Kidney International, Vol. 64 (2003), pp. 788–792

GENETIC DISORDERS–DEVELOPMENT

Refinement of the critical region for MCKD1 by detection of transcontinental haplotype sharing

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Background. Autosomal-dominant medullary cystic kidney disease type 1 (MCKD1) [OMIM 174000] is a hereditary nephropathy that leads to renal salt wasting and end-stage renal failure at a median age of 62 years. In a Welsh MCKD1 kindred we have recently demonstrated linkage to the *MCKD1* locus on chromosome 1q23.1 and refined the critical *MCKD1* region to <3.3 Mb.

Methods. In order to refine the candidate gene region for *MCKD1*, high-resolution haplotype analysis in three large kindreds with MCKD1 was performed.

Results. We report here on high-resolution haplotype analysis in this Welsh kindred, as well as in the Arizona kindred, which was used for the first definition of MCKD as a disease entity, and in a kindred from the Dutch/German border. We detected extensive haplotype sharing among all affected individuals of all three kindreds. Scrutinization of the genealogy of the Arizona kindred revealed an origin from Germany in the 17th century, thereby providing historical data for haplotype sharing by descent at the *MCKD1* locus.

Conclusion. Under the hypothesis of haplotype sharing by descent, we refined the critical genetic interval to <650 kb, thus enabling candidate gene analysis.

Medullary cystic kidney disease type 1 (MCKD1) (OMIM 174000) was first defined as a disease entity in an extensive 12-branch pedigree from Arizona, the founder of which lived in the early 1800s [1, 2]. MCKD1 shares the histologic features of renal tubular basement membrane disruption, corticomedullary cysts, and renal fibrosis, with other disease entities of the so-called "nephronophthisis-MCKD complex" [3, 4]. MCKD1 is therefore considered a model disease of renal fibrosis, which constitutes a critical feature of chronic renal insufficiency of all origins [5]. In a large Cypriot kindred, a gene for MCKD1 was localized to chromosome 1q by linkage analysis and restricted to an 8 cM interval [6, 7]. In a Welsh kindred we have recently demonstrated linkage to this *MCKD1* locus on 1q23.1, and refined to <3.3 Mb the critical *MCKD1* region and cloned this region in a combined yeast artificial chromosome (YAC) and P1-related artifical chromosome (PAC) contig [8]. Here, we report on extensive haplotype sharing in the Arizona kindred used in the first definition of MCKD [1, 2], in the Welsh kindred used for refinement of *MCKD1*, and in the German kindred. The hypothesis of haplotype sharing by descent was confirmed independently by historical data on the genealogy. Under this hypothesis we restricted the critical genetic interval to <650 kb.

The rhesus blood group B glycoprotein gene (*RhBG*) had been proposed as a candidate gene for MCKD1 [9]. Since the *RhBG* was localized within the newly refined critical *MCKD1* region, we performed mutational analysis of its 10 exons, not finding any evidence that this is the responsible gene for MCKD1.

METHODS

Patients

We studied three extended families originating from Arizona/USA (F284), Wales/United Kingdom (F327), and the border between The Netherlands and Germany (F629). Clinical inclusion criteria for all three kindred were as published for the Arizona MCKD kindred and the Welsh MCKD1 kindred [8]. Genomic DNA was isolated by standard methods directly from blood samples or from blood lymphocytes after Epstein-Barr virus (EBV) transformation. The study was approved by the ethics committee of the Albert-Ludwigs-University Freiburg.

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Key words: MCKD1, haplotype sharing, RhBG.

Received for publication February 19, 2003
and in revised form March 12, 2003
Accepted for publication April 22, 2003

