Research

Molecular genetics and structural genomics of the human protein kinase C gene module

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Abstract

Background: Protein kinase C (PKC) has become a major focus among cell biologists interested in second-messenger signal transduction and much has been learned about differences in the cellular localization and function of its different isotypes. In this study we systematically address the genomic locations and gene structures of the human PKC gene module.

Results: We first carried out fine chromosomal mapping of all nine PKC genes by fluorescence *in situ* hybridization (FISH), using cosmid and BAC probes. The PKC genes are found to be dispersed throughout the genome, and in some positions distinct from those previously reported: PKCα is at 17q24, PKCβ at 16p12, PKCγ at 19q13.4, PKCδ at 3p21.2, PKCε at 2p21, PKCζ at 1p36.3, PKCη at 14q22-23, PKCθ at 10p15 and PKCι at 3q26. For PKCι, an additional FISH signal mapped on Xq21.3 revealed a pseudogene (derived by retrotransposition). PKCγ, ζ and θ are found to map to the most distal positions on the chromosomes, potentially implicating telomere position effects in their expression. Using the complete human genome draft sequence and bioinformatics tools, we then carried out a systematic analysis of PKC gene structure, including determination of the occurrence of single-nucleotide polymorphisms corresponding to the PKC loci.

Conclusion: This resource of genomic information now facilitates investigation of the PKC gene module in structural chromosomal abnormalities and human disease locus mapping studies.

Background

The human protein kinase C (PKC) family of serine/threonine kinases comprises nine distinct isotypes with essential roles in cellular activation and differentiation in diverse cell types. They are, for example, known to phosphorylate several cellular proteins, leading to modulation of surface antigens, activation of other protein kinases, and induction of several key transcription factors, including aberrant signaling responses contributing towards malignant transformation [1]. Like many other signaling effectors, PKC is not a single entity but constitutes, at the gene level, nine different isotypes - PKCα, β, γ, δ, ε, ζ, η, θ and ι. PKC became the focus of attention among cell biologists interested in signal transduction and tumorigenesis after it was discovered that it is activated not only by the inositol phospholipid-derived second messenger diacylglycerol, but also by phorbol esters and other tumor promoters [2]. Members of the PKC family have been implicated in numerous cellular responses in a large variety of cell types [3]. PKC isotypes can be grouped into three subfamilies on the basis of their domain structure: the first subfamily includes the Ca^{2+}-dependent PKCα, β and γ isotypes. These kinases contain, in addition to the catalytic

C3 protein kinase domain that is shared by all members of the family, a phospholipid- and diacylglycerol/phorbol ester-binding C1 domain and a Ca^{2+}-binding C2 domain. PKCδ, ε, η and θ contain the conserved C1 domain but only a C2-like domain, defective in Ca^{2+} binding. PKCζ and ι completely lack the C2 domain and their C1 domain contains only one of the two cysteine-rich zinc-finger-like motifs present in all other isotypes (for a review see [4]).

The reasons for the heterogeneity of PKC isotypes are not yet fully understood. However, the differences between these distinct PKC isotypes with respect to cofactor dependencies, subcellular localization, and tissue distribution (including levels of expression in a given cell type), suggest that they may be independently regulated through coupling to distinct receptor signaling pathways and possess different substrate specificities [4]. In this context, the expression of more than one PKC isotype in a cell potentially provides it with the ability to mount heterogeneous responses to diverse extracellular signals. However, many gaps remain in our knowledge of the detailed functions of PKC in certain important cell types such as T cells. Nevertheless, the potential relevance of PKC isotype-specific function(s) in the etiology of a number of human diseases is much discussed ([2] and see below).

To date, human and other mammalian PKC loci have not been systematically characterized, and very little is known regarding structural differences between the isotype genes. We previously focused on the genomic structure of the PKCθ gene [5,6] and its function (for a review see [2]). We now report the complete chromosomal localization and analysis of the structure of the PKC loci. In a critical first step, we systematically defined the chromosomal locations of all nine human PKC loci using cloned contigs of cosmid and/or bacterial artificial chromosome (BAC) probes and fine chromosomal mapping by fluorescence *in situ* hybridization (FISH). In addition, making use of the complete human genome draft sequence and bioinformatics tools, we have determined the gene organization of the human PKC gene loci as a resource of structural, functional, and positional candidate genes usage. Given the multiplicity of reported PKC functions in numerous cell types, this structural genomic information on the PKC gene module may assist investigations of biological processes regulating cell proliferation, differentiation and survival.

Results and discussion
Chromosomal fine mapping of the nine PKC loci by FISH analysis
Genomic clones were isolated under high-stringency hybridization conditions from human genomic libraries using cDNA probes for the nine human PKC isotypes. Genomic PKC clones confirmed by polymerase chain reaction (PCR) (up to five clones per gene) were subsequently used to determine the human chromosomal location of the

PKC gene module using FISH. Fluorescent doublets were observed in the majority of the metaphases on each of the two sister chromatids at the positions indicated in Figure 1a, which shows the composite karyotype of the nine distinct PKC genes. No other chromosomal site exhibited significant fluorescent signals (data not shown). The fluorescence intensity of up to eight signal-carrying chromosomes was measured to create a highly significant average green-to-red profile. This profile was linearly interpolated to the size of standard chromosomal ideograms, allowing an objective assignment of fluorescent signals to a chromosomal band. The diagrammatic presentation of fluorescent spot distribution on metaphase human chromosomes is shown in Figure 1b.

Thus, the chromosomal location of all members has been fine-mapped to confirm and/or correct the existing data set. As Table 1 shows, out of the nine human PKC members, five isotypes - PKCα, β, δ, ζ and ι - had not been correctly assigned to individual human chromosomes in the existing literature [5,7-13]. A comparison of our chromosomal locations for PKC genes with the genome-draft assignments (HUGO), confirmed our results overall; however, our detailed FISH analysis provided enhanced mapping resolution.

