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# Localization of the human caveolin-3 gene to the D3S18/D3S4163/D3S4539 locus (3p25), in close proximity to the human oxytocin receptor gene

## Identification of the caveolin-3 gene as a candidate for deletion in 3p-syndrome

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**Abstract** **Caveolin-3, a muscle-specific caveolin-related protein, is the principal structural protein of caveolae membrane domains in striated muscle cell types (cardiac and skeletal). Recently, we identified an autosomal dominant form of limb girdle muscular dystrophy in humans that is due to mutations within exon 2 of the caveolin-3 gene (3p25). However, the detailed location of the human caveolin-3 gene and its position with regard to neighboring genes remains unknown. Here, we have isolated three independent BAC clones containing the human caveolin-3 gene. Using a PCR-based approach, we determined that these clones contain both exons 1 and 2 of the human caveolin-3 gene. In addition, we performed microsatellite marker analysis of these BAC clones, using a panel of 13 markers that are known to map within the 3p25 region. Our results indicate that these BAC clones contain the following three markers: D3S18, SHGC-1079 (also known as D3S4163) and D3S4539. Interestingly, D3S18 is a marker for two known human diseases, von Hippel-Lindau disease and 3p-syndrome. As D3S4163 and D3S4539 are known to map in the vicinity of the 3′ end of the human oxytocin receptor gene, we determined if these caveolin-3 positive BACs also contain the oxytocin receptor gene. We show that (i) these BACs contain all four exons of the oxytocin receptor gene and (ii) that the genes encoding caveolin-3 and the oxytocin receptor are located ∼7–10 kb apart and in the opposite orientation. As 3p-syndrome is characterized by cardiac septal defects and caveolin-3 is expressed primarily in the heart and skeletal muscle, caveolin-3 is a candidate gene that may be deleted in 3p-syndrome.**



### 1. Introduction

Caveolae are 50–100 nm vesicular invaginations of the plasma membrane [1]. It has been proposed that caveolae participate in vesicular trafficking events and signal transduction processes [2–5]. Caveolin, a 21–24 kDa integral membrane protein, is a principal component of caveolae membranes in vivo [6–10]. Caveolin is only the first member of a new gene family. As a consequence, caveolin has been re-termed caveolin-1 [11].

The mammalian caveolin gene family now consists of caveolins-1, -2 and -3 [4,5,11–13]. Caveolins-1 and -2 are co-expressed and form a hetero-oligomeric complex [14] in many cell types, with particularly high levels in adipocytes, whereas expression of caveolin-3 is muscle-specific and found in both cardiac and skeletal muscle [15]. The expression of caveolin-3 is induced during the differentiation of skeletal myoblasts and caveolin-3 is localized to the muscle cell plasma membrane (sarcolemma) where it forms a complex with dystrophin and its associated glycoproteins [15]. However, under certain conditions, caveolin-3 can be physically separated from the dystrophin complex [16]. This indicates that although caveolin-3 is dystrophin-associated, it is not absolutely required for the biogenesis of the dystrophin complex [16].

Caveolin-3 is most closely related to caveolin-1 based on protein sequence homology. Caveolin-1 and caveolin-3 are ∼65% identical and ∼85% similar (see Tang et al. for an alignment: [13]). However, caveolin-3 mRNA is expressed predominantly in muscle tissue types (skeletal muscle, diaphragm and heart) [13]. Identification of a muscle-specific member of the caveolin gene family has implications for understanding the role of caveolins in different muscle cell types (smooth, cardiac and skeletal), as previous morphological studies have demonstrated that caveolae are abundant in these cells. A number of studies have highlighted the importance of caveolae and caveolins in the pathogenesis of Duchenne's muscular dystrophy. More specifically, dystrophin has been localized to plasma membrane caveolae in smooth muscle cells using immuno-EM techniques [17] and skeletal muscle caveolae undergo characteristic changes in their size and distribution in patients with Duchenne's muscular dystrophy, but not in other forms of neuronal-based muscular dystrophies examined [18]. This indicates that muscle cell caveolae may play an important role in muscle membrane biology.