Haplotype analysis

Haplotype analysis was performed in 25 individuals (including 12 affected individuals), and inferred in 11 additional individuals (7 additional affected individuals), using 25 consecutive polymorphic microsatellite markers that span the critical *MCKD1* region in the following order: cen - D1S514 - D1S498 - D1S137P24e* - D1S305 - D1S98F1a* - D1S21N7c* - D1S29H23g* - D1S29H23e* - D1S29H23c* - D1S1595/S2140 - D1S243J18b* - D1S303 - D1S243J18c* - D1S336K24g* - D1S336K24f *- D1S33 6K24c* - D1S336K24h* - D1S336K24a* - D1S2624 - D1S284F21e* - D1S356J7e* - D1S85G21d* - D1S394 - D1S2125 - D1S2635 - tel. Marked above with asterisks are 16 novel polymorphic markers that were created by searching for microsatellite markers using a list of di-, tri-, and tetranucleotide repeats in a Basic Local Alignment Search Tool (BLAST) search [10] against the sequence of the minimal contig of the critical MCKD1 region [8, 11, 12]. Allele frequencies of the 9 polymorphic markers shared as haplotypes were tested in at least 58 control chromosomes. To display haplotype analysis, Cyrillic version 2.13 (Cherwell Scientific, Oxford, UK) was used. Only affected family members were evaluated for haplotype sharing due to the age-dependent penetrance of MCKD [8].

Mutational analysis of the candidate gene Rhesus blood group B glycoprotein

Mutational analysis was performed in the candidate gene Rhesus blood group B glycoprotein (*RhBG*). The following primers and conditions were employed: exon 1: 5′-TCCGTGAAACCTGCCCTGC-3′ and 5′-ATGG TCCCTCCCAAGACACC-3′; exon 2: 5′-TTTATAGG CTCGGCTCAAGG-3′ and 5′-TTGAAGGACAGGG CTAGATC-3′; exon 3: 5′-TGCTGTCCTGGCTTCAT GCC-3′ and 5′-ACTCCATCCCTCCCCAGAC-3′; exon 4: 5′-CTGCCTCTCACCCCACCTC-3′ and 5′-CACCC CGAGAGACAGTCACC-3′; exon 5: 5′-GGTAGGT GATTTGCCTGAAG-3′ and 5′-CTTTCTTGAGGGT CTCAGAG-3′; exon 6: 5′-AGAAGCAGTAGGTGT CACTG-3′ and 5′-ACCACCTCATCCTGTTGGAG-3′; exon 7: 5′-AACATGGAGTCTTTGGTACC-3′ and 5′-TGCTCCCAGAATATGGATG-3′; exon 8: 5′-TGT GAGTTCCAGTGCCATG-3′ and 5′-TGGCACAAA GTAGATGCTCC-3′; exon 9: 5′-TGGTCTTATGCCT CCTAGAC-3′ and 5′-AGGAGCAGCAAGGAAGG AAG-3′; exon 10: 5′-TTGCTGCTCCTTCTCCTCTG-3′ and 5′-TCTTGCAGCTGGAGGAATGG-3′; at annealing temperatures of 58°C (exons 5 and 7), 60°C (exons 2, 6, 8, and 10), 62°C (exons 3 and 9), and 66°C (exons 1 and 4). To examine the *RhBG* 1265-1271insC polymorphism, direct sequencing of exon 9 was performed. To detect the G227A (Gly76Asp) polymorphism, allele-specific polymerase chain reaction (PCR) was performed in 20 control chromosomes (primers: 5′-ATGGTCTTCG TGGGCTTTGG-3′ or 5′-ATGGTCTTCGTGGGCTT TGA-3′ and 5′-TTGAAGGACAGGGCTAGATC-3′ at an annealing temperature of 57°C).

RESULTS

High-resolution haplotype analysis of the critical genetic *MCKD1* region was performed with 25 consecutive polymorphic microsatellites of the critical *MCKD1* region (Fig. 1). Sixteen of these markers were newly generated for this purpose (data available from the authors). We detected extensive haplotype sharing for 9 consecutive markers among all 18 affected individuals of all three kindred: (*1*) the Arizona kindred (F284; USA) used in the initial definition of MCKD as a disease entity [1, 2]; (*2*) the Welsh kindred (F327; UK) used to refine *MCKD1* to <3.3 Mb [8]; and (*3*) the German kindred from the Dutch border (F629; Federal Republic of Germany) (Fig. 1). The shared haplotype was also present in 3 individuals of the youngest generation in pedigree F327 (UK) (Fig. 1) The status of affectedness in these individuals cannot be distinguished in these individuals due to their young age and age-dependant penetrance of MCKD.

