Immunogenetics (1997) 45: 422–427

ORIGINAL PAPER

Damián Heine-Suñer · Miguel Angel Díaz-Guillén
Fernando Pardo-Manuel de Villena
Mercedes Robledo · Javier Benítez
Santiago Rodríguez de Córdoba

A high-resolution map of the regulator of the complement activation gene cluster on 1q32 that integrates new genes and markers

Received: 12 December 1996 / Revised: 23 December 1996

Abstract Sixteen microsatellite markers, including two described here, were used to construct a high-resolution map of the 1q32 region encompassing the regulator of the complement activation (*RCA*) gene cluster. The *RCA* genes are a group of related genes coding for plasma and membrane associated proteins that collectively control activation of the complement component C3. We provide here the location of two new genes within the *RCA* gene cluster. These genes are *PFKFB2* that maps 15 kilobases (kb) upstream of the *C4BPB* gene, and a gene located 4 kb downstream of *C4BPA*, which seems to code for the 72 000 M_r component of the signal recognition particle (*SRP72*). Neither of these two genes is related structurally or functionally to the *RCA* genes. In addition, our map shows the centromere-telomere orientation of the *C4BPB/MCP* linkage group, which is: centromere-*PFKFB2*-*C4BPB*-*C4BPA*-*SRP72*-*C4BPAL1*-*C4BPAL2*-telomere, and outlines an interval with a significant female-male recombination difference which suggests the presence of a female-specific hotspot(s) of recombination.

The microsatellite data reported in this paper have been submitted to the genome data base (gdb) and have been assigned the accession numbers 4745387 (*D1S3705*) and 4745353 (*D1S3704*)

D. Heine-Suñer · M. A. Díaz-Guillén · S. Rodriguez de Cordoba (✉)
Departamento de Inmunología, Centro de Investigaciones Biológicas (CSIC), C/Velazeves 144, 28006, Madrid and Unidad de Patología Molecular Fundación Jimenez Diaz Avda. Reyes Católicos 2, 28040, Madrid, Spain

F. Pardo-Manuel de Villena
Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, 3307 North Broad St., Philadelphia 19140, PA, USA

M. Robledo · J. Benítez
Servicio de Genética, Fundación Jiménez Díaz, Avda. Reyes Católicos 2, 28040, Madrid, Spain

Introduction

Complement is a major defence and clearance system in the bloodstream. Its efficacy as an innate defence mechanism against microbial infections depends largely on a set of regulatory proteins that modulate complement activation and prevent non-specific damage to host tissues (reviewed in Law and Reid 1995). Many of these regulatory proteins interact with C3 or C4 derivatives and are encoded by closely linked genes that constitute the regulator of the complement activation (*RCA*) gene cluster (Rodríguez de Córdoba et al. 1985). In humans the *RCA* gene cluster is located on the long arm of chromosome 1 (1q32; Weis et al. 1987) and includes the genes coding for membrane cofactor protein (*MCP*, CD46), C3b/C4b receptor (*CR1*, CD35), C3dg receptor (*CR2*, CD21), decay-accelerating factor (*DAF*, CD55), C4b-binding protein (*C4BPA* and *C4BPB*), and factor H [(*HF1*) (Pardo-Manuel de Villena et al. 1990)]. Several lines of evidence indicate that these *RCA* genes share a common ancestor from which they originated by multiple events of gene duplication (Hourcade et al. 1989). Moreover, generation of new genes in the *RCA* gene cluster may be a still ongoing process. This is supported by the discovery of a number of genes in the human *RCA* gene cluster which are relatively-recent events of duplication. These duplicated genes have either an unknown function or are clearly pseudogenes and include: *C4BPAL1* and *C4BPAL2*, duplications of the *C4BPA* gene that originated 50–70 million years ago (Sanchez-Corral et al. 1993; Pardo-Manuel et al. 1995); *FHL1*, *FHR2*, and *FHR3* that are duplications of the *HF1* gene (Zipfel and Sherka 1994); and *MCPL1* and *CR1L1* that are duplications of *MCP* and *CR1*, respectively (Hourcade et al. 1990, 1992).

In accordance with their evolutionary origin, all of the human *RCA* genes are placed in tandem within two gene groups that have not been physically linked yet. The two groupings are an approximately 900 kilobase (kb) DNA segment that contains the following genes in a head-to-tail arrangement: 5′-*C4BPB*-*C4BPA*-*C4BPAL1*-*C4BPAL2*-*DAF*-*CR2*-*CR1*-*MCPL1*-*CR1L1*-*MCP*-3′ (Pardo-Manuel et

al. 1990), and a 650 kb DNA segment that contains *HF1* and *FHR2*, as well as the gene coding for the b-subunit of the coagulation Factor XIII (*F13B*; Rey-Campos et al. 1990; Sherka et al. 1995).