In particular, the reported localization of the PKCι gene locus to the X chromosome (Xq21.3 [13] and HUGO, see Table 1) could not be confirmed, as we detected an additional FISH signal at 3q26. Once isolated from the human genome draft sequence, the Xq21.3 chromosomal DNA sequence harboring PKCι was revealed to be a processed pseudogene similar to the authentic gene at 3q26. This PKCι Xq21.3 pseudogene contains an uninterrupted open reading frame (ORF) and no introns, consistent with an origin through retrotransposition. Interestingly, it is identical to the PKCι gene sequence except for one point mutation at the stop codon (Figure 2), which elongates the ORF by 27 amino acids. The PKCι Xq21.3 pseudogene may therefore be expressed. Because of the extremely high identity of the two prospective PKCι mRNAs,

Table I

Critical chromosomal regions of the PKC gene module

PKC	HUGO	Literature	FISH	Mouse
alpha	17q22-q23.2	17q22-23.2	17q24	11
beta	16p12-p13.1	16p11.2	16p12	
gamma	19q13.2-13.4	19q13.4	19q13.4	7
delta	3p21	3p	3p21.2	14
epsilon	2p21	2p21	2p21	
zeta	1p36.2-36.33	1	1p36.3	
eta	14q	14q22-23	14q22-23	12
theta	10p15	10p15	10p15	2
iota	Xq21.3 (pseudo)	Xq21.3 (pseudo)	3q26	
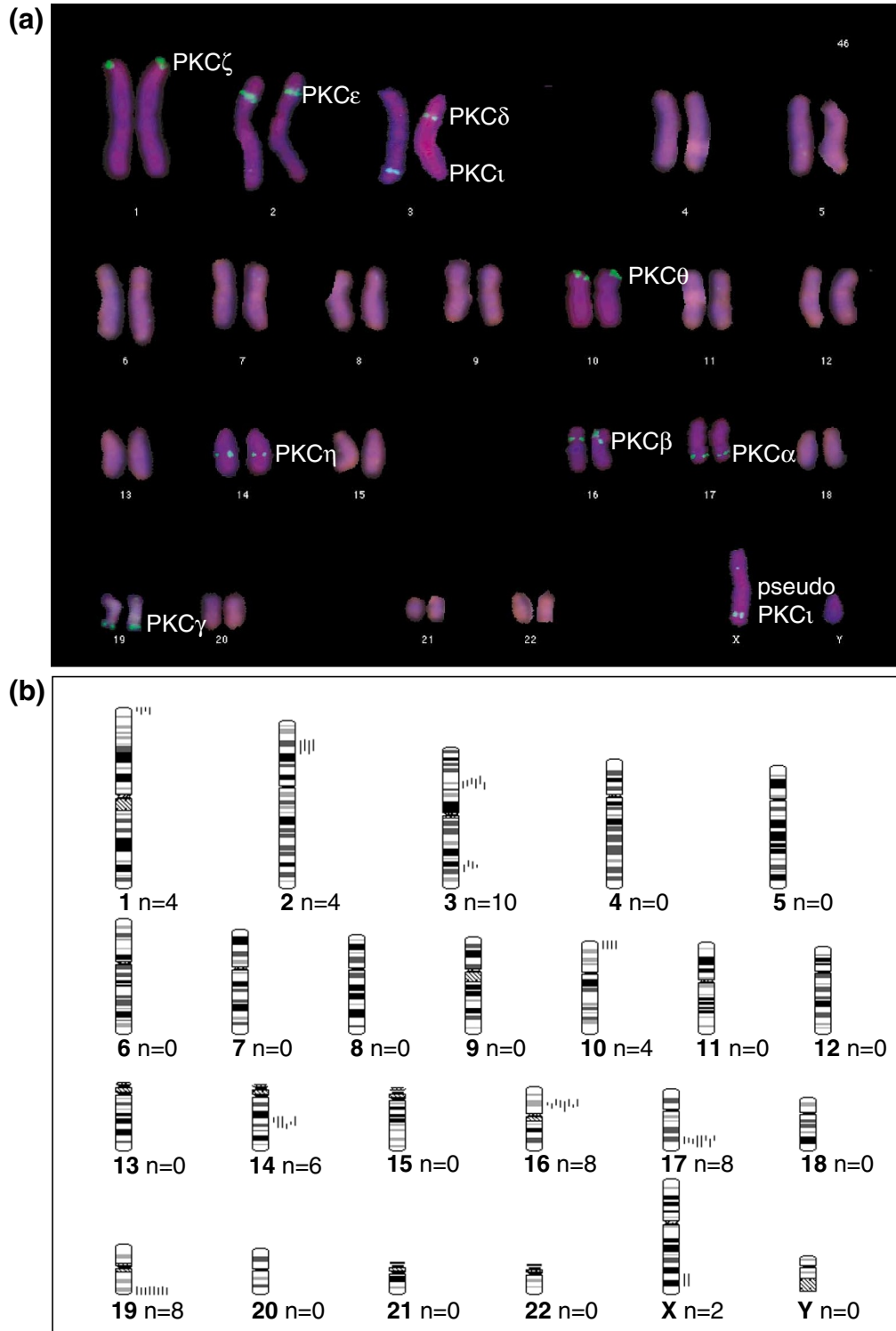
Figure 1

Chromosomal mapping of the nine human PKC gene loci by FISH to metaphase spreads. **(a)** Composite karyotype of the nine distinct PKC genes assigned by FISH (in green) to human metaphase spreads (DAPI/PI banding). The PKC gene product label indicates the assignment of the averaged fluorescence peak to the corresponding chromosome band. **(b)** Ideograms depicting the distribution of fluorescent spots (indicated by vertical bars at the side of the ideograms). Each mark represents the localization of one hybridization spot; n = number of metaphases scored.

