMS and CMT 1A breakpoints were positioned from our data (on SMS) and from the literature data. The region common to all unstable YACs is indicated as unstable region.

of the SMS microdeletion and the proximal breakpoint of the CMT duplication in the same region. NotI restriction analysis of the YACs (figure 3) suggested that this region is not bigger than 500 Kb. We could therefore assume that the CMT duplicated region is about 1.5 Mb and that the region, deleted in the SMS hybrid and present in the YAC contig is about 1 Mb.

To determine if the SMS and CMT breakpoints were the same, we localized the locus D17S458 distal to the SMS deletion breakpoint of the hybrid present in the hybrid panel described above (data not shown). From the data presented by Matsunami et al (7) and Pentao et al (9) we deduced that this locus (D17S458) is proximal to the CMT1A duplication. Therefore the locus 5G7 is neither duplicated in CMT1A nor deleted in the SMS hybrid we used. These results also confirmed our previous data indicating that probe mfd41 lies distally to another SMS breakpoint (26).

In order to extend these conclusions, we analyzed the chromosomes of three additional patients by FISH using the YAC 797G1 (U3 positive and 5G7 negative) as probe. From the data on normal chromosomes (fig 6A), we concluded that this YAC is not coligated. Using the same probe we analyzed the chromosomes of three SMS patients presenting visible microdeletions of different size (26). About 50 metaphases from each patients have been analyzed. All of them exhibit the same pattern, presented in fig 6B and 6C. The non deleted chromosome shows a normal pattern of hybridization. The deleted chromosome shows a considerably reduced hybridization signal (sometime no signal). It implies that: 1—the major part of the region containing U3 is deleted in the three patients. 2—the distal breakpoint of these three microdeletions lies in the the same region as the hybrid precedingly analyzed.

During this work we observed that, in certain conditions like over-growth, several YACs from the contig were unstable, presenting several deleted forms in the same yeast colony. The basis of this instability seemed not to be the size (for example YAC 961F10, 1.6 Mb, is perfectly stable) but rather the region

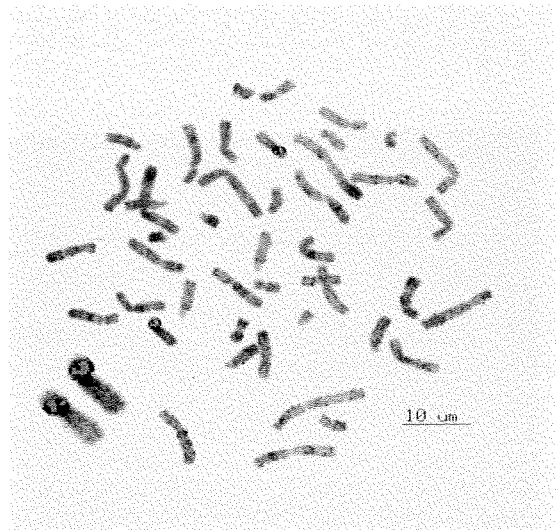


Figure 4. FISH mapping of the YAC contig. Metaphase chromosome spreads were hybridized with Alu-PCR products synthesized from four YACs (see material and methods) covering the contig (49G7, 828B9, 884G12 and 961F10) and with a chromosome 17 specific aliphoid probe (D17Z1). YACs were labelled with biotin-16-dUTP, D17Z1 with digoxigenin and chromosomes with propidium iodide. Chromosome stained with PI, have been pseudocoloured in grey. A magnification of the two chromosomes 17 is shown at the left bottom corner.

they contained. All the unstable YACs contained a region, indicated in fig 3, distal to U3 and proximal to 5G7. The localization of this region is provocatively the same as the region where we localized the distal breakpoints of the SMS deletions analysed in this work. This point will be discussed further in relation with the molecular mechanism leading to the CMT1A duplication.