The shared haplotype comprises the following 9 consecutive polymorphic markers: cen - D1S29H23g* - D1S 29H23e* - D1S29H23c* - D1S1595/S2140 - D1S243 J18b* - D1S303 - D1S243J18c* - D1S336K24g* - D1S 336K24f* - tel (Fig. 2). Asterisks denote newly generated markers.

DISCUSSION

Since allele frequencies were not available in the 9 polymorphic markers sharing haplotypes, we genotyped at least 58 healthy control chromosomes from Eurasian origin. The control group consisted of 29 healthy individuals, 26 of them originating from Germany, and, of the remaining 3 individuals, one was from The Netherlands, one from Hungary, and one from the Czech Republic. While allele frequencies for the shared alleles in 7 novel polymorphic markers ranged from 0.43 to 0.66, allele frequencies of published markers D1S1595/S2140 and D1S303 were 0.18 and 0.13, respectively (Fig. 2). The combined shared haplotype of 9 markers has a frequency of 2.9×10^{-4}. To further test haplotype sharing by descent we analyzed 16 additional polymorphic markers to either side of the shared haplotye *MCKD1* [6, 8]. We identified 3 centromeric and 4 telomeric markers among the three kindred that partially shared haplotypes (Fig. 2).

As an independent confirmation of haplotype sharing by descent we found, in the genealogy of the Arizona kindred, documentation that they originated in the 17th century and "fled to Holland then to England and to America." The German kindred F629 originated from