PKCι Xq21.3

CCTAGGTTTTCTTCTAGGGTTTTCATGGTTTTAGATCTAACATGTAAGTCTTTAATCCATCT
TGAATTGATTTTTGTATAAGGTGTAAGGAAGGGATCCAGTTTCAGCTTTCTACATATGGCTA
GCCAGTTTTCCCAGCACCATTTATTAAATAGGGGATCCTTTCCCCATGCTTGTTTTTCTCA
GGTTTGTCAAGGATCAGATGGTTGTAGATGTGTAAAATTTACCACCTTTTTTCTTTACATTT
TTGAGGTTTGTGTCCTGCTTAGGGAGATCTGTATTTTCTTCTAATTTTTATGTTTCCTTTTA
TATTTAAATTTTTGGTCCATACGGAATTATTTTCTTCCAAATGGCTAGCAAATTGTCCTAAT
GCTAATTATCAAATAATCCCCTCTTTAGCTCACTGATTTAACGTGCCATCTTTGGGCTGCTT
GGGCGAGGCGACCCTTGGGTCGGCGTTGCGGGTGAGGTGGGCAGGTAGGTGGGCGGATGGCC
GCGGTTCTCCGACAGGCGCGGGCGGCGGGGTCTCCCACGGCGTCCGAAGCCCCCTCCCCGCA
CCCCCGGCCTCCAGCGTTGAGGCGGGGGAGTGAGGAG**ATGCCGACCCAGAGGGACAGCAGCA**
CCATGTCCCACAGGGTTGCAGGCGGCTGCAGTGGGGACCATTCCCACCAGGTCTGGGTGAAA
GCCTACTACCGCGGGGATATCATGATAACACATTTGAACCTTCTGTCTCCTTTGAGGGCCT
TTACAATGTGGTTCGAGACATGTTCTTTTGACAACGAACAGCTCTTCACCATGAAATGGATA
GACGAGGAAGGAGACCCGTGTACAGTATCATCTCAGTTGGAGTTAGAAGAAGCCTTTACACT
TTATGAGCTAAACAAGGACTCTGAACTCTTGATTCATGTGTTCCCTTGTGTACCAGAACGTC
CCGGGATGCCTTGTCCAAGAGAAGATAAATCCATCTACCGTAGAGGTGCACGCCACTGGAGA
AAGCTTTATTGTGCCAATGGACACACTTTTCAAGCCAAGTGCTTCAACAGGCGTGCTGACTG
TGCCATCTGCACAGACTGAATATGGGGACTTGGATGCCAAGGATATAAGTGTATCAACTGCA
AACTCTTGGTTCATAAGAAGTGCCATAAACTCCTCACAATTGAATGTGGGCGGCATTCTTTG
CCACTGGAACCAATGATGCCCATGGATCAGTCATCCATGCATTCTGACCATGCACAGACAGT
AATTCCATATAATCCTTCAAGTCATGAGAGTTTGGATCAAGTTGGTGAAGAAAAAGAGGCAA
TGAACACCAGAGAAAGTGGCAAAGCTTCATCCAGTCTAGGTCTTCAGGATTTTGATTTGCTC
CAGGTAATAGGAAGAGGAAGTTAAGCCAAAGTACTGTTGGTTCCATTAAAAAAAAAAAAAAA
ACAGATCGTATTTATGCAATGAAAGTTGTGAAAAAAGAGCTTGTTAATGATGATGAGGATAT
TGATTGGGTGCAGACAGAGAAACATGTGTTTGAGCAGGCATCCAATCATCCTTTCCTTGTTG
GGCTGCAGTCTTGCTTTCAGACAGAAAGCAAGTTGTTCTTTGTTTGTTATAGAGTATGTAAATGGA
GGAGACCTAATGTTTCATATGCAGCGACAAAGAAAACTTCCTGAAGAACATGCCAGATTTTA
CTCTGCAGAAATCAGTCTAGCATTCAATTATCTTCATGAGTGAGGGATAATTTATAGAGATT
TGAAACTGGACAATGTATTACTGGACTCTGAAGGCCACATTAAACTCACTGACTACGGCATG
TGTTAAGGAAGGATTACGGCCAGGAGATACAACCAGCACTTTCTGTGGTACTCCTAATTACA
TTGCTCCTGAAATTTTAAGAGGAGGAGATTATGGTTTCAGTGTTGACTGGTGGGCTCTTGGA
GTGCTAATGTTTGAGACGATGGCAGGAAGGACTCCATTTGATACTGTTGGGGAGCTCCGATAA
CCCTGACCAGAACACAGAGGATTCTCTCTTCCCAAGTTATTGTGGAAAAACAAATTCACATAC
CACGTTCTCTGTCTGGAAAAGCTGCAAGTGTTCTGAAGAGTTTTCTTAACAAGGACCCTAAG
AAACGACTGGGTTGTCATCCTCAAACAGGATTTGCTGGATCAGTCTGAATTTGAAGGTTTTGGAGTATAT
AAATGTTGATTGGGATATGATGGAGCAAAAACAGGTGGTATCTCCCTTTAAACCAAGTATTT
CTGGGGAATTTGGTTTGGACAACTTTGATTCTCAGTTTACTAATGAACCTGTCTAGCTCACT
CCAGATGTCGATGACATTGTGAGAAAGATTGATCAGTCTGAATTTGAAGGTTTTGAGTATAT
CAATCCTCTTTTGATGTCTGCAGAAGAATGTGTC**CGA****TCCTCATTTTTCAACCATGTATTCCT**
ACTCATGTTGCCATTTAATGCATGGATAAACTTGCTGCCAGCCTGGATACAATTAACCATTT
TATATTTGCCACCTACAAAAAAACACCCAGTATCTTCTCTCGTAGACTACATGAATCAATTA
TTACATCTTTTTTTACTATGAAAAATAAATTAATACTAGCTTCCAGACAATCATGTCAAAATT
TAGTTGAACTGGTTTTTCAGTTTTTAAAAGGCCTACAGATGAGTAATGAAGTTATCTTTTTT
GTTTAAAAAAAAAAACAAAGAAAACCACTGCATTT

Figure 2
Amino-acid sequence of the PKCι Xq21.3 pseudogene. The uninterrupted ORF is in red. The point mutation reverting the wild-type TGA stop codon to CGA is shown in blue.

'highly conserved gene duplication' is most probably the result of an *in silico* error in the assembly of the draft sequence, confirming the existence of errors in the existing sequence.

PKCγ, ζ and θ are found to map to the most distal parts of their chromosomes (PKCγ at 19q13.4, PKCζ at 1p36.3 and PKCθ at 10p15), suggesting that there might be a telomeric position effect modifying these genes' expression throughout the replicative lifespan of human cells. However, there is no experimental evidence on this at present.

Genomic organization of the PKC gene module
Using HUGO and bioinformatics tools, we have dissected the genomic organization, that is the characterization of the exon/intron structure, of the nine PKC isotype genes (Figure 3). The PKC loci range in size from approximately 24.4 kilobases (kb) (PKCγ) to 480 kb (PKCα), and are composed of 14 to 18 coding exons and 13 to 17 introns, varying in size from 94 to 188,435 base pairs (bp). The exons are small to intermediate in size, ranging from 32 to 381 nucleotides. All intron-exon junctions appear to comply with the GT-AG rule. Considering the common evolutionary origin of this gene family (as shown by a cDNA-based phylogenetic tree of PKC and its closest relative, PKD, Figure 3a), it is not surprising that some of their exon structures are identical (and nearly homologous in their amino-acid sequence) within the PKC subfamilies (Figure 3b). The AUG translation initiation site for the ORFs of PKCα, β, γ, ε, ζ and η is located in exon 1, for PKCδ, θ and ι one intron is located in the 5ʹuntranslated region (5ʹUTR) and only the subsequent exons determine the functional domains of the PKC proteins. Nevertheless, the existence of additional exons within the 5ʹUTR cannot be excluded at this point.

this possibility could only be investigated further by using an antibody designed to detect the additional 27 amino acid sequence of a putative pseudogene protein product, and such an antibody is not yet available.