The particular mechanisms that underlie gene duplication are not clear, but unequal recombination certainly has a role. Interestingly, the 1q32 region has been found to contain a chromosome fragile site and to display frequent allelic loss in breast and male germ cell tumours (Mathew et al. 1994; Hoggard et al. 1995; Turecki et al. 1995). To determine whether frequent chromosome breakage and loss, as well as a high duplication rate of *RCA* genes, could be caused by common mechanisms, we developed new markers and constructed a high-resolution genetic map of the *RCA* region that we analyzed searching for recombination hotspots.

Materials and methods

New microsatellite isolation and typing

Several restriction endonucleases were used to digest the DNA of λEMBL3 phages of a genomic contig that includes the *C4BPB, C4BPA, C4BPAL1, C4BPAL2,* and *DAF* genes (Pardo-Manuel de Villena and Rodríguez de Córdoba 1995). $(CA)_n$ microsatellites were detected by Southern blot analysis of these digestions using a $(CA)_{10}$ dsDNA probe. Bands hybridizing with such a probe were subcloned into a pBluescript SK+ plasmid and sequenced on an ABI PRISM 377 automatic sequencer with a dye terminator cycle sequencing kit (Perkin-Elmer Cetus, Norwalk, CT).

Families and genetic markers

We typed a total of 18 Caucasian families and 128 individuals to construct a high-resolution map between the *F13B* gene and the microsatellite marker *D1S245*. Nine of these families were chosen because they were known to be informative for the segregation of the *RCA* genes (Rodríguez de Córdoba and Rubinstein 1986). All families are composed of both parents and at least three children. Two pedigrees include three generations. Markers for *CR1, DAF* and *HF1* are RFLP markers that were developed in our laboratory and used in previous studies (Rodríguez de Córdoba et al. 1985; Rey-Campos et al. 1987). The following $(CA)_n$ microsatellites were selected from previous publications: *D1S245, D1S249, D1S306, D1S412, D1S456, D1S477,* and *D1S510* (Gyapay et al. 1994); *CACNL1A3* (Gregg et al. 1993); *C4BPB* (Velasco et al. 1992); and *F13B* (Nishimura and Murray 1992); *REN* (Edwards et al. 1991). *D1S3705* and *D1S3704* are new microsatellites described in this paper.

PCR and RFLP markers conditions

Amplification was performed in a total volume of 10 µl containing 50 ng of genomic DNA, 5 pM of each primer, 1 unit of *Taq*-polymerase (Perkin-Elmer Cetus), 250 µM of dATP, dGTP and dTTP, 10 µM of dCTP, 0.1 µl of α32P-dCTP at 3000 Ci/mM, 1.5 mM MgCl2, 50 mM KCl, and 10 mM Tris-HCl (pH 8.3). Amplification conditions were one cycle at 94 °C for 4 min, 55 °C for 1 min, 72 °C for 1 min, followed by 30 cycles of 94 °C for 45 s and 55 °C for 40 s. Samples were resolved on 6% polyacrylamide sequencing gels. Restriction fragment length polymorphism (RFLP) analysis was performed as described in Rey-Campos and co-workers (1987).

Linkage analysis

Statistical support for the construction of the map was obtained as follows: We knew the position of *D1S412, D1S477, D1S510, D1S456, D1S249,* and *D1S245* with a lod score ≥ 3 (1000:1 odds) support from four previous maps (Murray et al. 1994; Gyapay et al. 1994; Sander et al. 1995; Dib et al. 1996). The consistent ordering of these markers with the same positions in several maps makes any errors in their positioning very unlikely. These maps allowed us to use the known order to position other markers using the CMAP function of the LINKAGE package (Lathrop et al. 1984). To do this we fixed the order of *D1S412, D1S477, D1S510, D1S456, D1S249,* and *D1S245* and inserted the rest of the markers in a stepwise fashion into this skeletal map using the CMAP program. In this manner we were able to determine the position within the map with a 1:1000 odds ratio the following markers: *CACNL1A3, F13B, PFKFB2 (D1S3705), C4BPB, C4BPA (D1S3704),* and *REN*. Although markers for *CR1, DAF,* and *HF1* showed a lod score lower than 3, we positioned them by a breakpoint analysis of the pedigrees. Microsatellites and RFLP gene information was used to determine the most likely haplotypes of the parents in each family by minimizing the number of double crossovers. This information allowed us to determine the position of the breakpoints in each recombinant individual. The genetic position found in this way for *CR1, DAF,* and *HF1* is in agreement with their physical position (Rey-Campos et al. 1988; Rey-Campos et al. 1990). Genetic distances were calculated with the CILINK function and converted to Haldane centimorgans with the MAPFUN utility of the LINKAGE program.

