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Biochimica et Biophysica Acta 1447 (1999) 307–312

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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Short sequence-paper

Genetic linkage and radiation hybrid mapping of the three human $GABA_C$ receptor ρ subunit genes: *GABRR1*, *GABRR2* and *GABRR3*

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Received 2 June 1999; received in revised form 23 July 1999; accepted 24 August 1999

Abstract

$GABA_C$ receptors mediate rapid inhibitory neurotransmission in retina. We have mapped, in detail, the human genes which encode the three polypeptides that comprise this receptor: ρ1 (*GABRR1*), ρ2 (*GABRR2*) and ρ3 (*GABRR3*). We show that *GABRR1* and *GABRR2* are located close together, in a region of chromosome 6q that contains loci for inherited disorders of the eye, but that *GABRR3* maps to chromosome 3q11-q13.3. Our mapping data suggest that the ρ polypeptide genes, which are thought to share a common ancestor with $GABA_A$ receptor subunit genes, diverged at an early stage in the evolution of this gene family.

Keywords: Chromosomal mapping; γ-Aminobutyric acid type C receptor; Gene family; Human; Linkage; Radiation hybrid

$GABA_C$ (γ-aminobutyric acid type C) receptors are agonist-gated chloride ion channels that are predominantly found on retinal bipolar cells [1–3]. Complementary DNA (cDNA) cloning experiments in mammals [4–6] have revealed the existence of three polypeptides (named ρ1, ρ2 and ρ3) that exhibit 28–42% sequence identity with subunits of the $GABA_A$ receptor, which is the major inhibitory neurotransmitter receptor in brain and retina. Expression studies in *Xenopus laevis* oocytes and in mammalian cells [4,7–10] have demonstrated that the ρ subunit can form homomeric and heteromeric ion channels with pharmacological properties similar to those of native $GABA_C$ receptors. The human $GABA_C$ receptor ρ1 (*GABRR1*) and ρ2 (*GABRR2*) subunit genes have previously been mapped to chromosome 6q14-q21 using a panel of human-hamster somatic cell hybrids [5]. No sequence or chromosomal mapping data have yet been provided for the human ρ3 polypeptide gene (*GABRR3*).

To obtain clones from which to generate polymorphic markers and sequence tagged sites (STSs) for the three $GABA_C$ receptor genes, human bacteriophage λ genomic libraries were screened with a 477 bp human ρ1 subunit cDNA fragment and a 410 bp rat ρ3 subunit cDNA fragment (B.E. Albrecht and M.G. Darlison, unpublished data). One clone (λhGR4.ρ1) that hybridized to the ρ1 subunit probe was shown, by subcloning and sequencing, to contain two exons (178 nucleotides in total that are

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0167-4781/99/$ – see front matter
PII: S0167-4781(99)00167-0

Fig. 1. Sequence of part of the human $GABA_C$ receptor ρ3 subunit gene (*GABRR3*). The nucleotide and deduced amino acid sequences of an exon of *GABRR3*, which encodes the majority of the intracellular loop, the fourth membrane-spanning domain (underlined) and at least part of the 3′ untranslated region, have been aligned with the corresponding parts of the rat ρ3 subunit cDNA and its encoded product [6] using the computer program GAP (Wisconsin Package, Version 10.0, January 1999, Genetics Computer Group, 575 Science Drive, Madison, WI); dots denote gaps that have been introduced to maximize the alignment score. Note that the human nucleotide sequence is numbered arbitrarily while the numbering of the rat cDNA sequence corresponds to that given in [6]. Positions at which the two nucleotide sequences are identical are indicated by vertical bars, and the sequences of the primers that identify a *GABRR3*-specific STS (see text) are boxed. The human *GABRR3* gene sequence has been given the EMBL accession number Y18994.