In addition to the PKCι locus at chromosome 3q26 (and the Xq21.3 pseudogene) another PKCι genomic sequence is found in the draft human genome, which had been mapped (in the database) to chromosome 12 (BAC KlonRP11-147C2). An alignment of intron placement in the chromosome 12 and chromosome 3 PKCι genes indicated the highest possible homology in their genomic organization; for example, the chromosome 12 PKCι gene contains (as well as the 17 introns) 18 exons identical in size and sequence to those of the 3q26 PKCι gene identified by FISH (data not shown). As no FISH signal for a PKCι sequence could be observed on chromosome 12, this apparent

These structural genomic data can be used to represent the phylogenetic relationships of the PKC genes. The organization of the regulatory and catalytic subdomains of PKC has been remarkably preserved during evolution: using the exon structure of PKCα as a reference, exons 10-15 within the catalytic domain share the highest similarity in size, organization and primary structure among conventional (c)PKC and novel (n)PKCs, but not atypical (a)PKCs. aPKCζ and ι are very distinct from cPKC and nPKC within the exon structure of the catalytic domain. Along this line, aPKCι and aPKCs appear particularly conserved between each other in both their regulatory and catalytic domain structures. Within the regulatory domain, the subfamilies cPKC, nPKC and aPKC are clearly distinct from each other, and there is even a split within the nPKCs into the D-forms, PKCδ and θ, and the E-forms, PKCε and η. Also, cPKCα and cPKCβ appear more closely related

Figure 3 *(see the figure on the next page)*
The evolutionary relationships and structures of the nine distinct human PKC genes. **(a)** A cDNA-based phylogenetic tree of PKC isotypes and their closest relative, PKD. **(b)** Evolutionary comparison between the PKC genomic gene sequences revealed subfamily-specific genomic organization. Within the subfamilies (grouped in rows), the color-coding indicates different collections of conserved exon sizes between loci.

(a)

```
                              PKCα
                          ┌── PKCγ
                          └── PKCβ
                              PKCε
                          ┌── PKCζ
                          └── PKCδ
              PKC             PKCη
                          ┌── PKCι
                          └── PKCθ
              PKD             PKDν
                          └── PKDμ
```

(b)

PKCα

Exons (in bp)		Introns (in bp)	
1	199	1	3071
2	**32**	2	188435
3	**83**	3	123722
4	**112**	4	3916
5	*129*	5	41599
6	**157**	6	1034
7	**135**	7	514
8	*97*	8	44976
9	138	9	2663
10	*174*	10	3101
11	**92**	11	2803
12	**63**	12	900
13	**139**	13	31226
14	**81**	14	12799
15	**108**	15	1864
16	153	16	14893
17	364		

Gene : ~ 480000 bp

PKCγ

Exons (in bp)		Introns (in bp)	
1	309	1	1029
2	**32**	2	150576
3	**83**	3	40548
4	**112**	4	3173
5	*129*	5	16152
6	**157**	6	1211
7	**135**	7	18772
8	*97*	8	10765
9	147	9	30702
10	*174*	10	17424
11	**92**	11	2156
12	**63**	12	6209
13	**139**	13	4182
14	**81**	14	268
15	**108**	15	5522
16	*141*	16	28712
17	577		

Gene : ~ 340000 bp

PKCβ

Exons (in bp)		Introns (in bp)	
1	365	1	498
2	**32**	2	966
3	**83**	3	5387
4	**112**	4	136
5	132	5	1656
6	**157**	6	678
7	**135**	7	344
8	88	8	286
9	30	9	4563
10	153	10	328
11	189	11	1605
12	**92**	12	94
13	**63**	13	129
14	**139**	14	2323
15	**81**	15	1481
16	**108**	16	1573
17	*141*	17	249
18	406		

Gene : ~ 24400 bp

PKCδ

Exons (in bp)		Introns (in bp)	
1	39	1	16975
2	134	2	1039
3	**200**	3	1430
4	**61**	4	176
5	**163**	5	94
6	**32**	6	1388
7	**86**	7	245
8	**130**	8	1292
9	101	9	631
10	97	10	226
11	101	11	139
12	*174*	12	263
13	**92**	13	644
14	**63**	14	1317
15	**139**	15	199
16	**189**	16	626
17	**129**	17	2106
18	174		

Gene : 30894 bp

PKCη

Exons (in bp)		Introns (in bp)	
1	85	1	65066
2	127	2	3821
3	**200**	3	3498
4	**61**	4	8871
5	**163**	5	1144
6	**32**	6	99
7	**86**	7	5213
8	**130**	8	5540
9	110	9	765
10	118	10	1551
11	161	11	4274
12	*174*	12	14587
13	**92**	13	1947
14	**63**	14	5490
15	**139**	15	14592
16	**189**	16	10954
17	**129**	17	2447
18	156		

Gene : ~ 152000 bp

PKCε

Exons (in bp)		Introns (in bp)	
1	363	1	>148000
2	*64*	2	>133000
3	160	3	2387
4	*35*	4	1285
5	86	5	4169
6	*130*	6	16723
7	143	7	2995
8	97	8	??
9	200	9	??
10	*174*	10	63746
11	*155*	11	58730
12	*139*	12	5809
13	*189*	13	8376
14	147	14	24982
15	162		

Gene : > 472500 bp

PKCζ

Exons (in bp)		Introns (in bp)	
1	403	1	68785
2	*64*	2	51823
3	151	3	2430
4	*35*	4	3437
5	89	5	1589
6	*130*	6	2254
7	128	7	3821
8	144	8	186
9	*174*	9	27818
10	*155*	10	43418
11	*139*	11	1193
12	*189*	12	17147
13	144	13	1798
14	321		

Gene : ~ 228000 bp

PKCι

Exons (in bp)		Introns (in bp)	
1	71	1	??
2	**122**	2	922
3	**90**	3	2966
4	**51**	4	77354
5	**86**	5	8817
6	132	6	1685
7	82	7	3271
8	53	8	1865
9	189	9	5016
10	**98**	10	13433
11	**87**	11	2345
12	**136**	12	110
13	**88**	13	1508
14	120	14	737
15	**80**	15	390
16	**90**	16	9183
17	**116**	17	223
18	474		