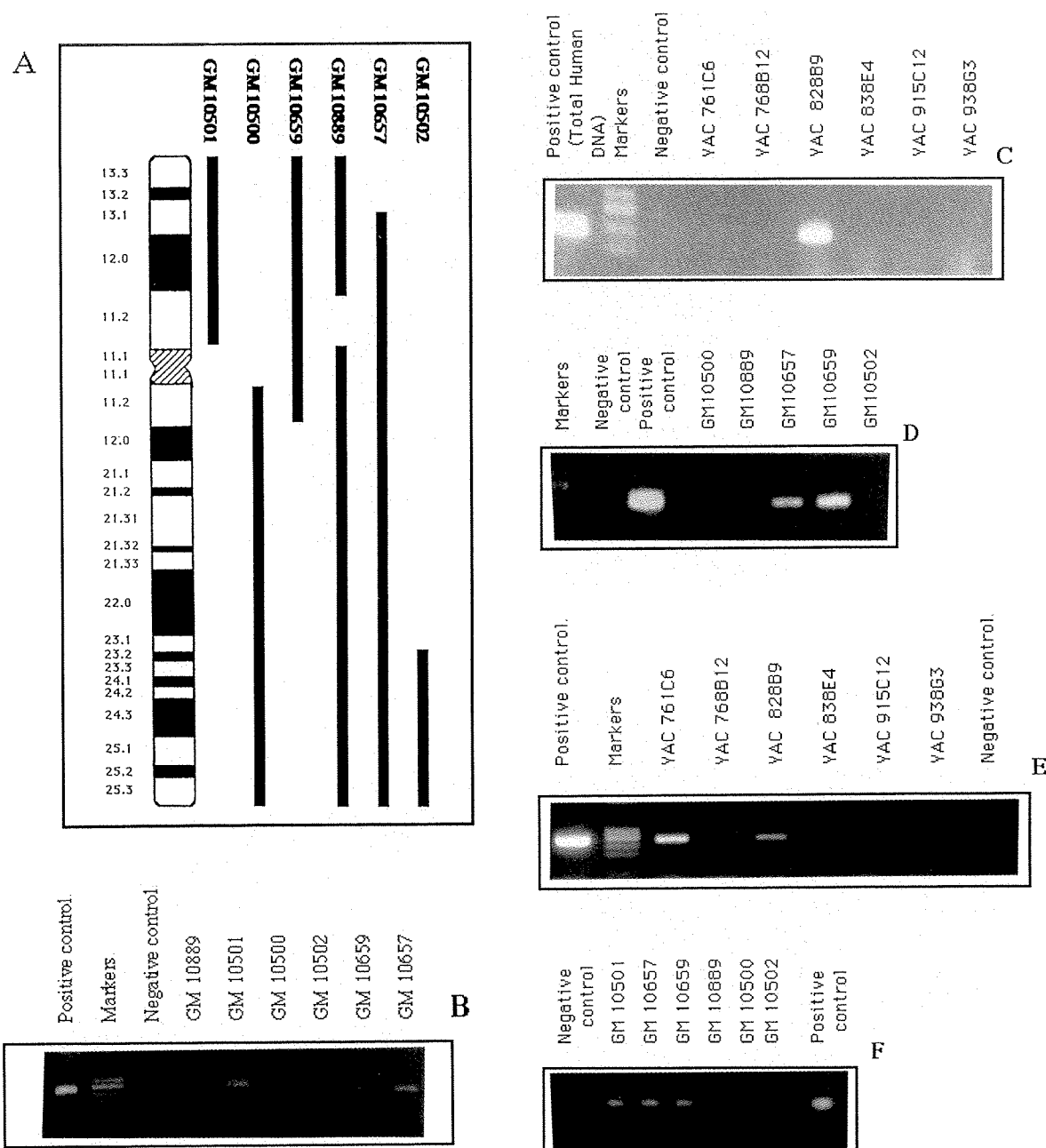


Figure 5. Characterization of new STS in the SMS region with PCR assay. Description of the somatic cell hybrid panel (A). Mapping of D17S837(AFM164wa9) on the somatic cell hybrid panel (B). The microsatellite D17S832 and D17S833 give the same pattern on this panel. Mapping of D17S805 (C) and U3 (E) on a YAC panel. Mapping of D17S805 (D) and U3 (F) on the somatic cell hybrid panel. The PCR amplification reactions have been performed as described in materials and methods using 200ng of YACs or hybrids DNA.