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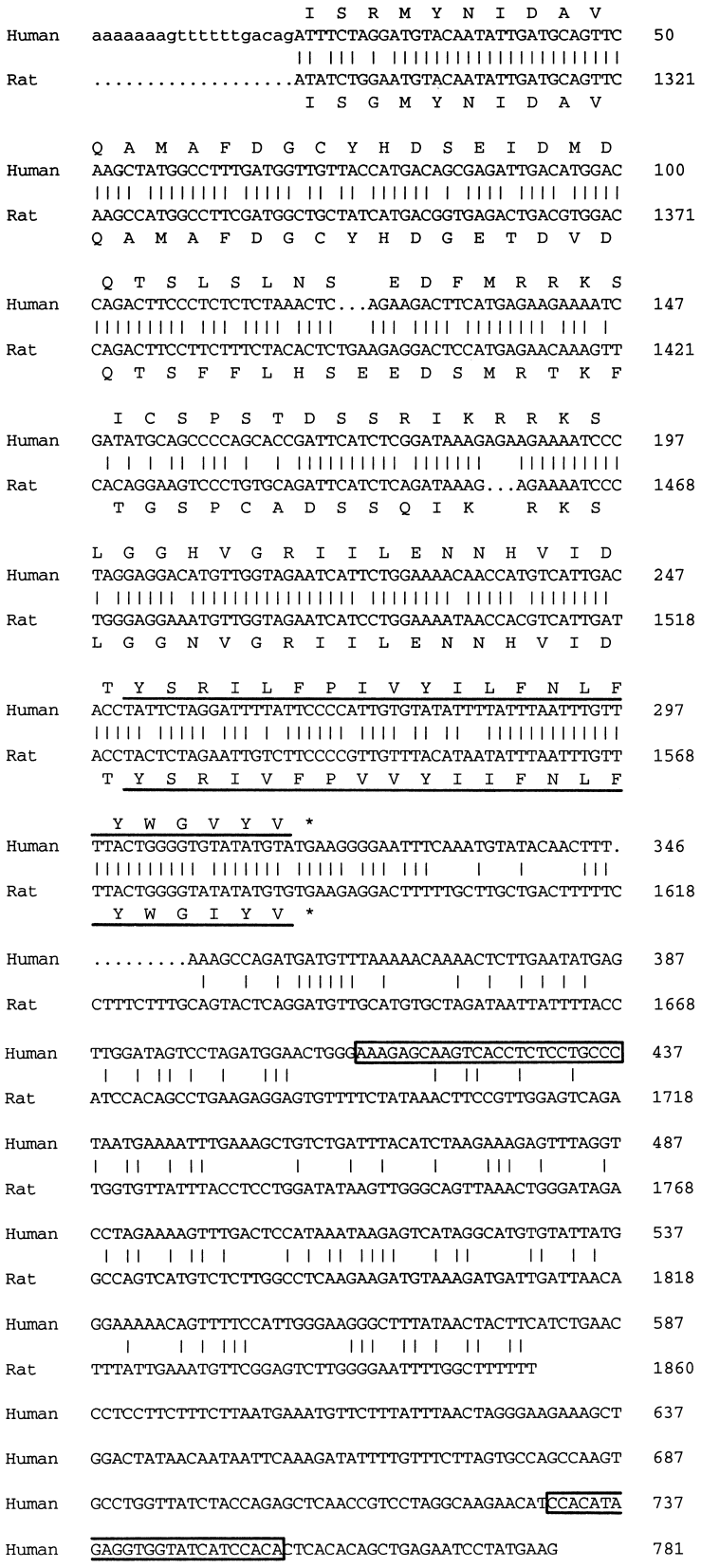
identical to part of the sequence of the human cDNA) of *GABRR1* that encode part of the amino-terminal extracellular domain. Another clone (λhGR3.ρ3) that hybridized to the ρ3 subunit probe was similarly analyzed and shown to possess an exon that specifies the majority of the intracellular loop, all of the fourth membrane-spanning domain and at least part of the 3′ untranslated region of a $GABA_A$/$GABA_C$ receptor-like subunit (Fig. 1). This partial polypeptide sequence of 99 residues is only 30% and 31% identical, respectively, to the corresponding portions of the human $GABA_C$ receptor ρ1 and ρ2 subunits. In contrast, it is 80% identical to the equivalent part of the rat ρ3 subunit [6]. Since this partial sequence includes the majority of the intracellular loop, a region which is highly divergent between different $GABA_A$ and $GABA_C$ receptor polypeptides, and since the coding region of λhGR3.ρ3 exhibits 84% identity to the corresponding portion of the rat ρ3 subunit cDNA sequence (Fig. 1), we conclude that λhGR3.ρ3 contains at least part of the human ρ3 subunit gene (*GABRR3*).