Gene : > 472500 bp

PKCθ

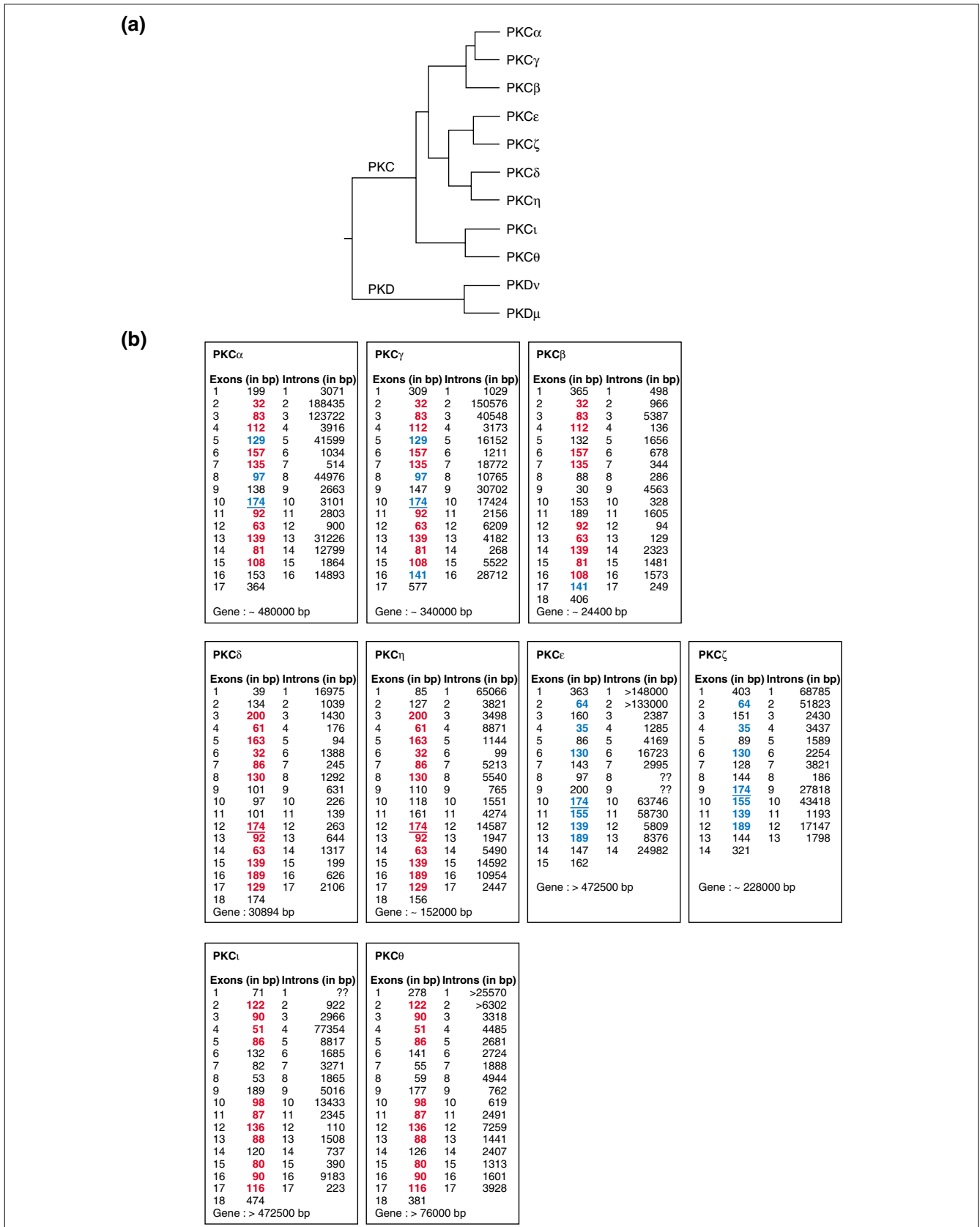| Exons (in bp)		Introns (in bp)	
1	278	1	>25570
2	**122**	2	>6302
3	**90**	3	3318
4	**51**	4	4485
5	**86**	5	2681
6	141	6	2724
7	55	7	1888
8	59	8	4944
9	177	9	762
10	**98**	10	619
11	**87**	11	2491
12	**136**	12	7259
13	**88**	13	1441
14	126	14	2407
15	**80**	15	1313
16	**90**	16	1601
17	**116**	17	3928
18	381		

Gene : > 76000 bp

Figure 3 *(see legend on the previous page)*

to each other than to cPKCγ. Overall, the data suggest that gene duplication followed by intron loss and/or insertion and exon shuffling have been important in the evolution of the PKC gene family. This careful *in silico* dissection of the gene fine structure is also a prerequisite to an efficient search for potential abnormalities in the PKC genes.

Alternative processing of PKC gene transcripts

The existence of alternative splice variants of PKC within certain tissues is an attractive option for providing further heterogeneity in PKC, reflecting the diverse pathways of signal transduction within these cells.

Alternative splicing is currently known for at least one human PKC isotype. Two distinct PKCβ cDNA clones have been isolated that encode sequences of 671 and 673 amino acids, respectively, which differ from each other only in the carboxy-terminal regions of approximately 50 amino acids [14,15]. Characterization of the PKCβ chromosomal gene provided direct evidence of the existence of two adjacent carboxy-terminal exons that could be alternatively spliced to generate two types of PKCβ [16]. Importantly, these splice variants, PKCβI and βII, appear to be expressed in a tissue-selective manner, suggesting that their functions are different.

PKC splicing variants are known in rodents

A mouse variant of PKCδ - PKCδII - has recently been described [17]. This has a 78 bp (26 amino acid) insertion into the caspase-3-sensitive site in the V3 domain of the original PKCδI sequence, which renders the PKCδII isoform insensitive to caspase-3. PKCδI is detected in most tissues, whereas PKCδII was expressed selectively in the testis, ovary, thymocytes, brain and kidney [18].

In addition, a carboxy-terminal truncated form of PKCδIII exists in rats, which has an 83 bp out-of-frame insertion at the same site in the V3 region [18]. Genomic DNA analysis revealed that the difference between mouse PKCδII and rat PKCδIII is due to a different sequence at the critical 5vdonor splice sites (data not shown). PKCδIII, which represents just the regulatory domain, might show a dominant-negative effect against PKCδI, and thus alternative splicing involving this variant might modulate signaling pathways.

Two forms of rat PKCζ RNA, with different 5v ends, have been reported. The major form (PKCζ wild type) is expressed ubiquitously, whereas the smaller form - protein kinase M ζ (PKMζ) - which encodes just the catalytic domain of the enzyme without most of its regulatory domain, is predominant in normal brain and certain rat prostate tumors. The rat PKCζ gene locus appears to contain two alternative promoters from which it can be transcribed to give two transcripts with 5v-end heterogeneity [19].

Finally, a PKCθII cDNA clone has been isolated from mouse testis, encoding a unique 5vsequence of 20 amino acids and the PKCθI (= wild type) sequence of 444 amino acids. The transcription of PKCθII RNA is initiated from the PKCθII-specific exon, which is located between exons 7 and 8 of the PKCθI gene, indicating that alternative splicing is the mechanism by which PKCθII is generated. PKCθII is expressed exclusively in the testis in an age-dependent manner with sexual maturation. Consistent with its lack of a C1 regulatory domain, PKCθII is constitutively active and may have a crucial role in spermatogenesis [20].

The determination of single-nucleotide polymorphisms within the human PKC gene module

The authentic PKC gene loci present in a single copy in the human genome have been analyzed in detail for single-nucleotide polymorphisms (SNPs) using resources from the National Center for Biotechnology Information website [21]. Among the 11 SNPs within the coding regions of the PKC gene module currently known from these databank searches, only two (one each in PKCδ and PKCη, respectively) give rise to non-synonymous codons and create missense mutations (Table 2). These missense mutations do not exert an effect on kinase function in any obvious way, even though an effect cannot be ruled out at this time. Overall, the human PKC genes appear not to be highly variable regions as characterized by SNP analysis; however, the available exonic SNPs appear sufficient to be used for haplotyping purposes in linkage and/or association studies.