Finally the presence of loci known to be present in the distal region of SMS (D17S71 and D17S445) was checked in the YAC contig. No YAC was found to be positive, indicating that these loci are located proximally to our contig and confirming the genomic data of Pentao et al (9). In contrast, using PCR, we mapped microsatellite sequences D17S261 (mfd41(27)) and D17S793 (AFM165zd4(23)) in the CMT duplicated region, between loci D17S455 (5H5) and D17S122 (VAW409). This could be helpful in developing diagnostic tests for CMT1A duplication.

DISCUSSION

First of all, it seems important to recall YAC contig construction strategies. Basically two different approaches could be applied. Both start with YAC screening with an STS. The first approach relies on the combination of chromosomal walking using end probes and detection of potential overlapping by fingerprinting. This approach is useful when the average distance between STS in the region is bigger than the average length of the YACs in the library. The second approach is based on the presence of at

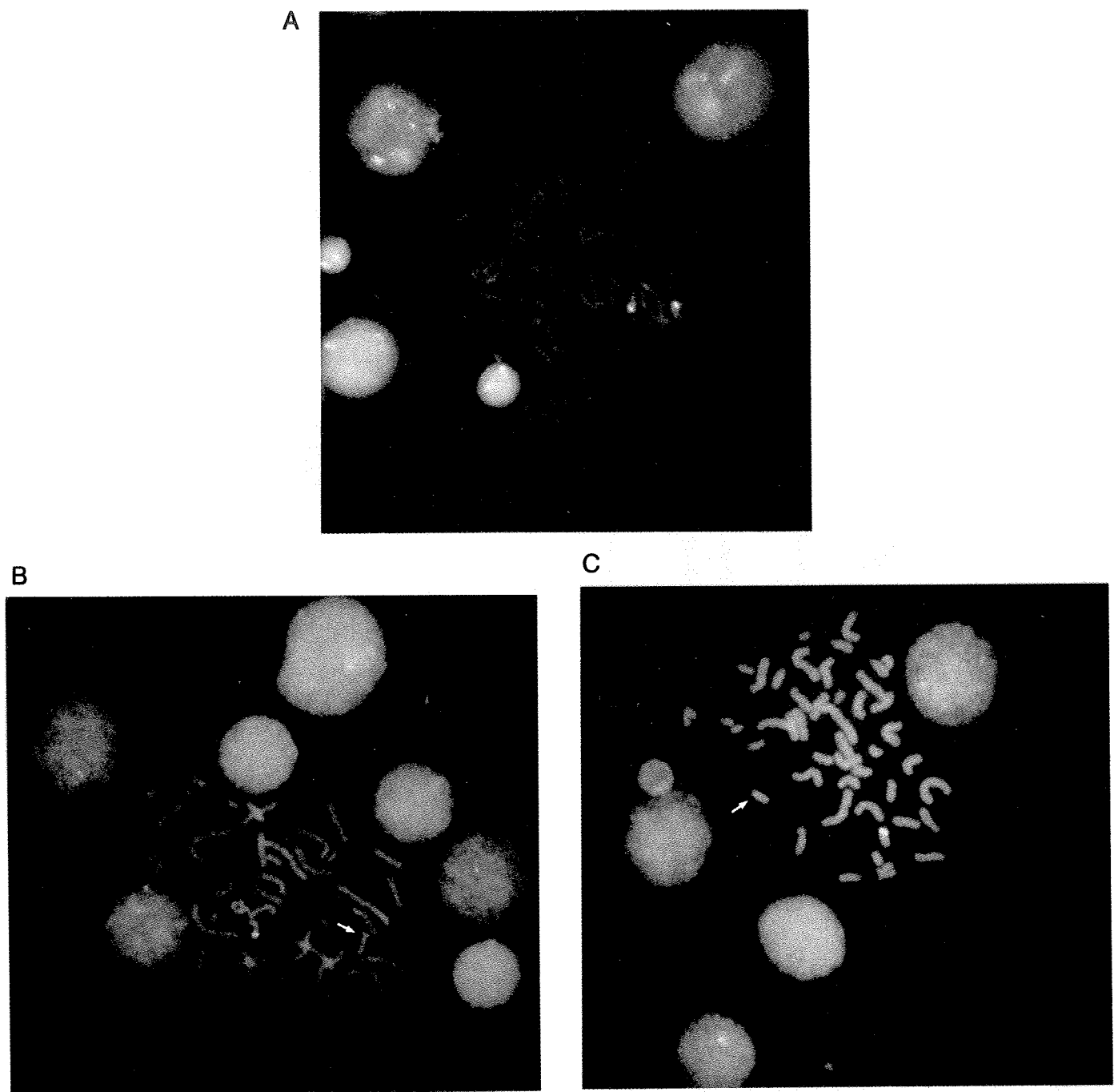


Figure 6. FISH mapping of the distal breakpoints of SMS patients. Metaphase chromosome spreads from a normal individual (A) and from a SMS patient (patient E from the paper by Moncla et al, 26; B and C) were hybridized with Alu-PCR products synthesized from the YAC 797G1 (U3 positive and 5G7 negative). YAC DNA was labelled with biotin-16-dUTP and chromosomes with propidium iodide. The hybridization pattern for the two other SMS patients tested was the same. We may note that the weaker hybridization on the deleted chromosome (indicated by an arrow) is also visible on the interphase nuclei.

least two STS in the same YAC. This implies screening the library again with the proximal and distal STS present on the YACs and then, step by step, extending the contig. This method is very powerful when applied to regions saturated in STS, or with 'big insert' YAC libraries.

Thus, using the 'Mega YACs' CEPH library (Ougen et al, in preparation), we constructed the contig described above (about 5 Mb) with only five STS and obtained data on the physical map

of the 17p11.2-p12 region, which could be summarized as follows:

—The region duplicated in CMT1A patients is about 1.5 Mb. This is in agreement with the previous results (7–9). Moreover our mapping data are in agreement with the genomic physical map constructed by Pentao et al (9) using PFGE analysis (for example the localization of the locus D17S124). Comparison of the data showed that no big rearrangements have occurred in the

YACs. This region is covered by only a few YACs (for example YAC 884F4 contains about 70% of the CMT1A duplicated region). This could be helpful to pick up new genes in the region.