In collaboration with Minetti and colleagues, we have recently identified an autosomal dominant form of limb girdle muscular dystrophy (LGMD-1C) in two Italian families that is due to a deficiency in caveolin-3 expression [19]. Analysis of their genomic DNA reveals two distinct mutations in the caveolin-3 gene at 3p25, (i) a 9 bp micro-deletion that removes the sequence TFT from the caveolin-scaffolding domain and (ii) a mis-sense mutation that changes a proline to a leucine (P→L) in the transmembrane domain [19]. Both mutations lead to a loss of ∼90% of caveolin-3 protein expression.

These results indicate that dramatic reductions in caveolin-3 can produce a disease phenotype in humans. However, the detailed location of the human caveolin-3 gene and its position with regard to neighboring genes remains unknown.

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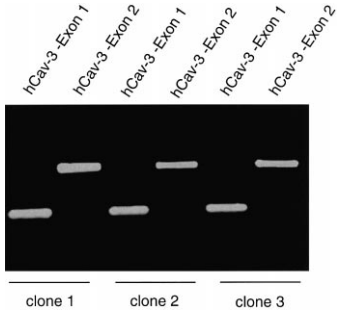


Fig. 1. Caveolin-3 positive BAC clones contain exons 1 and 2 of the human caveolin-3 gene. The presence of the exons of caveolin-3 within a given BAC clone was determined by PCR using the primer pairs listed in Table 1. Note that all three BAC clones contain both exons 1 and 2 of the human caveolin-3 gene.

Knowledge of the detailed localization of the caveolin-3 gene might implicate caveolin-3 in the pathogenesis of other human diseases. Here, we have shown that the human caveolin-3 gene is located in close proximity to three known microsatellite markers (D3S18, SHGC-1079 (also known as D3S4163) and D3S4539) and the oxytocin receptor gene. Interestingly, D3S18 is a marker for two known human diseases, von Hippel-Lindau disease and 3p-syndrome.

## 2. Materials and methods

### 2.1. Isolation of human genomic BAC clones

A probe corresponding to the full-length cDNA of human caveolin-3 was used to screen a human genomic BAC library (Release I, Genome Systems), as we described previously for human caveolins-1 and -2 [20,21]. The presence of the exons of the human caveolin-3 gene within a given BAC clone was verified by PCR analysis and DNA sequencing.

### 2.2. Microsatellite marker analysis

Isolated human genomic BAC clones containing the human caveolin-3 gene were subjected to microsatellite marker analysis by PCR with the following 13 Généthon markers: D3S18, D3S1304, D3S1597, D3S3591, D3S601, D3S3601, D3S3691, D3S3728, D3S587, D3S4539, D3S726, D3S732 and SHGC-1079 (D3S4163). Généthon primer pairs were obtained from Research Genetics.

### 2.3. Analysis of the oxytocin receptor gene

The presence of the exons of the human oxytocin receptor gene within a given caveolin-3 positive BAC clone was determined by PCR analysis. Primers were designed based on the known sequence of the intron-exon boundaries of the human oxytocin receptor gene [22] and are listed in Table 3.

## 3. Results

### 3.1. Isolation of BAC clones containing the human caveolin-3 gene

The human cDNA encoding caveolin-3 was used to screen a human genomic BAC library to obtain corresponding clones containing the human caveolin-3 gene. Through this screening approach, a total of three independent positive clones were isolated. All three clones contain both exons 1 and 2 of the human caveolin-3 gene (Fig. 1 and Table 1).