Chromosomal location of fine-mapped PKC gene loci with human disease loci

Using the Cancer Genome Anatomy Project [22], a comparison of the chromosomal location of these nine fine-mapped PKC loci with human disease loci revealed chromosomal aberrations/breakpoints in these regions in, for example, patients with malignant disease (Figure 4). In particular, the locations 17q24 (PKCα), 19q13 (PKCγ), 3p21 (PKCδ), 2p21 (PKCε), 1p36 (PKCζ) and 3q26 (PKCι) are noteworthy, as deletions or balanced translocations involving these defined chromosomal regions are frequently described in naturally occurring malignancies. Thus, in theory, PKC-family genes could be affected as possible disease candidate genes in accidental recombinations during intrachromosomal rearrangement or even interchromosomal translocations, as in the deleterious joining of *abl* sequences to the immunoglobulin or *bcr* loci that leads to malignancy (via gain-of-function). However, many other genes involved in the regulation of cell activation, proliferation and apoptosis are also included in these chromosomal regions. Nevertheless, our mapping results suggest that some PKC isotypes could be candidate genes involved in human cancer.

Members of the PKC family have been consistently implicated in aberrant signaling responses contributing to malignant transformation, for example, as intracellular receptors for tumor-promoting phorbol esters, which have been shown to protect various cells, including T cells, from apoptosis [23,24]. As a result of this potentially transforming capacity of PKC-

Table 2

SNPs of the PKC gene module

| PKC | cDNA | | Protein | | | RefSNP |
	Position	GenBank accession	Position	Protein accession	Codon position	Cluster ID(rs#)
beta	C 2167 T (3ᵥUTR)	X06318.1		Untranslated region		rs424741
delta	C 1182 T (exon 12)	L07860.1	S 375 F	NP_006245.1	2	rs1056998
	A 445 G (exon 5)	L07860.1	Q 129 Q	NP_006245.1	3	rs1135095
epsilon	T 1587 C (exon 11)	X65293.1	H 524 H	NP_005391.1	3	rs1143691
	C 1776 T (exon 12)	X65293.1	S 587 S	NP_005391.1	3	rs1143692
eta	G 678 A (exon 5)	M55284.1	R 172 H	NP_006246.1	2	rs1042559
	A 1837 G (exon 13)	M55284.1	N 558 N	NP_006246.1	3	rs1088680
	G 2285 C (3ᵥUTR)	M55284.1		Untranslated region		rs1139537
theta	T 2206 A (3ᵥUTR)	L07032.1		Untranslated region		rs1065081
	T 2554 C (3ᵥUTR)	L07032.1		Untranslated region		rs663532
	C 2636 T (3ᵥUTR)	L07032.1		Untranslated region		rs2453

Data retrieved from [21].

family genes, the aberrantly high expression levels of distinct PKC isotypes found in most tumor cell lines argue for a functional link between PKC and oncogenesis (G.B., unpublished work). Chronic recruitment of PKCθ (by an undefined mechanism) into the membrane fraction of malignant cells has been reported in cell lines derived from patients with T-cell leukemia [25]. Other, more definitive, results show that both Bcr-Abl and PKCι activity are necessary for resistance to apoptosis in hematopoietic K562 cells, supporting a functional role for PKCι in the survival of leukemia cells [26]. Recent studies, including our own [23,27], have directly linked distinct PKC isotypes to molecular pathways regulating apoptosis. But in spite of the putative role of PKCs in cellular growth control and differentiation, no clinical example of a causative role of PKCs in primary cell malignancy has yet been published. It remains to be seen whether the genetic dissection of naturally occurring malignant somatic cell mutants will eventually establish a link between PKCs and clinical disease.

A directed search for potential loss-of-function (and most likely recessive) mutations in the PKC loci, potentially associated with distinct genetic syndromes, will now be initiated. Along with information from biochemical work on signal transduction, tissue-specific expression patterns and phenotypes of the currently established mouse loss-of-function PKC isotype knockout lines ([28-33] and G.B., unpublished work), the genomic fine mapping reported here will enable genetic studies in defined groups of patients to search for functional PKC polymorphisms or mutations associated with familial genetic defects or abnormalities. Given the enormous increase in genetic and molecular databases, bioinformatics approaches should continue to improve and develop into useful tools for evaluating hypotheses, with only the very promising ones being subjected to empirical testing. This human genetic,

and therefore long-term, approach may aid in delineating the basic physiological and possible pathophysiological functions of the PKC gene module, and may illuminate whether and eventually how PKCs are involved in human genetic disease.

Materials and methods

Cosmid and BAC clones were isolated from human single-chromosome libraries from the Human Genome Mapping Project (HGMP) Resource Center (Los Alamos National Laboratory and the Laurence Livermore National Laboratory) as described in [34,35]. FISH analysis was carried out essentially as described in [5,36]. Comparative genomic hybridization (CGH) analysis software (MacProbe 3.3) was used to statistically determine the chromosomal localization of the hybridized probe. Long-range PCR was carried out using the DNA polymerase Elongase™ as described by the manufacturer (Gibco/BRL, Gaithersburg, USA).

FISH and detection by immunofluorescence was carried out on metaphase chromosome preparations from phytohemagglutinin-stimulated human lymphocytes from healthy probands. For each slide (area 18 x 18 mm), a total of 200 ng biotinylated DNA probe. Post-hybridization washes were carried out to a stringency of 0.1x SSC at 45°C. The biotinylated probe was detected using FITC-avidin conjugates (Vector Laboratories, Burlingame, USA) and one round of signal amplification. The data were recorded as a digital fluorescence image and processed by the MacProbe 3.3 software (PSI, London, UK) on a Leitz Aristoplan microscope equipped with integrating color CCD camera (Xbyion Electronic Systems, Cedar Knolls, USA). For each probe a minimum of 20 metaphases were analyzed by recording fluorescent spots on chromosomes and comparing them with images of DAPI-banded chromosomes.

PKCα

ATG

Expression information from cDNA sources:
Brain, colon, eye, kidney, liver, ovary, parathyroid, tonsil, uterus, colon_ins, head_neck, ovary, uterus_tumor

Pituitary tumor, invasive [PRKCA, SP294GLY]
In four invasive pituitary tumors, Alvaro *et al.* [38] found a substitution of a negatively charged aspartic acid by anapolar glycine.