—The region present in the above described YACs contig, and deleted in the SMS hybrid we used, is about 1 Mb. Moreover FISH analysis of the chromosomes of 3 additional SMS deletions indicates that their distal breakpoints lie in the same region (in term of hundred Kilobases). As pointed out, the two STS (D17S71 and D17S445) previously mapped distally in the SMS critical region (13) were not located in our contig. However we isolated several YACs containing these loci and covering about 1–1.5 Mb of genomic DNA (data not shown). We could thus estimate the size of the SMS critical region to be over 2–2.5 Mb.

The proximal end of the CMT1A duplication and the distal end of the four SMS microdeletions we analysed, lie in the same genomic region, presumably less than 500 Kb. We may note that the extent of three microdeletions, cytogenetically estimated, is not the same (26). If the distal breakpoints are close together (as suggested in this work), it implies that the variations in size of the deleted regions occurred at the proximal breakpoint (between D17S29 and D17S58, 26). Naturally these data do not demonstrate that all the distal SMS breakpoints occurred in the same region, but that the region between U3 and 5G7 could be a 'rearrangements hot spot'. We may note that no one of the deletion tested so far extends toward the CMT1A/HLPP region (27).

We observed that in certain growth conditions (like overgrowth) some of the YACs (containing a genomic fragment localized between the loci U3 and 5G7) from the region showed deletions and some not (data not shown), indicating that this unstable genomic region is not distributed randomly (and is not associated with the length of the insert in the YAC) but rather includes the same segment in all YACs. This localization could be provocatively superposed to the potential localization of the four SMS breakpoints we analyzed. An interesting question is: does this reflect a genomic instability in the human genome, potentially associated with a pathological phenotype? The available data are too few to conclude definitively, however two additional examples of such instability strengthen the above hypothesis. All the YACs containing a genomic segment proximal to the locus PGK1 on the X chromosome are unstable and show several deleted forms (this lab). Recently Tumer et al showed that the breakpoints of two genomic rearrangements, associated with the Menkes syndrome were localized in this region (28). It has also been noted that the YACs from the region containing the gene for the Steinert disease were also unstable (29). However additional examples of instability in the same region in the human genome (associated with diseases) and in the corresponding YACs are necessary to conclude.