Results

New genes within the RCA gene cluster

Random sequencing of a 350 kb λEMBL3 phage contig that includes the *C4BPB, C4BPA, C4BPAL1, C4BPAL2,* and *DAF* genes (Pardo-Manuel de Villena and Rodríguez de Córdoba 1995), allowed us to identify and characterize two new genes within this region, of which the structure and sequence will be reported elsewhere. These two genes are the heart isozyme of the 6-Phosphofructo-2-kinase/Fructose-2,6-biophosphatase bifunctional enzyme (*PFKFB2*) (reviewed in Pilkis et al. 1995), and a gene that shares a very high similarity to the C*anis familiaris* 72000 *Mr* component of the signal recognition particle (*SRP72*; reviewed in Lutcke 1995), which suggests that it is the human homologue. Neither of these two genes has a structural, functional, or evolutionary relationship with the *RCA* genes. *PFKFB2* is involved in the regulation of a key step in glycolysis and the *SRP72* product is part of a multimeric complex which mediates the attachment of ribosomes and translocation of secretory or membrane proteins into the rough endoplasmatic reticulum. The *PFKFB2* gene was previously mapped to 1q31 by in situ hybridization with a rat gene probe (Hilliker et al. 1991). However, we found this gene to be located 15 kb upstream of the *C4BPB* gene, which places it in the 1q32 instead of the 1q31 region.

New microsatellites within the RCA gene cluster

PFKFB2 contains two $(CA)_n$ dinucleotide microsatellites within introns 7 and 9. BLAST and BIOSCAN similarity

Table 1 Characterization of the new microsatellite markers

Microsatellite	Repeat	Location	Primer sequences	Allele sizes	Allele frequencies	Het	PIC
DIS3705	$(CA)_n$	Intron 9 of the *PFKFB2* gene	F: gggaaaaaataccaatagccaagtc R: catacacatgagtctgtgcattgca	146 148 156	0.50 0.002 0.47	0.48	0.37
DIS3704	$(CA)_n$	Intron 1 of the *C4BPA* gene	F: catggttatgcacgttgtcc R: cttagagcttgggcaaactg	152 154 156 158 164 168 170 172	0.028 0.485 0.057 0.014 0.114 0.171 0.085 0.042	0.70	0.68

Het: Expected heterozygosity

searches indicated that the microsatellite found within intron 7 had already been characterized and included in the Genethon genetic map with the name *D1S2727* (Dib et al. 1996). The microsatellite within intron 9 had not been previously described and has been assigned the name *D1S3705* (Table 1). The *C4BPA* gene also contains a previously undescribed $(CA)_n$ microsatellite, located within the first intron and named *D1S3704* (Table 1). Another microsatellite, which we include in the genetic map and call here *C4BPB*, is located 29 nucleotides downstream of the 3′ end of the *C4BPB* gene, within the 4.17 kb interval between the *C4BPB* and *C4BPA* genes. The *C4BPB* microsatellite had been previously reported and was described by Velasco and co-workers (1993). Table 1 shows primer sequences, polymorphism, and heterozygosity rates for the *D1S3704* and *D1S3705* microsatellites. Heterozygosity rates were calculated by typing a total of 76 chromosomes for the *C4BPA* microsatellite and 96 for the *PFKFB2* microsatellite. Physical positions for *D1S2727*, *D1S3704*, *D1S3705*, and *C4BPB* are shown in Figure 1. Although, *D1S2727* was not typed in this study, its position in the map reported by Dib and co-workers (1996) is in general agreement with the map reported here.

High-resolution map of the RCA region

Sixteen different markers, including those described here for the first time, were used to construct a genetic linkage map of the *RCA* region. Nine of these markers correspond