A *Sau*3AI restriction digest of the *GABRR1*-specific λ clone, λhGR4.ρ1, was subsequently blotted and hybridized with a mixture of three microsatellite repeat oligonucleotides ($(AG)_{12}$, $(GT)_{12}$ and $(CA)_{12}$; Bios, New Haven, CT). A positively hybridizing approx. 1 kb fragment was subcloned and sequenced and found to contain an $(AC)_{14}$ repeat. Oligonucleotide primers, the sequences of which flank this motif (F: 5′-GGGAAGTAGAGGGCTAAGCGAATGT-3′ and R: 5′-CCCTAGGGCAACTGTTGTCAAACTT-3′), were synthesized and used in polymerase chain reaction (PCR) amplifications (see [11]) at an annealing temperature of 59°C. The polymorphic nature of the $(AC)_{14}$ repeat was demonstrated using DNA from the parents of the reference panel of families of the Centre d'Étude du Polymorphisme Humain (CEPH). The size of the predominant allele was 196 bp (details of the polymorphism have been submitted to the CEPH database). The marker is of moderate informativity, having a calculated heterozygosity of 0.41.

To refine the mapping of *GABRR1*, the GABRR1 microsatellite repeat locus was genotyped in the 40 families of the CEPH reference panel and subjected to linkage analysis using CRIMAP version 2.4 [12]. Ten AFM markers located in the proximal portion of chromosome 6q, in the interval from D6S284 to D6S301, were selected to form a framework map. The order of markers in the CEPH/Généthon linkage map [13] was first confirmed using the FLIPS option of CRIMAP. Subsequently, GABRR1 and three Marshfield markers (D6S251/MFD131, D6S252/MFD171 and D6S249/MFD97; genotypes obtained from the Cooperative Human Linkage Centre, Iowa City, IA) were placed simultaneously in the fixed framework map using the ALL option of CRIMAP; this was followed by recalculation of the map distances using the FIXED option (Fig. 2). The marker order was supported by the absence of any close double recombinants, and no alternative marker orders were found whose likelihood lay within lod 2 of the best order. GABRR1 was localized between Généthon markers AFM286za5 (D6S445) and AFM311wa5 (D6S458) and showed no recombination with AFM317zb1 (D6S462).

Radiation hybrid mapping in the Genebridge 4 (GB4) panel [14] was used to map *GABRR1* and *GABRR2*. For the latter, the previously published primers Rho2i-5′ and Rho2i-3′ [5] were employed in the PCR to generate a *GABRR2* amplification product of 299 bp from genomic DNA. This fragment contains an exon that encodes part of the second and all of the third membrane-spanning domain of the ρ2 polypeptide. The GABRR1 genetic marker