Balanced chromosomal abnormalities		
Band	**Abnormality**	**Morphology**
17q22	t(15;17)(q22;q22)	Acute promyelocytic leukemia (FAB type M3)
17q22	t(15;17)(q26;q22)	Acute promyelocytic leukemia (FAB type M3)
17q22	t(17;19)(q22;p13)	Acute lymphoblastic leukemia, NOS , FAB type L1
17q22	t(3;17)(q26;q22)	Chronic myeloid leukemia, t(9;22)
17q23	t(9;17)(q34;q23)	Lymphoblastic lymphoma

PKCγ

ATG

Expression information from cDNA sources:
Blood, bone, brain, CNS, colon, eye, foreskin, germ cell, heart, kidney, lung, lymph, prostate, spleen, tonsil, brain, breast, lung, marrow, nervous normal pnet, skin, uterus, uterus tumor

Balanced chromosomal abnormalities		
Band	**Abnormality**	**Morphology**
16p11	t(16;21)(p11;q22)	Acute myeloid leukemia, NOS
		Acute myeloblastic leukemia without maturation (FAB type M1, M2, M4, M5a, M5b, M7)

PKCβ

ATG

Expression information from cDNA sources:
Brain, stomach

Mutations:
Single nucleotide substitutions (Ser 659 Arg; CGC-AGC, Exon 18); [39]

Phenotype: Retinitis pigmentosa

Balanced chromosomal abnormalities			
Band	**Abnormality**	**Morphology**	**Site**
19q13	inv(19)(p13q13)	Astrocytoma, grade III-IV	Brain
19q13	inv(19)(p13q13)	Hairy cell leukemia	
19q13	t(10;19)(q24;q13)	Adenoma	Thyroid
19q13	t(11;19)(q13;q13)	Benign mesenchymal tumor, special type	Liver
19q13	t(11;19)(q23;q13)	Acute myelomonocytic leukemia (FAB type M4)	
19q13	t(12;19)(q13;q13)	Acute myeloblastic leukemia with maturation (FAB type M2)	
19q13	t(14;19)(q32;q13)	Chronic lymphocytic leukemia	
19q13	t(14;19)(q32;q13)	Follicular lymphoma	
19q13	t(14;19)(q32;q13)	Peripheral B-cell neoplasm, NOS	
19q13	t(19;22)(q13;q11)	Chronic myeloid leukemia, aberrant translocation	
19q13	t(19;22)(q13;q11)	Diffuse large B-cell lymphoma	
19q13	t(1;19)(q21;q13)	Acute lymphoblastic leukemia, NOS, FAB type L1	
19q13	t(2;19)(p13;q13)	Epithelial disorder	Thyroid
19q13	t(5;19)(q13;q13)	Adenoma	Thyroid
19q13	t(6;19)(p22;q13)	Chronic myeloid leukemia, t(9;22)	
19q13	t(8;19)(q22;q13)	Acute myeloblastic leukemia with maturation (FAB type M2)	
19q13	t(9;22;19)(q34;q11;q13)	Chronic myeloid leukemia, aberrant translocation	

PKCδ

ATG

Expression information from cDNA sources:

Blood, brain, breast, colon, germ cell, heart, kidney, lung, lymph, ovary, parathyroid, pooled,prostate, testis, tonsil, uterus, whole embryo

Balanced chromosomal abnormalities			
Band	**Abnormality**	**Morphology**	**Site**
3p21	t(1;3)(p34;p21)	Acute lymphoblastic leukemia, FAB type L1	
3p21	t(3;10)(p21;p13)	Diffuse large B-cell lymphoma	
3p21	t(3;12)(p21;p13)	Peripheral B-cell neoplasm, NOS	
3p21	t(3;14)(p21;q32)	Diffuse large B-cell lymphoma	
3p21	t(3;22)(p21;q11)	Chronic myeloid leukemia, aberrant translocation	
3p21	t(3;3)(p21;q27)	Follicular lymphoma	
3p21	t(3;5)(p21;p15)	Adenoma	Salivary gland
3p21	t(3;7)(p21;q35)	Acute myelomonocytic leukemia (FAB type M4)	
3p21	t(3;8)(p21;q12)	Adenoma	Salivary gland
3p21	t(3;9;22)(p21;q34;q11)	Chronic myeloid leukemia, aberrant translocation	

PKCε

ATG

Expression information from cDNA sources:
Blood, brain, kidney, ovary, head_neck, muscle, placenta_normal, tongue

Balanced chromosomal abnormalities			
Band	**Abnormality**	**Morphology**	**Site**
2p21	t(2;11)(p21;q23)	Acute myeloid leukemia, NOS ; FAB type M4	
2p21	t(2;11)(p21;q23)	Refractory anemia	
2p21	t(2;12)(p21;p13)	Acute lymphoblastic leukemia, NOS	
2p21	t(2;16)(p21;q24)	Adenocarcinoma	Ovary
2p21	t(2;9;22)(p21;q34;q11)	Chronic myeloid leukemia, aberrant translocation	

Figure 4 *(see the second part of the figure and the legend on the next page)*

PKCι

ATG

Expression information from cDNA sources:
B-cells, blood, brain, breast, CNS, colon, eye, fetal brain, germ cell, heart, kidney, lung, lymph, neural, ovary, parathyroid, placenta, pooled, prostate, testis, tonsil, uterus, whole embryo

Balanced chromosomal abnormalities

Band	Abnormality	Morphology	Site
1p36	inv(1)(p22p36)	Adenocarcinoma	Breast
1p36	t(1;1)(p36;q25)	Diffuse large B-cell lymphoma	
1p36	t(1;13)(p36;q14)x2	Rhabdomyosarcoma	Soft tissue
1p36	t(1;17)(p36;q21)	Acute promyelocytic leukemia (FAB type M3)	
1p36	t(1;18)(p36;p11)	Diffuse large B-cell lymphoma	
1p36	t(1;2)(p36;p24)	Leiomyoma	Uterus, corpus
1p36	t(1;3)(p36;q21)	Acute myeloid leukemia, NOS	
1p36	t(1;3)(p36;q21)	Acute promyelocytic leukemia (FAB type M3, M4, M6)	
1p36	t(1;3)(p36;q21)	Chronic myelomonocytic leukemia	
1p36	t(1;3)(p36;q21)	Refractory anemia with excess of blasts	
1p36	t(1;7)(p36;q32)	Refractory anemia with excess of blasts	
1p36	t(1;7)(p36;q34)	Acute myeloblastic leukemia with maturation (FAB type M2)	
1p36	t(1;9;22)(p36;q34;q11)	Chronic myeloid leukemia, aberrant translocation	

PKCζ

ATG

Expression information from cDNA sources:
Blood, breast, CNS, colon, ear, eye, foreskin, germ cell, heart, kidney, lung, lymph, placenta, pooled, prostate, stomach, testis, tonsil, uterus, whole embryo, bladder, breast,cervix, colon, connective tissue, head neck, leiomios, lung, lung tumor, nervous tumor

Balanced chromosomal abnormalities

Band	Abnormality	Morphology
14q22	t(5;14)(q35;q22)	Chronic myeloid leukemia, t(9;22)

PKCη

ATG

Expression information from cDNA sources:
Brain, colon, germ cell, heart, lung, lymph, muscle, prostate, testis, thyroid, connective tissue, ovary

Balanced chromosomal abnormalities

Band	Abnormality	Morphology
10p15	t(10;11)(p15;q23)	Acute monoblastic leukemia (FAB type M5)