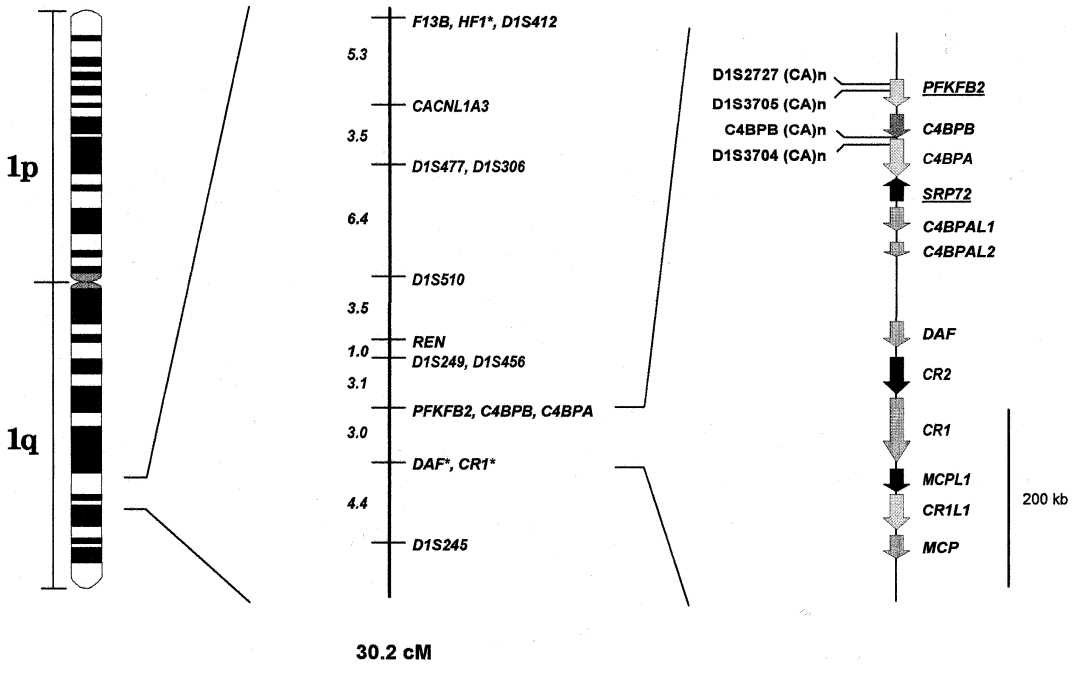

Fig. 1 From *left* to *right*: Cytogenetic map of human chromosome 1, sex-averaged genetic map of *F13B-D1S245* region, and physical map between *PFKFB2* and *MCP*. Brackets indicate the region to which each map locates in the previous map. All markers except those with an *asterisk* were positioned with a lod ≥ 3 support. *HF1*, *CR1*, and *DAF* were positioned by breakpoint analysis, as described in Materials and methods. Physical map of the *C4BPB/MCP* interval (Pardo-Manuel de Villena et al. 1996) has been modified to include the *PFKFB2* and *SRP72* genes (*underlined*) and microsatellites *D1S2727*, *D1S3704*, *D1S3705*, and *C4BPB*