I S R M Y N I D A V
Human aaaaaaagttttttgacagATTTCTAGGATGTACAATATTGATGCAGTTC 50
Rat .....ATATCTGGAATGTACAATATTGATGCAGTTC 1321
I S G M Y N I D A V
Q A M A F D G C Y H D S E I D M D
Human AAGCTATGGCCTTTGATGGTTGTTACCATGACAGCGAGATTGACATGGAC 100
Rat AAGCCATGGCCTTCGATGGCTGCTATCATGACGGTGAGACTGACGTGGAC 1371
Q A M A F D G C Y H D G E T D V D
Q T S L S L N S E D F M R R K S
Human CAGACTTCCCTCTCTCTAAACTC...AGAAGACTTCATGAGAAGAAAATC 147
Rat CAGACTTCCTTCTTTCTACACTCTGAAGAGGACTCCATGAGAACAAAGTT 1421
Q T S F F L H S E E D S M R T K F
I C S P S T D S S R I K R R K S
Human GATATGCAGCCCCAGCACCGATTCATCTCGGATAAAGAGAAGAAAATCCC 197
Rat CACAGGAAGTCCCTGTGCAGATTCATCTCAGATAAAG...AGAAAATCCC 1468
T G S P C A D S S Q I K R K S
L G G H V G R I I L E N N H V I D
Human TAGGAGGACATGTTGGTAGAATCATTCTGGAAAACAACCATGTCATTGAC 247
Rat TGGGAGGAAATGTTGGTAGAATCATCCTGGAAAATAACCACGTCATTGAT 1518
L G G N V G R I I L E N N H V I D
T Y S R I L F P I V Y I L F N L F
Human ACCTATTCTAGGATTTTATTCCCCATTGTGTATATTTTATTTAATTTGTT 297
Rat ACCTACTCTAGAATTGTCTTCCCCGTTGTTTACATAATATTTAATTTGTT 1568
T Y S R I V F P V V Y I I F N L F
Y W G V Y V *
Human TTACTGGGGTGTATATGTATGAAGGGGAATTTCAAATGTATACAACTTT. 346
Rat TTACTGGGGTATATATGTGTGAAGAGGACTTTTTGCTTGCTGACTTTTTC 1618
Y W G I Y V *
Human .....AAAGCCAGATGATGTTTAAAAACAAAACTCTTGAATATGAG 387
Rat CTTTCTTTGCAGTACTCAGGATGTTGCATGTGCTAGATAATTATTTTACC 1668
Human TTGGATAGTCCTAGATGGAACTGGGAAAGAGCAAGTCACCTCTCCTGCCC 437
Rat ATCCACAGCCTGAAGAGGAGTGTTTTCTATAAACTTCCGTTGGAGTCAGA 1718
Human TAATGAAAATTTGAAAGCTGTCTGATTTACATCTAAGAAAGAGTTTAGGT 487
Rat TGGTGTTATTTACCTCCTGGATATAAGTTGGGCAGTTAAACTGGGATAGA 1768
Human CCTAGAAAAGTTTGACTCCATAAATAAGAGTCATAGGCATGTGTATTATG 537
Rat GCCAGTCATGTCTCTTGGCCTCAAGAAGATGTAAAGATGATTGATTAACA 1818
Human GGAAAAACAGTTTTCCATTGGGAAGGGCTTTATAACTACTTCATCTGAAC 587
Rat TTTATTGAAATGTTCGGAGTCTTGGGGAATTTTGGCTTTTTT 1860
Human CCTCCTTCTTTCTTAATGAAATGTTCTTTATTTAACTAGGGAAGAAAGCT 637
Human GGACTATAACAATAATTCAAAGATATTTTGTTTCTTAGTGCCAGCCAAGT 687
Human GCCTGGTTATCTACCAGAGCTCAACCGTCCTAGGCAAGAACATCCACATA 737
Human GAGGTGGTATCATCCACACTCACACAGCTGAGAATCCTATGAAG 781