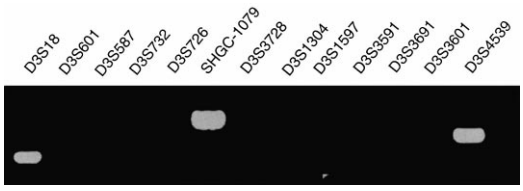


Fig. 2. The human caveolin-3 gene is located in close proximity to three microsatellite markers: D3S18, SHGC-1079 (also known as D3S4163) and D3S4539. Primers corresponding to 13 Généthon microsatellite markers from the 3p25 region were used to perform PCR analysis employing the human genomic caveolin-3 BAC clones as the template. Note that three PCR products of the expected size (see Table 2) were observed and correspond to the markers D3S18 SHGC-1079 (also known as D3S4163) and D3S4539.

### 3.2. Microsatellite marker analysis of caveolin-3 positive BAC clones

To more precisely determine the location of the human caveolin-3 gene, we next performed microsatellite marker analysis on the corresponding human genomic BAC clones. As 3p25 corresponds to a region that is frequently deleted in von Hippel-Lindau disease and 3p-syndrome, numerous microsatellite markers have been used for LOH analysis of this region. Through literature searches, we identified the 13 most commonly used markers as follows: D3S18, D3S1304, D3S1597, D3S3591, D3S601, D3S3601, D3S3691, D3S3728, D3S587, D3S4539, D3S726, D3S732 and SHGC-1079 (D3S4163). These markers, corresponding primer sequences and the expected size of their PCR products are as indicated in Table 2.

Fig. 2 shows that three microsatellite markers were detected (D3S18, SHGC-1079 (also known as D3S4163) and D3S4539) and are present in all three caveolin-3 genomic clones. A search of the GenBank data base indicated that the markers SHGC-1079 (also known as D3S4163) and D3S4539 are located at the 3′ end of the human oxytocin receptor gene at 3p25 [23]. These results indicate that the human caveolin-3 gene must be located near the human oxytocin receptor gene.

### 3.3. The human caveolin-3 gene is located in close proximity to the human oxytocin receptor gene

Given our results from the microsatellite marker analysis, we next determined if our caveolin-3 positive BAC clones contain the human oxytocin receptor gene.

Primers were designed to PCR amplify the four exons of the human oxytocin receptor gene. These primers and the expected size of their PCR products are as indicated in Table 3. All four exons of the human oxytocin receptor gene were detected (Fig. 3) and are present in all three caveolin-3 genomic clones (data not shown).

As the average insert size of these human genomic BAC clones is ~100 kb, these data indicate that the genes encoding

Table 1
Primer pairs used to amplify the exons of the human caveolin-3 gene and the expected size of their PCR products

| hCav-3 gene | Forward primer (5→3) | Reverse primer (5→3) | Expected size (bp) |
|---|---|---|---|
| hCav-3 Ex 1 | ATGGCAGAAGAGCACACAGAT | CTTGACTATGTCCTCGTTAAT | 114 |
| hCav-3 Ex 2 | GTGGATTTTGAAGACGTGATC | TTAGACCTCCTTCCGCAGCAC | 342 |

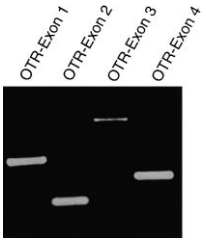


Fig. 3. Caveolin-3 positive BAC clones contain all four exons of the human oxytocin receptor gene. Primers corresponding to the four exons of the human oxytocin receptor gene were used to perform PCR analysis employing the human genomic caveolin-3 BAC clones as the template. These primers and the expected size of their PCR products are as indicated in Table 3. Note that all four exons of the human oxytocin receptor gene were detected and are present in all three caveolin-3 genomic clones (data not shown).