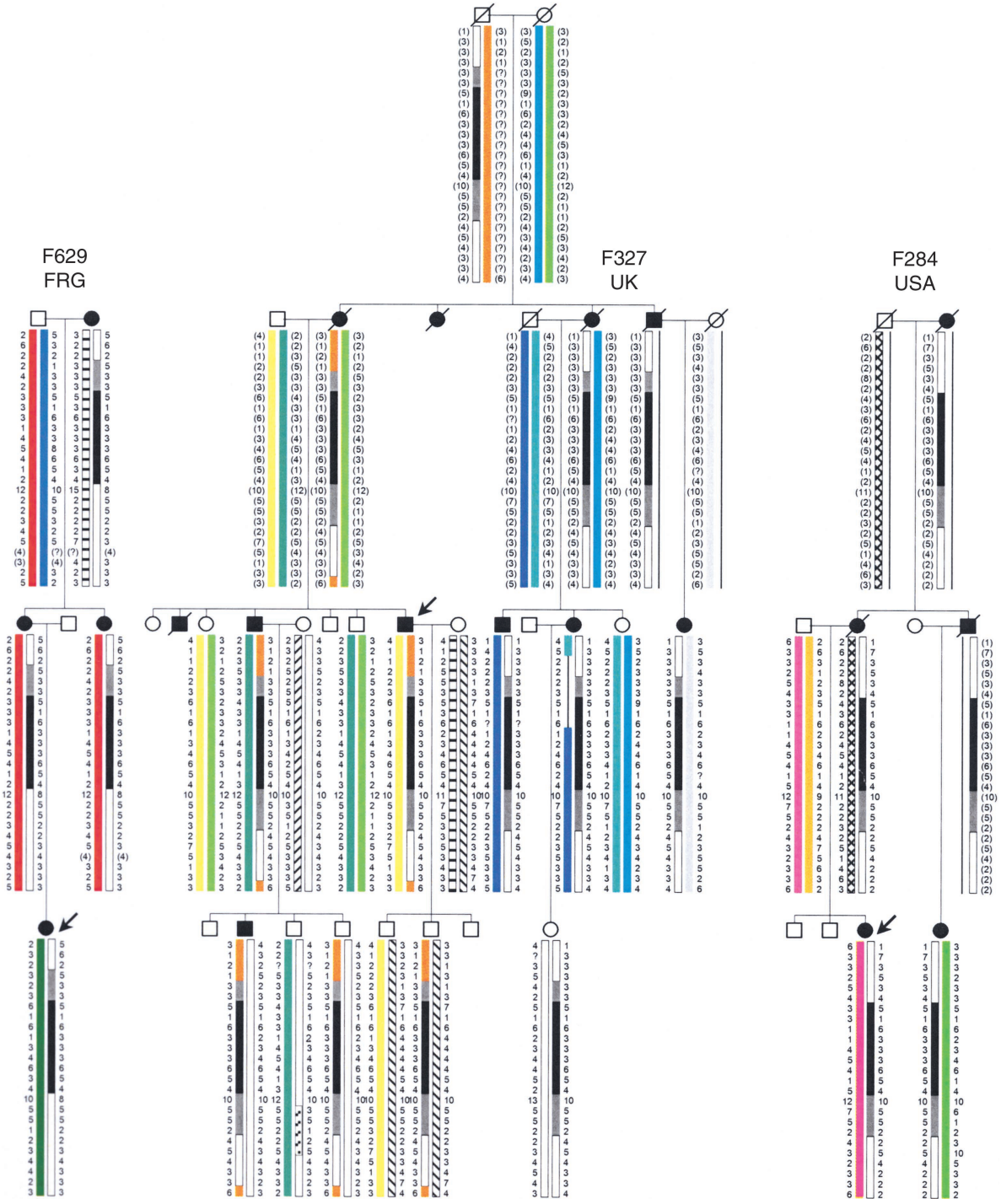

Fig. 1. Haplotyping results from the Arizona kindred (F284) used for initial description of MCKD, the Welsh kindred (F327) used to refine *MCKD1* to <3.3 Mb. Paternal haplotypes are drawn to the left, maternal haplotypes to the right. Marker order from top to bottom is: cen - D1S514 - D1S498 - D1S137P24e* - D1S305 - D1S98F1a* - D1S21N7c - D1S29H23g* - D1S29H23e* - D1S29H23c* - D1S1595/S2140 - D1S243J18b* - D1S303 - D1S243J18c* - D1S336K24g* - D1S336K24f* - D1S336K24c* - D1S336K24h* - D1S336K24a* - D1S2624 - D1S284F21e* - D1S356J7e* - D1S85G21d* - D1S394 - D1S2125 - D1S2635 - tel. Newly generated markers are denoted by asterisks; former flanking markers are underlined; and novel flanking markers are encased in boxes. Haplotypes of the genetic markers are shown as differently colored bars. There is consistent haplotype sharing for 9 markers (black bars) among all 18 affected individuals of all 3 kindred. For 6 to 7 additional surrounding markers, partial haplotype sharing (gray bars) among some kindred is shown (see also Fig. 2). Black symbols indicate individuals with affected status, white symbols indicate individuals with unaffected or unknown status, the latter due to age-dependant penetrance of MCKD1. Circles denote females; squares denote males; a slash through a symbol denotes a deceased individual; and arrows point out individuals in Figure 2 who have been tested in mutational analysis for *RhBG*. Inferred alleles are shown in parentheses.