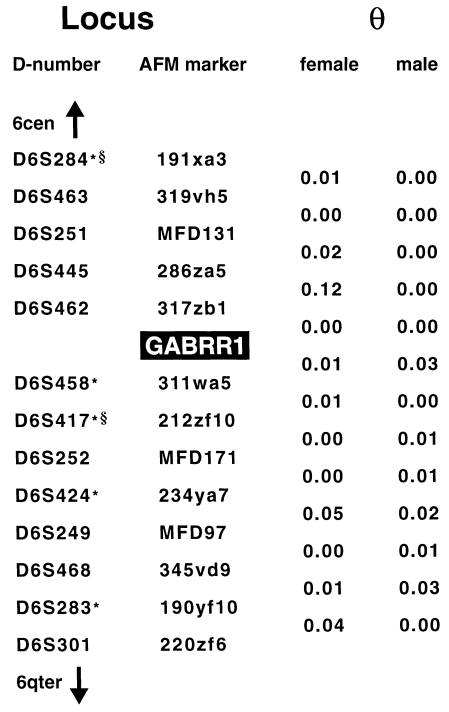


Fig. 2. Linkage mapping of GABRR1 on human chromosome 6q. The figure shows a linkage map of part of chromosome 6q, based on the Généthon linkage map of CEPH markers [13], with GABRR1 and three Marshfield (MFD) markers inserted in their maximum likelihood locations. Sex-specific recombination fractions between markers are shown. *These markers were haplotyped with other AFM markers from the same interval in the Généthon linkage map. §Fluorescence in situ hybridization (FISH)-mapped markers flanking *GABRR1* in the radiation hybrid map (see text).

and the *GABRR2*-specific STS were initially localized to human chromosome 6 using a single chromosome somatic cell hybrid panel [15]. Subsequently, they were typed in the available hybrids of the GB4 panel. Hybrids yielding a band of the expected size were scored as 1, those yielding no band were scored as 0, and either unclear or inconsistent results were scored as 2. The scores were submitted to the mapping server at the Whitehead Institute (http://www.genome.wi.mit.edu).

The GABRR1 marker was localized between framework markers AFMb298zg9 and AFM310xa9 on chromosome 6q, and its GB4 data vector showed no differences from that of AFM185xd10 (not shown). None of these markers is present in the Généthon linkage map. No differences were detected between the vectors for the *GABRR2*-specific STS used here and another *GABRR2*-specific marker, the Whitehead Institute expressed sequence tag, WI-7717; both were localized approx. 4 cR (equivalent to approx. 1.1 Mb) telomeric to *GABRR1*. The nearest flanking markers present in both linkage and radiation hybrid maps were D6S284/AFM191xa3 and D6S275/AFM158ya11 (haplotyped with D6S417; Fig. 2), which map to the cytogenetic intervals 6q13-q15 and 6q16.3, respectively (Genome DataBase). Thus, on the basis of map distances and cytogenetic mapping data, *GABRR1* and *GABRR2* are located towards the telomeric end of the interval 6q13-q16.3.

No microsatellite marker was detected within clone λhGR3.ρ3. Thus, a 343 bp *GABRR3*-specific STS was generated by designing primers (F: 5′-AAAGAGCAAGTCACCTCTCCTGCCC-3′ and R: 5′-TGTGGATGATACCACCTCTATGTGG-3′) which recognize part of the 3′ untranslated region of the gene (Fig. 1). An annealing temperature of 59°C was used in the PCR, and the STS was mapped to chromosome 3 using the single chromosome hybrid panel. Radiation hybrid mapping further localized the STS between framework markers CHLC.GATA68D03 (D3S2459) and WI-5968 (D3S3099), at a distance of 7.9 cR from the former. The closest flanking framework markers for which cytogenetic map locations are available in the Genome DataBase are WI-5486 (D3S2970; 3q11-q13.3) and AFM259va9 (D3S1572; 3q13). In summary, our mapping data refine the localization of the human $GABA_C$ receptor ρ1 and ρ2 polypeptide genes, *GABRR1* and *GABRR2*, to chromosome 6q13-q16.3, and reveal that, in contrast, the ρ3 subunit gene, *GABRR3*, maps to chromosome 3q11-q13.3.