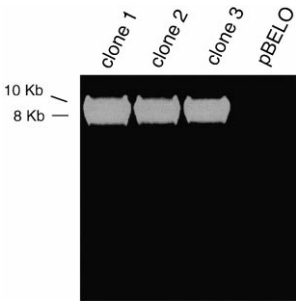


Fig. 4. The distance between the caveolin-3 gene and the oxytocin receptor gene is ~7–10 kb. Primer pairs based on the 3′ ends of the last encoding exons of the human caveolin-3 and oxytocin receptor genes were used to perform PCR analysis of the caveolin-3 positive genomic clones. Note that a single band of ~7–10 kb was observed in all three genomic clones (lanes 1–3). In addition, this band was not observed when the same reaction was carried out with the empty BAC vector (lane 4), clearly indicating that this amplification product was specific.

human caveolin-3 and the oxytocin receptor must be located at a maximum distance of ~100 kb from each other.

In this regard, it is interesting to note the historical context in which the first cDNA isolated for caveolin-3 was cloned [13]. Searches of the GenBank database with the human caveolin-1 protein sequence revealed a striking homology after translation with a DNA sequence entry presumed to encode the 3′ end of the rat oxytocin receptor gene (accession number U15280) [13]. This homologous DNA sequence was PCR-amplified from rat genomic DNA and used as a probe to screen a rat heart cDNA library. The isolated clones were expressed in a muscle-specific manner and were designated caveolin-3, because of their close homology with caveolins-1 and -2 [13]. We now know that this originally identified rat genomic sequence represents exon 2 of the rat caveolin-3 gene. Re-analysis of this rat genomic DNA sequence data indicates that the second exon of the rat caveolin-3 gene is located ~3.5 kb downstream of the last exon of the rat oxytocin receptor gene and in the opposite orientation. This would suggest that a similar organization may exist for the corresponding locus in humans.

To test this hypothesis directly, we designed primer pairs based on the 3′ ends of the last exons of the human caveolin-3 and oxytocin receptor genes. PCR-based analysis of these caveolin-3 positive genomic clones using these primers revealed a single band of ~7–10 kb. In addition, this band was not observed when the same reaction was carried out with the empty BAC vector, clearly indicating that this amplification product was specific (Fig. 4). These results suggest that the distance between the ends of the human caveolin-3 and oxytocin receptor genes is ~7–10 kb and that they are indeed present in the opposite orientation (Fig. 5), as predicted based on analysis of the rat genomic sequence data.

## 4. Discussion

Here, we have determined that the human caveolin-3 gene is located within close proximity to three microsatellite markers located at 3p25, D3S18, SHGC-1079 (also known as D3S4163) and D3S4539. Interestingly, D3S18 is a marker for two known human diseases, von Hippel-Lindau disease

Table 2
Commonly used 3p25 microsatellite markers, primer sequences and the expected size of their PCR products

| Marker | Forward primer (5→3) | Reverse primer (5→3) | Expected size (bp) |
|---|---|---|---|
| D3S18 | CACAAGTGATGCCTTGTAGCTG | CAGTAGTGTCCTGTATTTAGTG | 200 |
| D3S1304 | TTCGCTCTTTGATAGGC | ATTTCATTTGTAATTTACTAGCAG | 253–269 |
| D3S1597 | AGTACAAATACACACAAATGTCTC | GCAAATCGTTCATTGCT | 162–178 |
| D3S3591 | CCAACTATGTTTTGGGTCTG | TGTGCCCAGTTAGATGATG | 148–152 |
| D3S601 | GTTGGCTATGGGTAGAATTGG | CAGGGTAGCCTTGATCTAAGT | 190 |
| D3S3601 | CAGTTACCTTGATAGACTGGTAGT | GAGATTTAGTTGACTCACCCAC | 239–247 |
| D3S3691 | TCTCAGCAATAGCAAACATCAGG | TTGAAACCAGGGTGACAAATACATC | 250–254 |
| D3S3728 | CTGAGGTGGGAGGTTCACTT | TTGCCACAAAATGTGCG | 222–224 |
| D3S587 | TTCCCCTGCACAAGCTG | AAGCCTCACAATCATGGTGG | 125–143 |
| D3S4539 | GCGCAGTGGACCTATTAGAA | GTATCTTGAAACCTTGCGGA | 236 |
| D3S726 | ACTTATTACCTAGCTCCCCt | ACAGATGGTTCCAAGCCAGA | 200 |
| D3S732 | GGTCTGGGTTATACCATGCCT | ACTCAATGTCTTTCCTTCCTC | 250 |
| SHGC-1079 (D3S4163) | GGCGTATGTTTGTGTATAAGGTACC | ACCCAAGTCCAGAACACTG | 319 |