Recently Pentao et al described a middle repetitive element involved in the CMT1A duplication (9). Is the same element involved in the SMS microdeletion? The answer is probably not, because this element is distal to the locus 5G7 (from the map presented by these authors), a locus which is distal to the four SMS breakpoints we analyzed.

—The SMS critical region was previously defined as distal to D17S29/D17S258 and proximal to D17S122/D17S261 including loci D17S71 and D17S445 (13,30). In this work we show that genes coding for U3 small nuclear RNA are localized in this region and deleted in a SMS hybrid (the total deletion of the snU3 cluster in the SMS hybrid has been verified using southern blots hybridization, data not shown). As no animal model for SMS

has been proposed so far (like Trembler mouse for CMT), the only way to propose candidate genes is to map these genes in the critical region, and snU3 is the first expressed sequence to be localized in a SMS deletion.

Although the molecular mechanisms involved in its function remain unclear, U3 sn RNA is known to be involved in pre-rRNA processing (31), thus representing a potentially important target for a control of ribosome production in eukaryotic cells. In humans, the 4–6 U3 gene copies are clustered in a single locus in the p11–p12 region of chromosome 17 (25) and the partial or complete deletion of one allele of the U3 locus might be relevant to the SMS phenotype. The haploid genome of yeast contains two variant U3 copies. While haploids with the double deletion are not viable, mutants deleted of a single U3 copy exhibit a dosage compensation, with no particular phenotype (32). In mammals, the effects of U3 gene dosage remain unknown. However we know that there are two snU3 clusters in the mouse (one of which is located in an evolutionarily conserved syntenic region of the murine chromosome 11(25)). The deletion of only one cluster in the mouse may have no visible phenotypic effect, whereas in humans the presence of only one locus could produce a phenotypic effect when one allelic cluster is deleted. Therefore the deletion of one copy of the snU3 cluster could be at least partially involved in the SMS phenotype. But how could the presence of only one copy of a locus, encoding a ubiquitous product like snU3, produce a rather specific phenotype?

Taking into account that the rate of U3 gene transcription in exponentially growing mammalian cells reaches the highest levels known for an RNA polymerase II gene so far (33), one might presume that the deletion of one copy of the U3 locus has significant effects when the demand for ribosome production is particularly high. Thus, tissues showing an especially high rate of protein biosynthesis could be preferentially affected, as frequently seen in metabolic diseases affecting the central nervous system. For example, the formation of the morphogenetic pattern proceeds through cell migration and the local distribution of those cells which remained able to divide in a given embryonic territory (this may be under the control of transcriptional factors as homeotic genes). The derangement of the protein machinery could affect this process, leading to morphogenetic anomalies like facial malformations. Another possible target could be the development of particular territories of the central nervous system (or the establishment of neuronal connexions) which could be perturbed by a decreased rate of ribosome synthesis. This may explain, at least partially, the mental retardation and the speech delay observed in SMS patients. Naturally snU3 deletion may also be involved in relatively aspecific traits such as slow growth. Moreover a gene also involved in the mechanism of RNA processing (small nuclear ribonucleoprotein polypeptide N, SNRPN) has been proposed recently as a candidate gene which could be implicated in the Prader–Willi syndrome (34).

In a further step, to check the hypothesis of a limiting rate of ribosome synthesis, we will analyse cultured cells of SMS patients for potential alterations in their pre-rRNA processing. To support our hypothesis we will also study the spatial distribution of snU3 RNA during the development in an animal model like the mouse. Regional variations affecting the putative target tissues affected in the SMS syndrome would be a good indication of the involvement of this gene in the SMS phenotype.

However, it seems very likely that the SMS phenotype, which is complex, is conferred by the deletion of several genes. Therefore, we are currently testing the SMS deletions for the

presence of other genes, which could also be involved in this disease.