The predominant expression of *GABRR1* and *GABRR2* in retina makes them interesting candidate genes for diseases of the eye that map to the interval 6q13-q16.3. Stargardt disease type 3 (STGD3) is one such disease, and its localization proximal to D6S252 [16] matches that of *GABRR1* and, on the basis of its proximity, *GABRR2*. The critical interval for the CORD7 (dominant cone-rod dystrophy) locus may also overlap with 6q13-q16.3 [17], as may that for a locus underlying retinitis pigmentosa in some families [18]. The polymorphic microsatellite marker reported here, GABRR1, should prove of use in link-

age studies of these disorders. To date, no diseases of the eye have been mapped to the region of chromosome 3 containing *GABRR3*.

The sequence similarity of the three ρ polypeptides indicates that the corresponding genes originated from a common ancestor during vertebrate evolution, and the close proximity of the ρ1 and ρ2 subunit genes suggests that they emerged via a local duplication event. In contrast, *GABRR3* may have arisen by duplication of a *GABRR1*/*GABRR2* progenitor, perhaps during the last putative tetraploidization event in the lineage leading to mammals (see [19]). Mapping studies have shown that many of the $GABA_A$ receptor subunit genes are localized in four clusters on human chromosomes 4, 5, 15, and the X, and that these clusters exhibit a conserved organization, consistent with two rounds of duplication of a primordial cluster [11]. Since none of the ρ polypeptide genes localize to the $GABA_A$ receptor subunit gene clusters, we suggest that $GABA_C$ receptor genes diverged from $GABA_A$ receptor genes at an early stage in the evolution of this family.

This work was supported by grants from the Wellcome Trust (grant no. 033981/Z91/Z to K.J.J.) and the Deutsche Forschungsgemeinschaft (SFB232/B7 to M.G.D.), and by an award from the Fonds der Chemischen Industrie (to M.G.D.). We thank the Human Genome Mapping Project Resource Centre, Cambridge, and their staff, for access to biological resources and computing facilities, and for their friendly advice and help. We also thank Prof. Robert Williamson (previously of St. Mary's Hospital Medical School, London) for access to CEPH reference family DNAs.

References

[1] A. Feigenspan, H. Wässle, J. Bormann, Pharmacology of GABA receptor Cl^- channels in rat retinal bipolar cells, Nature 361 (1993) 159–162.

[2] J. Bormann, A. Feigenspan, $GABA_C$ receptors, Trends Neurosci. 18 (1995) 515–519.

[3] G.A.R. Johnston, $GABA_C$ receptors: relatively simple transmitter-gated ion channels?, Trends Neurosci. 17 (1996) 319–323.

[4] G.R. Cutting, L. Lu, B.F. O'Hara, L.M. Kasch, C. Montrose-Rafizadeh, D.M. Donovan, S. Shimada, S.E. Antonarakis, W.B. Guggino, G.R. Uhl, H.H. Kazazian Jr., Cloning of the γ-aminobutyric acid (GABA) ρ1 cDNA: a GABA receptor subunit highly expressed in the retina, Proc. Natl. Acad. Sci. USA 88 (1991) 2673–2677.

[5] G.R. Cutting, S. Curristin, H. Zoghbi, B. O'Hara, M.F. Seldin, G.R. Uhl, Identification of a putative γ-aminobutyric acid (GABA) receptor subunit rho_2 cDNA and colocalization of the genes encoding rho_2 (GABRR2) and rho_1 (GABRR1) to human chromosome 6q14-q21 and mouse chromosome 4, Genomics 12 (1992) 801–806.

[6] T. Ogurusu, R. Shingai, Cloning of a putative γ-aminobutyric acid (GABA) receptor subunit ρ3 cDNA, Biochim. Biophys. Acta 1305 (1996) 15–18.