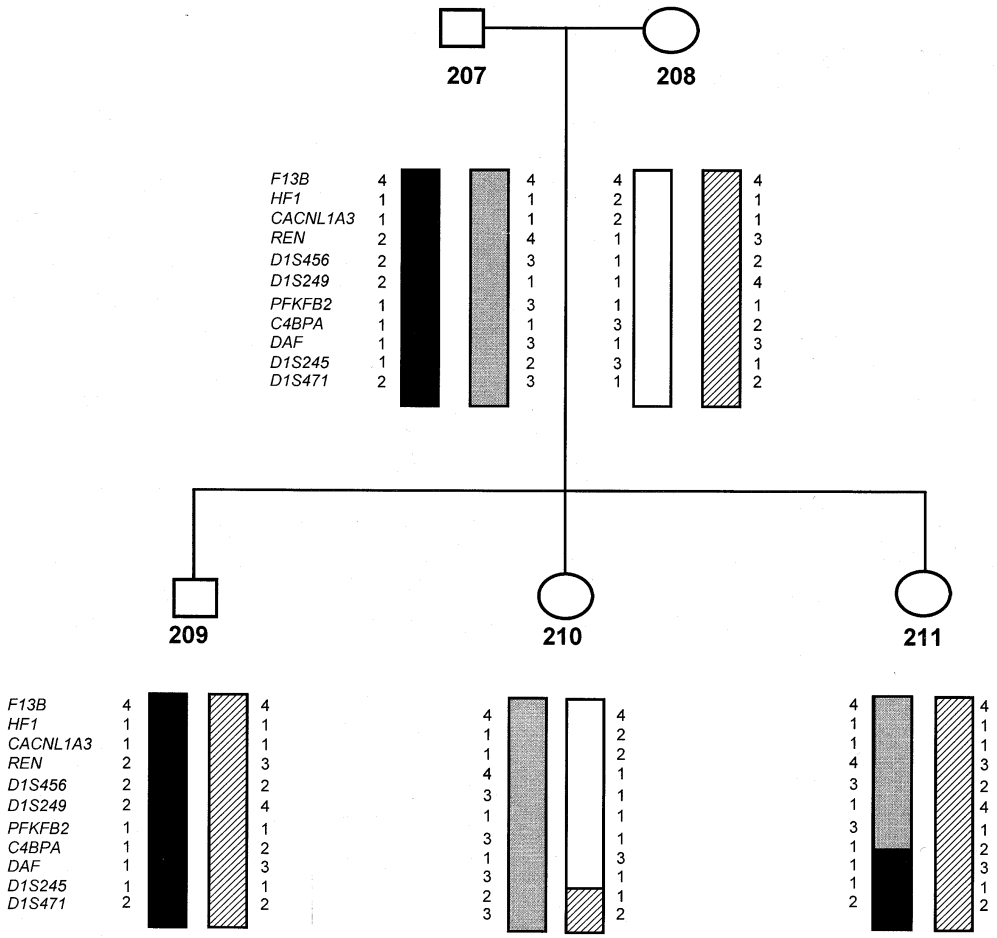

Fig. 2 Pedigree of a family in which a recombination event was detected between *DAF* and *PFKFB2 (D1S3705)*. An additional microsatellite, *D1S471*, was added to insure the correct typing of this family. *D1S471* and *D1S245* show no recombination and are physically linked (Chumakov et al. 1995; Dib et al. 1996)

to genes. Six of these gene-related markers are microsatellites that map within, or very close to genes and three correspond to RFLPs hybridized with gene-specific probes. The other seven markers are not known to be gene related. Thirteen of these markers were positioned with a lod score ≥ 3 support into the sex-averaged skeletal map provided in Figure 1. Only *DAF, CR1,* and *HF1* could not be placed with this lod score support into the map. However, we were able to order these markers by determining the most likely parental haplotypes and the precise location of the breakpoints by minimizing recombination events and double crossovers. For example, Figure 2 shows a pedigree with two breakpoints: one located between *DAF* and *PFKFB2* and the other between *DAF* and *D1S245*. The position of *DAF* within the genetic map and physical map data allows us to determine that the *C4BPB/MCP* cluster has an orientation centromere-*D1S249-PFKFB2-C4BPB-C4BPA-SRP72-C4BPAL1-C4BPAL2-DAF-CR2-CR1-MCPL1-CR1-L1-MCP-D1S245*-telomere. We detected no recombination events between *DAF* and *CR1*, or between *HF1* and *F13B* in the families analyzed. Such lack of recombination is in consonance with the fact that *CR1* and *DAF* are separated by less than 200 kb (Rey-Campos et al. 1987), and that *HF1* and *F13B* have been both mapped to a 650 kb DNA fragment (Rey-Campos et al. 1990).

The total length of the sex averaged map is of 30.2 Haldane cM (Fig. 1). Sex-specific maps (Fig. 3), show an overall lower recombination rate (20.8 cM) in males than in females (39.7 cM). The genetic distance found for the interval between *C4BPB/MCP* and *HF1/F13B* was found to be 22.5 cM, which is larger than that of 7 cM previously reported (Rodríguez de Córdoba et al. 1988). We have no obvious explanation for such a difference. However, since our current results are based on a much larger number of informative meioses and markers, we consider the later estimate to be more accurate. Recombination differences beween sexes were found in four intervals: *F13B-CACNL1A3, CACNL1A3-D1S477, D1S477-D1S510, D1S249-PFKFB2 (D1S3705)*. The interval between *CACNL1A3* and *D1S510* was found to have a significantly higher recombination rate in females than in males ($\chi^2 = 4.1$; $P < 0.05$). In addition, the *D1S249-PFKFB2 (D1S3705)* was found to recombine in females (6.6 cM), but no recombinants were found in males. On the other hand, males show a higher recombination rate than females between *F13B* and *CANCL1A3*. Differences between sexes in these two last intervals were not found to be statistically significant.

Discussion

We present here a high resolution map of the 1q32 region, including the *RCA* gene cluster, which integrates two new