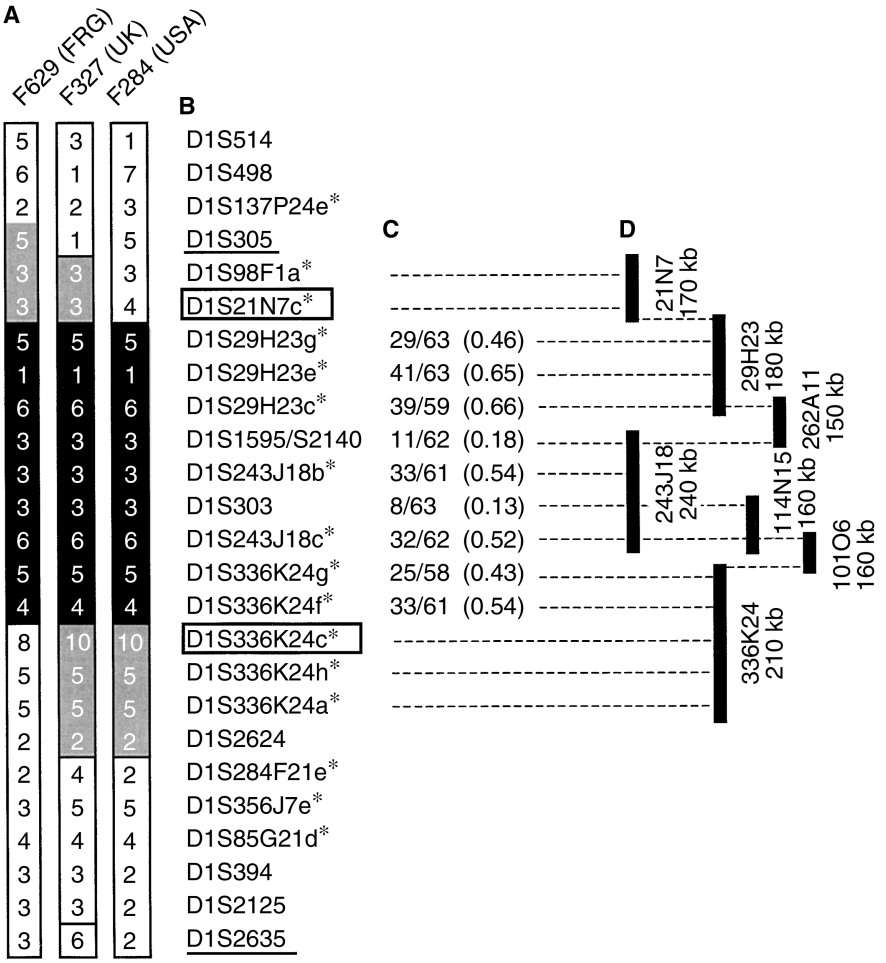

Fig. 2. Refinement of the *MCKD1* locus by demonstration of haplotype sharing; cloning of the critical region in a BAC contig. (*A*) Haplotypes cosegregating with the affected status of patients (indicated by arrows in Figure 1) from the 3 MCKD1 kindred (indicated at top). The haplotype of 9 consecutive markers shared by all affected members of the 9 MCKD1 families is shown on a black background. The partially shared haplotype of 6 to 7 additional surrounding markers is shown on a gray background. The centromere is at the top, the telomere is at the bottom. (*B*) Twenty-five microsatellite markers at the *MCKD1* locus. Newly generated markers are denoted by asterisks. Markers flanking the published 3.3 Mb interval (8) are underlined. Newly defined flanking markers delimiting the haplotype shared in MCKD1 (A) are shown in boxes. (*C*) Allele frequencies of 9 markers from shared haplotypes as determined in at least 58 chromosomes of healthy control individuals are indicated as absolute numbers and as percentages in parentheses. (*D*) Minimal BAC contig of the critical *MCKD1* region. BAC sizes are given in kb. Physical map positions (12) of newly defined flanking markers D1S21N7c and D1S336K24c are 153,176 kb and 153,778 kb, respectively, thus refining the critical *MCKD1* interval to <650 kb.

the German/Dutch border, while the Welsh kindred F327 originated from the area around Bristol, which was an important harbor used to immigrate to the United States in the 17th century. Under the hypothesis of haplotype sharing by descent, a lack of sharing defines D1S21N7c and D1S336K24c as novel flanking markers. Because the physical map positions of these newly defined flanking markers are 153,176 kb and 153,778 kb, respectively, we thus refined the critical *MCKD1* interval to less than 650 kb (Fig. 2) (*http://www.ensembl.org*).

CONCLUSION

The rhesus blood group B glycoprotein gene (*RhBG)* has been suggested as a candidate gene for MCKD1 [9]. Since the *RhBG* was localized within the newly refined critical *MCKD1* region, we performed mutational analysis of its 10 exons. In an affected individual of MCKD1 family F327 (Fig. 1, arrow), we found a one-nucleotide insertion (1265-1271insC) in the heterozygous state. However, this insertion did not cosegregate with the affected status in family F327 and was found in 6 out of 20 control chromosomes through direct sequencing of exon 9 (data not shown). In addition, a nucleotide exchange G227A (Gly76Asp) was identified in the heterozygous state in the same individual. This substitution G227A was present in 10 out of 20 control chromosomes analyzed by allele-specific PCR (data not shown), and therefore must represent an innocuous polymorphism. Thus, we were unable to confirm *RhBG* as a candidate gene for MCKD1.

ACKNOWLEDGMENTS

We thank all members of the MCKD families for their participation. The excellent technical assistance of Barbara Schönfeld and Anita Imm is gratefully acknowledged. Dr. Fuchshuber was supported by a grant from the German Research Foundation (DFG Fu 202/2-1) and the Fritz-Thyssen-Stiftung (1999-2061). F.H. was a Heisenberg Scholar of the German Research Foundation (Hi 381/7-2).

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