MATERIALS AND METHODS

YAC screening

The 'Mega YACs' CEPH library (containing YACs with large inserts, 1 Mb average length) was screened by PCR using the previously described STS developed from DNA markers 5G7 (7), VAW412 (7), VAW411(7). We designed the following primers from the sequence of the probes: VAW409R (D17S122) 5' GTAGTCATTGTAACCTCTTATGC and 5' GGAACACAAAGAA-GGCCATTG, and U3: 5' GCTGTCAATTCAGTATTATGCTAAT and 5' AATG-ATCCCTGAAAG TATAGTCTT.

YAC characterization

After being identified as positive, the YAC clones were characterized by PFGE on a Biorad CHEF DRII. The agarose plugs and the pulse field electrophoresis were prepared as described in the instruction manuals. The DNAs were fractionated in 1% agarose gel in $0.5 \times$ TBE at 200V/160mA with a pulse time from 45 to 120s at 15°C for 24 hours. Under these conditions DNA fragments in the 2000 Kb range were resolved. After electrophoresis, gels were stained in ethidium bromide, and transferred to Pall Biodyne B membrane by standard methods.

PCR and IRS-PCR amplification

Each reaction tube contained 200ng of DNA (YAC or hybrid cell lines), primers 0.5mmol/l, Tris-HCl 10mM pH 8.8, potassium chloride 50mM, magnesium chloride 1.5mM, non ionic detergent 0.1% and 0.3U of Taq DNA polymerase (Northumbria Biologicals Limited). The PCR were performed on a Perkin Elmer Cetus DNA thermal cycler with the following conditions: 94°C for 3 min followed by 30 or 40 cycles (the specific conditions for each primer are given in figure legends) with a final extension of 72°C for 7 min.

IRS-PCR does not differ significantly from 'simple copy' PCR in materials requirements. The primer (GTGAGCCACCGCGCCCGGCC (35)) was the Alu 5 primer. The PCR products are separated on 0.8% agarose gel in TBE $1 \times$ buffer, and stained with ethidium bromide.

Hybridization

Filters were prehybridized for at least 1 hour in SSC $5 \times$, denhart's solution $5 \times$, SDS 0.5% at 65°C. The PCR products or plasmids inserts used as probes were labelled by random priming method without spermine or ethanol precipitation to remove unincorporated dNTPs. The probe was added to the prehybridized filter (without changing the prehybridization solution) and hybridized at 65°C overnight. The filter was washed in SSC $2 \times$ SDS 0.1% first during 15 min, then in SSC $1 \times$ SDS 0.1% during 15 min. A final wash was done in SSC $0.1 \times$ SDS 0.1% during 2–10 min. All the washes were done at 65°C. The filters were exposed on Hyperfilm MP Amersham film with an intensifying screen for 2 days. The filters were stripped by NaOH 1N during 30 min and in SSC \times SDS 0.5%, Tris 0.2N during the same time. The complete suppression of repeated sequences was carried out during the prehybridization with sonicated human placenta DNA (100mg/ml).

FISH

FISH was carried out on metaphasic chromosomes using inter-Alu PCR products from YACs as described by Lengauer et al (36), labelled with biotin-16-dUTP by nick translation. D17Z1 was labelled with digoxigenin-11-dUTP. Hybridization, washing and detection was carried out as previously described (36) with the following modifications: 250 ng of Alu-PCR products from individual YACs were pooled and labelled, 25 ng of the pooled and labelled Alu-PCR products were preannealed with 1 μ g of Cot1 DNA (BRL/life technologies) in 10 μ l for 30 min. Signals were visualized on a confocal microscope.

Hybrid panel

The following hybrids were used in this study: DHA4 (GM 10889) is a clonal cell line from a SMS patient (27) retaining only a del(17)(p11.2) human chromosome 17. The other hybrids JW-4(17p13.1–17qter), 88H5(17pter–p11.2), SP-3(17q11.2–qter), P12.3.B(pter–q12), MH-41(q23.1–qter) were described in more details in Van Tuinen et al (24).

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