[7] S. Shimada, G. Cutting, G.R. Uhl, γ-Aminobutyric acid A or C receptor? γ-Aminobutyric acid ρ1 receptor RNA induces bicuculline-, barbiturate-, and benzodiazepine-insensitive γ-aminobutyric acid responses in *Xenopus* oocytes, Mol. Pharmacol. 41 (1992) 683–687.

[8] T. Kusama, C.E. Spivak, P. Whiting, V.L. Dawson, J.C. Schaeffer, G.R. Uhl, Pharmacology of GABA ρ1 and GABA α/β receptors expressed in *Xenopus* oocytes and COS cells, Br. J. Pharmacol. 109 (1993) 200–206.

[9] T. Kusama, T.-L. Wang, W.B. Guggino, G.R. Cutting, G.R. Uhl, GABA ρ2 receptor pharmacological profile: GABA recognition site similarities to ρ1, Eur. J. Pharmacol. 245 (1993) 83–84.

[10] R. Shingai, K. Yanagi, T. Fukushima, K. Sakata, T. Ogurusu, Functional expression of GABA ρ3 receptors in *Xenopus* oocytes, Neurosci. Res. 26 (1996) 387–390.

[11] M.E.S. Bailey, D.A. Matthews, B.P. Riley, B.E. Albrecht, M. Kostrzewa, A.A. Hicks, R. Harris, U. Müller, M.G. Darlison, K.J. Johnson, Genomic mapping and evolution of human $GABA_A$ receptor subunit gene clusters, Mamm. Genome 10 (1999) 839–843.

[12] E. Lander, P. Green, Construction of multilocus genetic linkage maps in humans, Proc. Natl. Acad. Sci. USA 84 (1987) 2363–2367.

[13] C. Dib, S. Fauré, C. Fizames, D. Samson, N. Drouot, A. Vignal, P. Millasseau, S. Marc, J. Hazan, E. Seboun, M. Lathrop, G. Gyapay, J. Morisette, J. Weissenbach, A comprehensive genetic map of the human genome based on 5,264 microsatellites, Nature 380 (1996) 152–154.

[14] G. Gyapay, K. Schmitt, C. Fizames, H. Jones, N. Vega-Czarny, D. Spillett, D. Muselet, J.-F. Prud'Homme, C. Dib, C. Auffraym, J. Morisette, J. Weissenbach, P.N. Goodfellow, A radiation hybrid map of the human genome, Hum. Mol. Genet. 5 (1996) 339–346.

[15] D.P. Kelsell, L. Rooke, D. Warne, M. Bouzyk, L. Cullin, S. Cox, L. West, S. Povey, N.K. Spurr, Development of a panel of monochromosomal somatic cell hybrids for rapid gene mapping, Ann. Hum. Genet. 59 (1995) 233–241.

[16] E.M. Stone, B.E. Nichols, A.E. Kimura, T.A. Weingeist, A. Drack, V.C. Sheffield, Clinical features of a Stargardt-like dominant progressive macular dystrophy with genetic linkage to chromosome 6q, Arch. Ophthalmol. 112 (1994) 765–772.

[17] R.E. Kelsell, K. Gregory-Evans, C.Y. Gregory-Evans, G.E.

Holder, M.R. Jay, B.H.F. Weber, A.T. Moore, A.C. Bird, D.M. Hunt, Localization of a gene (*CORD7*) for a dominant cone-rod dystrophy to chromosome 6q, Am. J. Hum. Genet. 63 (1998) 274–279.

[18] A. Ruiz, S. Borrego, I. Marcos, G. Antiñolo, A major locus for autosomal recessive retinitis pigmentosa on 6q, determined by homozygosity mapping of chromosomal regions that contain gamma-aminobutyric acid-receptor clusters, Am. J. Hum. Genet. 62 (1998) 1452–1459.

[19] L.G. Lundin, Evolution of the vertebrate genome as reflected in paralogous chromosomal regions in man and the house mouse, Genomics 16 (1993) 1–19.