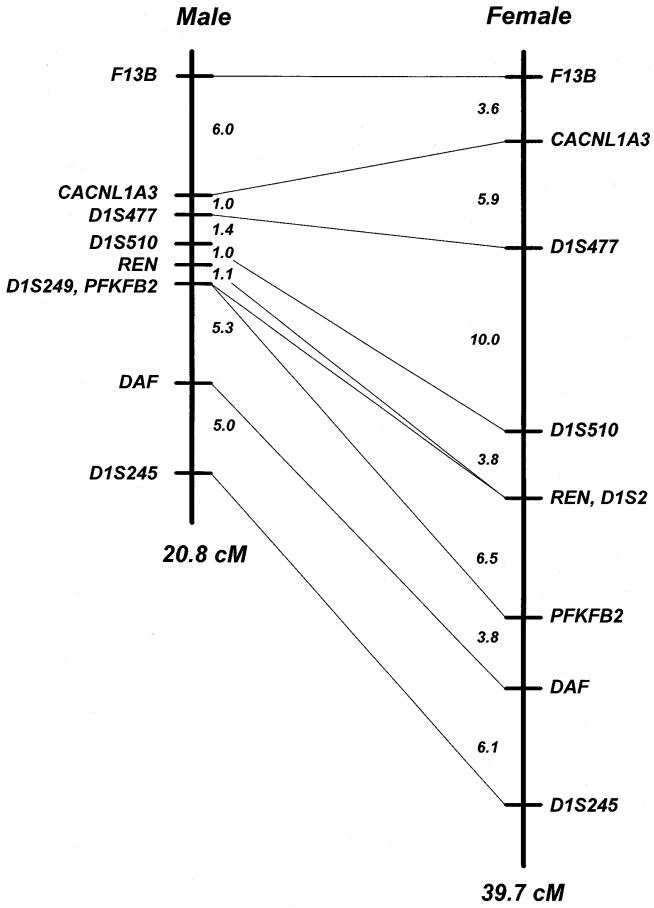

Fig. 3 Sex-specific maps of the *F13B-D1S245* interval

genes and two new microsatellite markers. The map refines and expands the physical map of the region and outlines the position of two potential hotspots.

The *RCA* genes have undergone multiple duplication events during evolution, some which are relatively recent. Hotspots of recombination have been associated with de novo duplications and deletions in several genetic diseases like Duchenne muscular dystrophy (DMD), hereditary neuropathy with liability to pressure palsies (HNPP), and Charcot-Marie-Tooth neuropathy type 1A (CTM1A) (Oudet et al. 1992; Reiter et al. 1996). Hotspots of recombination are defined as regions of the genome of a known size that recombine more frequently than would be expected for the physical distance involved. The *PFKFB2-CR1* interval is the only genetic interval for which we know an approximate physical size. We have observed in this interval of 500 kb a recombination fraction of 3 cM, which exceeds that expected for the physical distance (1 cM per 1000 kb). This is very suggestive, as a recombination hotspot in the *PFKFB2-CR1* may help to explain the high frequency of gene duplication events in this location. However, a larger sample size will be required to definitely establish whether there is a hotspot within this segment.

Recombination hotspots can also be sex-specific as is the case of a hotspot described within the major histocompatibility complex of the mouse (Shiroishi et al. 1991). In this study, we found significant differences between males and females in the interval between *CACNL1A3* and *D1S510,* and such a difference suggests that a female-specific hotspot or hotspots are active within this interval. No data are available regarding gene duplication events in this genomic region. However, this region has been associated with allelic loss in breast cancer and male germ cell tumours (Hoggard et al. 1995; Mathew et al. 1994).

Recently, several efforts have been made to develop a physical contig of the human genome and integrate cytogenetic, genetic and physical data (Chumakov et al. 1995; Bray-Ward et al. 1996). These maps, however, are still incomplete in many regions of the genome and present numerous gaps. For example, a recently described YAC contig of the human genome presents at least four gaps in the 1q32 region between *F13B* and *D1S245* (Chumakov et al. 1995). A high resolution genetic map provides a good starting point to be able to complete a physical map of the *RCA* region by providing a high density of markers that will allow the construction of correct physical contigs without chimerisms or gaps. The map presented here adds to the information about the organization of the 1q32 genomic region and is relevant not only to the *RCA* genes, but also to several types of cancer. As mentioned above, loss of heterozygosity has been reported for the 1q32 region around *D1S249* and *REN* in breast cancer and male germ cell tumors (Hoggard et al. 1995; Mathew et al. 1994). This loss is considered to indicate the presence of a tumour suppressor gene within the region that is deleted. A detailed map and new markers around *REN* and *D1S249* may facilitate the localization and cloning of such a gene.

Acknowledgments We thank Jose Luis San Millán for his help and advice in the use of the LINKAGE program. We also thank Soledad Vara de Rey and Alicia Barroso for their excellent technical assistance. This work was supported by grants from the Spanish Comisión Interministerial de Ciencia y Tecnología (SAL 96/0055; SAF 96/0162) and Fondo de Investigaciones Sanitarias (FIS 95/0885). In addition, this study is based upon work supported by the North Atlantic Treaty Organization under the grant awarded to D. H.-S. in 1996.