Table 3
Primer pairs used to amplify the exons of the oxytocin receptor (OTR) gene

| OTR gene | Forward primer (5→3) | Reverse primer (5→3) | Expected size (bp) |
|---|---|---|---|
| hOTR Ex 1 | TGTTAAGGCTCTGGGACC | CTGCACCGAGTCCGCAGG | 383 |
| hOTR Ex 2 | TGGAAGCCGCTGAACATC | CTCCTCTGAGCCACTGCA | 96 |
| hOTR Ex 3 | GGTGGACTCAGCAGATCC | CTTCCTTGGGCGCGTTGG | 1 060 |
| hOTR Ex 4 | CCTCGGCCTTCATCATCG | CGCCGTGGATGGCTGGGA | 240 |

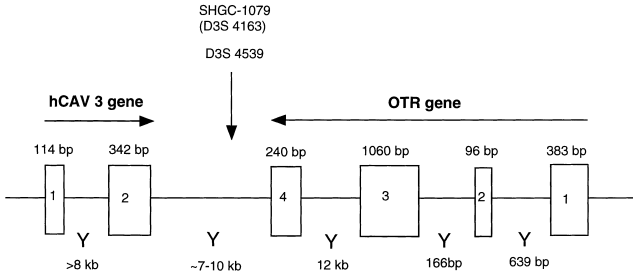


Fig. 5. Overall organization of the caveolin-3/oxytocin receptor locus. The organization of the human caveolin-3 gene and the human oxytocin receptor gene was as described previously [19,22,28,29]. Our current findings demonstrate that the distance between the ends of the human caveolin-3 and oxytocin receptor genes is ~7–10 kb and that they are indeed present in the opposite orientation. See text for details.

and 3p-syndrome. As D3S4163 and D3S4539 are known to map in the vicinity of the 3′ end of the human oxytocin receptor gene, we also determined if these caveolin-3 positive BACs contain the oxytocin receptor gene. In this regard, we find that the genes encoding caveolin-3 and the oxytocin receptor are located ~7–10 kb apart and in the opposite orientation.

3p-syndrome, a hemizygous deletion of 3p25-pter, is characterized by growth retardation, specific cranio-facial features (microcephaly, ptosis, micrognathia), mental retardation and cardiac septal defects [24]. In addition, trisomy 3p is associated with psychomotor and mental retardation, short neck and congenital heart defects, such as atrial septal defects and mitral valve clefts [25]. These results suggest that a gene that is both expressed in the heart and the brain may be involved in the pathogenesis of these congenital defects. Interestingly, caveolin-3 is expressed primarily in skeletal muscle fibers, cardiac myocytes and brain astrocytes [13,15,26]. In addition, we show here that the human caveolin-3 gene is localized near the D3S18 locus and the D3S18 marker is specifically deleted in 3p-syndrome. Taken together, these results suggest that caveolin-3 is a candidate gene that may be deleted in 3p-syndrome.

A form of dilated cardiomyopathy in humans has also been mapped to the 3p25 region using linkage analysis [27]. The closest known marker for this disease is D3S1304 [27] and this marker was not present in our caveolin-3 positive BAC clones (Fig. 2). However, since the genetic analysis of dilated cardiomyopathy is still in the early stages, our results do not rule out a role for caveolin-3 in the pathogenesis of dilated cardiomyopathy.

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