PKCθ

ATG

Expression information from cDNA sources:
Bladder, blood, bone, bone marrow, brain, breast, cervix, denis_drash, eye, germ cell, heart, kidney, lung, ovary, placenta, pool, prostate, small intestine, spleen, testis, cell line, tonsil

Balanced chromosomal abnormalities

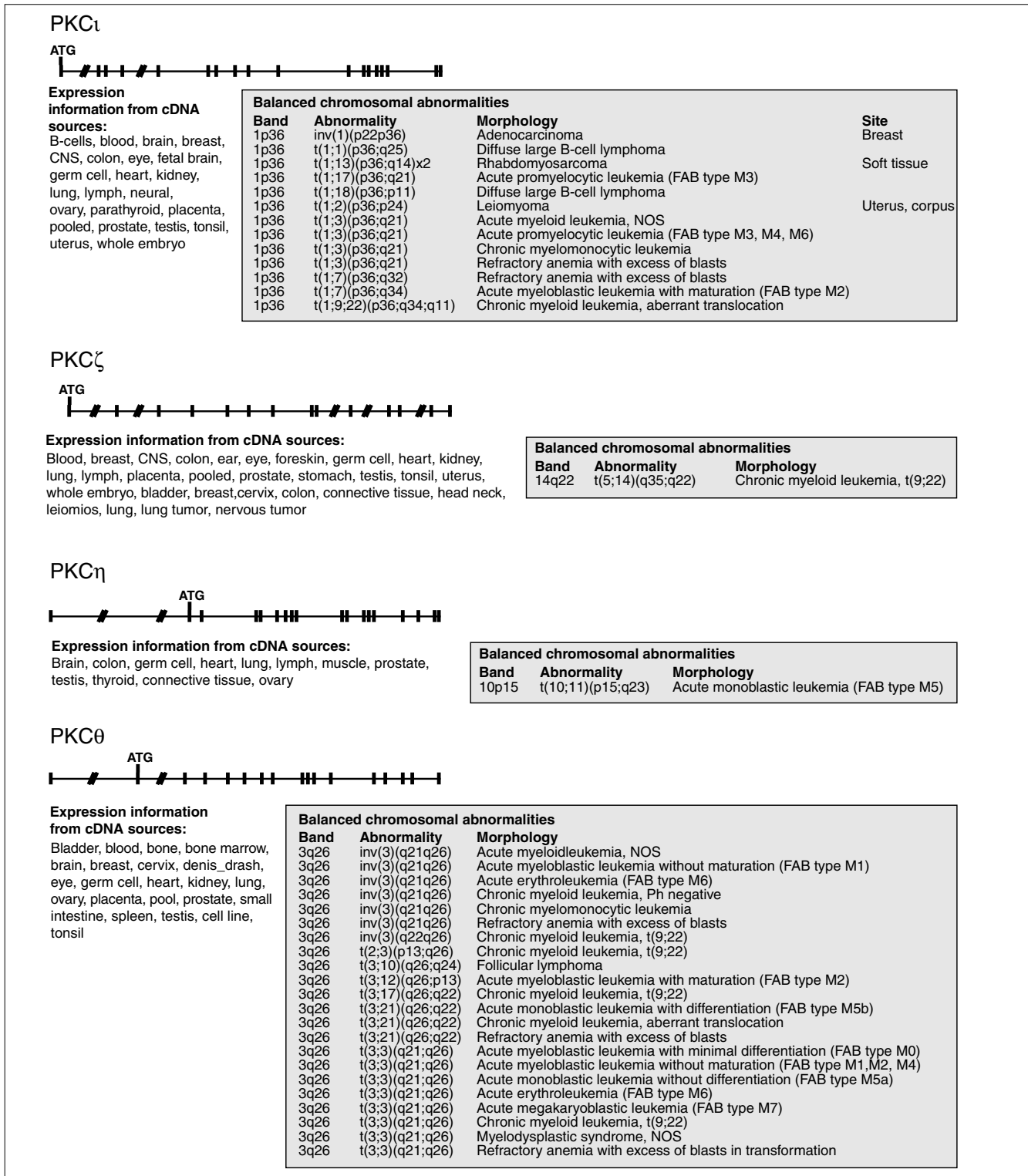| Band	Abnormality	Morphology
3q26	inv(3)(q21q26)	Acute myeloidleukemia, NOS
3q26	inv(3)(q21q26)	Acute myeloblastic leukemia without maturation (FAB type M1)
3q26	inv(3)(q21q26)	Acute erythroleukemia (FAB type M6)
3q26	inv(3)(q21q26)	Chronic myeloid leukemia, Ph negative
3q26	inv(3)(q21q26)	Chronic myelomonocytic leukemia
3q26	inv(3)(q21q26)	Refractory anemia with excess of blasts
3q26	inv(3)(q22q26)	Chronic myeloid leukemia, t(9;22)
3q26	t(2;3)(p13;q26)	Chronic myeloid leukemia, t(9;22)
3q26	t(3;10)(q26;q24)	Follicular lymphoma
3q26	t(3;12)(q26;p13)	Acute myeloblastic leukemia with maturation (FAB type M2)
3q26	t(3;17)(q26;q22)	Chronic myeloid leukemia, t(9;22)
3q26	t(3;21)(q26;q22)	Acute monoblastic leukemia with differentiation (FAB type M5b)
3q26	t(3;21)(q26;q22)	Chronic myeloid leukemia, aberrant translocation
3q26	t(3;21)(q26;q22)	Refractory anemia with excess of blasts
3q26	t(3;3)(q21;q26)	Acute myeloblastic leukemia with minimal differentiation (FAB type M0)
3q26	t(3;3)(q21;q26)	Acute myeloblastic leukemia without maturation (FAB type M1,M2, M4)
3q26	t(3;3)(q21;q26)	Acute monoblastic leukemia without differentiation (FAB type M5a)
3q26	t(3;3)(q21;q26)	Acute erythroleukemia (FAB type M6)
3q26	t(3;3)(q21;q26)	Acute megakaryoblastic leukemia (FAB type M7)
3q26	t(3;3)(q21;q26)	Chronic myeloid leukemia, t(9;22)
3q26	t(3;3)(q21;q26)	Myelodysplastic syndrome, NOS
3q26	t(3;3)(q21;q26)	Refractory anemia with excess of blasts in transformation

Figure 4 *(continued from the previous page)*

Comparison of the human PKC gene loci with chromosomal localization of balanced aberrations in tumors using data from the Cancer Genome Anatomy Project [37]. In addition to the chromosomal abnormality data listed to the right of each gene locus structure diagram, information on the tissue expression of the gene (from expressed sequence tag (EST) databases) and on known human mutations [38,39] is given. Several of the PKC loci are noteworthy, as deletions or translocation events in this chromosomal region are described in the breakpoint map of recurrent chromosomal rearrangements in human neoplasia [22,37]. Cytogenetic studies on recurrent balanced chromosomal aberrations in all hematological malignancies and solid tumors published are collected in [37].

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