References

Bray-Ward, P., Menninger, J., Lieman, J., Desai, T., Mokady, N., Banks, A., and Ward, D.C. Integration of the cytogenetic, genetic, and physical maps of the human genome by FISH mapping of CEPH YAC clones. *Genomics 32:* 1–14, 1996

Chumakov, I.M. Rigault, P., Le Gali, I., Bellanne-Chantelot, C., Billault, A., Guillou, S., Soularue, P., Guasconi, G., et al. A YAC contig of the human genome. *Nature 377 (supplement),* 1995

Dib, C., Faure, S., Fizames, C., Samson, D., Drouot, N., Vignal, A., Millasseau, P., Marc, S., Hazan, J., Seboun, E., Lathrop, M., Gyapay, G., Morissette, J, and Weissenbach, J. A comprehensive map of the human genome based on 5,264 microsatellites. *Nature 380:* 152–154, 1996

Edwards, A., Civitello, A., Hammond, H.A., and Caskey, C.T. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *Am J Hum Genet 49:* 746–756, 1991

Gregg, R.G., Couch, F., Hogan, K., and Powers, P.A. Assignement of the human gene for the a1 subunit of the skeletal muscle DHP-

sensitive Ca2+ channel (*CACNL1A3*) to chromosome 1q31-32. *Genomics 15:* 107–112, 1993

Gyapay, G., Morisette, J., Vignal, A., Dib, C., Fizames, C., Millasseau, P., Marc, S., Bernardi, G., Lathrop, M., and Weissenbach, J. The 1993–94 Genethon human genetic linkage map. *Nat Genet 7:* 246 255, 1994

Hilliker, C.E., Darville, M.I., Aly, M.S., Chikri, M., Szpirer, C., Marynem, P., Rousseau, G.G., and Cassiman, J.J. Human and rat chromosomal localization of two genes for 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase by analysis of somatic cell hybrids and in situ hybridization. *Genomics 10:* 867–873, 1991

Hoggard, N., Brintnell, B., Howell, A., Weissenbach, J., and Varley, J. Allelic imbalance on chromosome 1 in human breast cancer. II. Microsatellite Repeat Analysis. *Genes Chromosom Cancer 12:* 24–31, 1995

Hourcade, D., Miesner, D.R., Bee, C., Zeldes, W., and Atkinson, J.P. Duplication and divergence of the amino-terminal coding region of the complement receptor 1 (*CR1*) gene . *J Biol Chem 265:* 974–980, 1990

Hourcade, D., García, A.D., Post, T.W., Taillon-Miller, P., Holers, V.M., Wagner, L.M., Bora, N.S., and Atkinson, J.P. Analysis of the human regulators of complement activation (*RCA*) gene cluster with yeast artificial chromosomes (YAC's). *Genomics 12:* 289–300, 1992

Hourcade, D., Holers, V.M., and Atkinson, J.P. The regulators of complement activation (*RCA*) gene cluster. *Adv Immunol 45:* 381–411, 1989

Lathrop, G.M., Lalouel, J.M., Julier, C., and Ott, J. Strategies for multilocus analysis in humans. *Proc Natl Acad Sci USA 81:* 3443–3446, 1984

Law, S. K.A. and Reid, K B. M. *Complement*, Oxford University Press, Oxford, 1995

Lutcke, H. Signal recognition particle (SRP), a ubiquitous initiator of protein translocation. *Eur J Biochem 228:* 531–549, 1995

Mathew, S., Murty, V.V. Bosl, G.J., and Chaganti, R.S. Loss of heterozygosity identifies multiple sites fo allelic deletions on chromosome 1 in human male germ cell tumors. *Cancer Res 54:* 6265–6269, 1994

Murray, J.C., Buetow, K. H., Weber, J.L., Ludwigsen, S., Scherpbier-Heddema, T., Manion, F., Quillen, J., Sheffield, V.C., Sunden, S., Duyk, G.M., Weissenbach, J., Gyapay, G., Dib, C., Morissette, G., Lathrop, M., Vignal, A., White, R., Matsunami, N., Gerken, S., Melis, R., Albertsen, H., Plaetke, R., Odelberg, S., Ward, D., Dausset, J., Cohen, D., and Cann, H. A comprehensive human linkage map with centimorgan density. *Science 265:* 2049–2065, 1994

Nishimura, D.Y. and Murray, J.C. A tetranucleotide repeat for the *F13B* locus. *Nucleic Acids Res 20:* 1167, 1992

Oudet, C., Hanauer, A., Clemens, P., Caskey, T., and Mandel, J.L. Two hotspots of recombination in the *DMD* gene correlate with the deletion prone regions. *Hum Molec Genet 1:* 599–603, 1992

Pardo-Manuel de Villena, F., Heine-Suñer, D., and Rodríguez de Córdoba, S. Ordering of the human regulator of complement activation gene cluster on 1q32 by two-colour FISH. *Cytogenet Cell Genet 72:* 339–341, 1996

Pardo-Manuel de Villena, F. and Rodríguez de Córdoba, S. *C4BPAL2:* a second duplication fo the *C4BPA* gene in the human *RCA* gene cluster. *Immunogenetics 41:* 139–143, 1995

Pardo-Manuel, F., Rey-Campos, J., Hillarp, A., Dahlback, B., and Rodríguez de Córdoba, S. Human genes for the α and β chains of complement C4b-binding protein are closely linked in head-to-tail arrangement. *Proc Natl Acad Sci USA* 87: 4529–4532, 1990

Pilkis, S.J., Claus, T.H., Kurland, I.J., and Lange, A.J. 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase: a metabolic signaling enzyme. *Annu Rev Biochem 64:* 799–835, 1995

Reiter, L.T., Murakami, T., Koeuth, T., Pentao, L., Muzny, D.M., Gibbs, R.A., and Lupski, J.R. A recombination hotspot responsible for two inherited peripheral neuropathies is located near a *mariner* transposon-like element. *Nat Genet 12:* 288–297, 1996

Rey-Campos, J., Baeza-Sanz, D., and Rodríguez de Córdoba, S. Physical linkage of the human genes coding for complement factor H and coagulation factor XIII B subunit. *Genomics 7:* 644–646, 1990

Rey-Campos, J., Rubinstein, P., and Rodríguez de Córdoba, S. A physical map of the human regulator of complement activation gene cluster linking the complement genes *CR1, CR2, DAF,* and *C4BP. J Exp Med 167:* 664, 1988

Rey-Campos, J., Rubinstein, P., and Rodríguez de Córdoba, S. Decay Accelerating Factor. Genetic polymorphism and linkage to the *RCA* (Regulator of Complement Activation) gene cluster in humans. *J Exp Med 166:* 246–252, 1987

Rodríguez de Córdoba, S., and Rubinstein, P. Quantitative variations of the C3b/C4b receptor (CR1) in human erythrocytes are controlled by genes within the regulator of complement activation (*RCA*) gene cluster. *J Exp Med 164:* 1274, 1986

Rodríguez de Córdoba, S., Lublin, D., Rubinstein, P., and Atkinson, J. The human genes for three complement components that regulate the activation of C3 are tightly linked. *J Exp Med* 161: 1189–1195, 1985

Rodríguez de Córdoba, S., Rey-Campos, J., Dykes, D.D., McAlpine, P.J., Wong, P., and Rubinstein, P. Coagulation factor XIII B subunit is encoded by a gene linked to the regulator of complement activation (*RCA*) gene cluster in man. *Immunogenetics 28:* 452–454, 1988

Sánchez-Corral, P., Pardo-Manuel de Villena, F., Rey-Campos, J., and Rodríguez de Córdoba, S. *C4BPAL1,* a member of the human regulator of complement activation (*RCA*) gene cluster that resulted from the duplication of the gene coding for the α-chain of the C4b-binding protein. *Genomics 17:* 185–193, 1993

Sander, A., Murray, J. C., Scherpbier-Heddema, T., Buetow, K.H., Weissenbach, J., Zingg, M., Ludwig, K., and Schmelzle, R. Microsatellite-based fine mapping of the Van der Woude syndrome locus to an interval of 4.1 cM between *D1S245* and *D1S414. Am J Hum Genet 56:* 310–318, 1995

Sherka, C., Moulds, J.M., Taillon-Miller, P., Hourcade, D., and Zipfel, P.F. The human factor H-related gene 2 (*FHR2*): structure and linkage to the coagulation factor XIIIb gene. *Immunogenetics* 42: 268–274, 1995

Shiroishi, T., Sagai, T., Hanzawa, N., Gotoh, H., and Moriwaki, K. Genetic control of sex-dependent meiotic recombination in the major histocompatibility complex of the mouse. *EMBO J 10:* 681–686, 1991

Turecki, G., Smith, M.A., and Mari, J.J. Type I bipolar disorder associated with a fragile site on chromsome 1. *Am J Med Genet 60:* 179–182, 1995

Velasco, E., Sanchez-Corral, P., Moreno, F., Rodríguez de Córdoba, S. Dinucleotide repeat polymorphism between the human *C4BPA* and *C4BPB* gene loci (1q32). *Hum Mol Genet 1:* 552, 1992

Weis, J.H., Morton, C.C., Bruns, G.A., Weis, J.J., Klickstein, L.B., Wong, W.W., and Fearon, D. T. A complement receptor locus: genes encoding C3b/C4b receptor and C3d/Epstein-Barr virus receptor map to 1q32. *J Immunol* 138: 312–319, 1987

Zipfel, P.E. and Sherka, C. Complement factor H and related proteins: an expanding family of complement regulatory proteins? *Immunol Today 15:* 121–126, 1994