Original articles

A novel genetic locus for low renin hypertension: familial hyperaldosteronism type II maps to chromosome 7 (7p22)

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Abstract

Familial hyperaldosteronism type II (FH-II) is caused by adrenocortical hyperplasia or aldosteronoma or both and is frequently transmitted in an autosomal dominant fashion. Unlike FH type I (FH-I), which results from fusion of the *CYP11B1* and *CYP11B2* genes, hyperaldosteronism in FH-II is not glucocorticoid remediable. A large family with FH-II was used for a genome wide search and its members were evaluated by measuring the aldosterone:renin ratio. In those with an increased ratio, FH-II was confirmed by fludrocortisone suppression testing. After excluding most of the genome, genetic linkage was identified with a maximum two point lod score of 3.26 at θ=0, between FH-II in this family and the polymorphic markers D7S511, D7S517, and GATA24F03 on chromosome 7, a region that corresponds to cytogenetic band 7p22. This is the first identified locus for FH-II; its molecular elucidation may provide further insight into the aetiology of primary aldosteronism.

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Aldosterone is a steroid hormone produced exclusively in the zona glomerulosa of the adrenal cortex.[1] It is the major circulating mineralocorticoid in humans.[2] The principal regulators of its synthesis and secretion are the renin-angiotensin system and potassium ion concentrations.[3] Primary hyperaldosteronism is biochemically characterised by suppressed plasma renin and a plasma aldosterone:renin activity ratio (ARR) of >25.[4 5] Although once thought to be rare and not worth looking for, unless patients were hypokalaemic, recent evidence suggests that primary aldosteronism is probably the commonest potentially curable or specifically treatable form of hypertension, with most patients being normokalaemic.[3 4] The majority of cases of primary aldosteronism are thought to be sporadic. Primary aldosteronism occurs most commonly from the fourth decade onwards[3] and may be the result of an adrenal adenoma or bilateral adrenocortical hyperplasia.[4 5] Patients with familial hyperaldosteronism (FH) are usually detected at a younger age than sporadic cases.[6 7]

Two types of FH have been described. FH type I (FH-I) (*Mendelian inheritance in man* (MIM) No 103900), or glucocorticoid remediable aldosteronism (GRA), arises from the formation of a hybrid gene owing to the unequal crossing over of the *CYP11B1* and *CYP11B2* genes during meiosis.[8 9] Hyperaldosteronism in FH-I is inherited in an autosomal dominant manner; genetic screening for FH-I is now available.[10] In FH type II (FH-II), hyperaldosteronism is non-glucocorticoid remediable and in some kindreds it is associated with aldosteronoma formation.[11] Sporadic cases and families with FH-II have been described in several countries, although the largest number of kindreds published is from Australia.[11 12]

The largest kindred with FH-II reported to date is a family with seven affected members from Queensland, Australia, which was previously described in detail.[13] Linkage between FH-II in this family and the *CYP11B2*/*CYP11B1* locus on chromosome 8 was not found.[13 14] Furthermore, the entire long arm of chromosome 8 was excluded from harbouring the disease locus.[13] Other loci harbouring potential candidate genes were also excluded, including that of the angiotensin II receptor type 1 gene.[15] For the purposes of the present study, we screened additional members of this kindred and performed a genome wide search for linking loci.

Methods

CLINICAL STUDIES

The studies were approved by the review boards of the participating institutions. The DNA studies were approved by the Office for Human Subject's Research, National Institutes of Health, Bethesda, MD, the clinical and other molecular studies by the Ethics Committee of the University of Queensland, Brisbane, Australia. Written informed consent for DNA collection was obtained from all subjects. Clinical

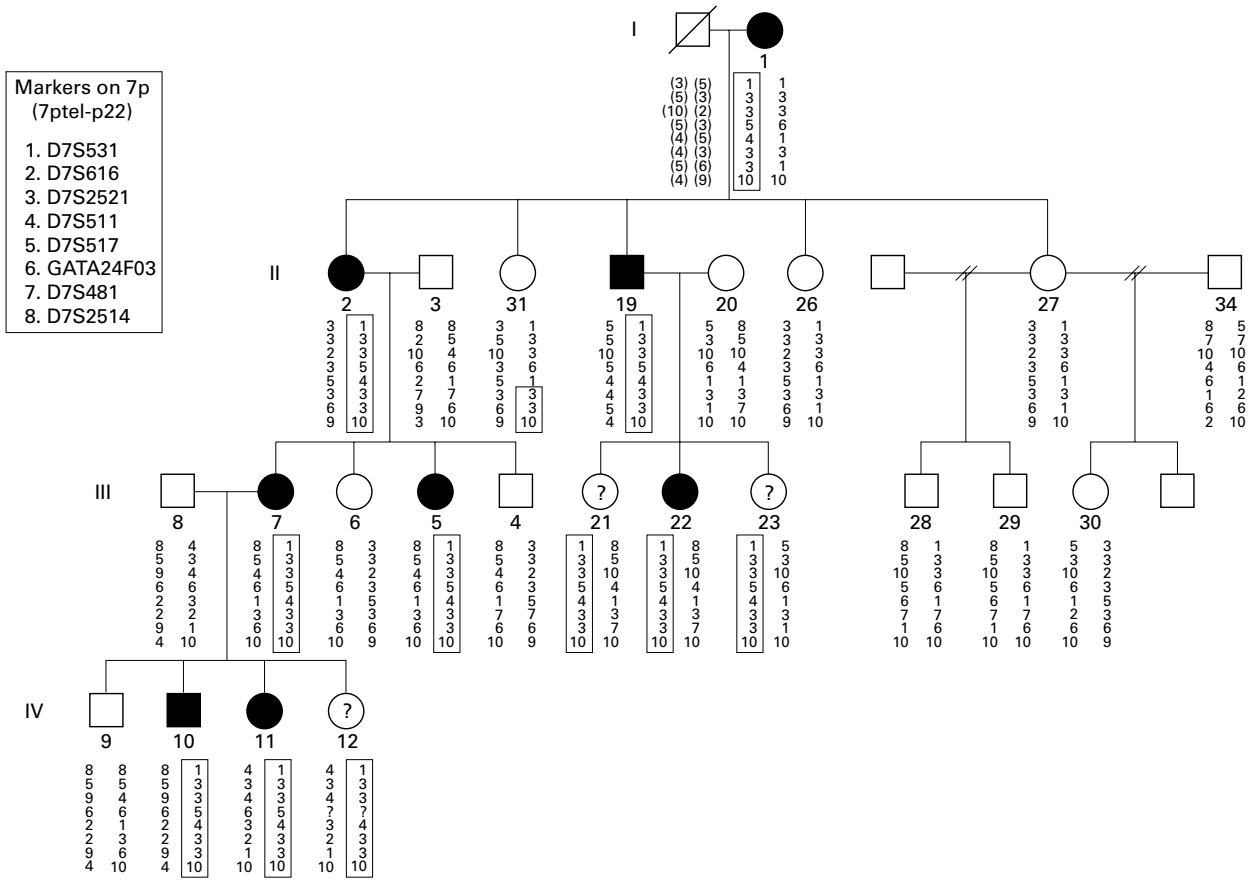

Figure 1 Haplotypes for eight markers from 7ptel-7p22 for the genetically informative part of a family that was previously described in detail.[13] Boxes represent the disease bearing chromosome; the order of the markers is according to information from the available databases. The recombination in subject 31 localises the FH-II genetic locus distal to GATA24F03 towards the telomeric end of 7p.

evaluation was performed at the Hypertension Unit, Greenslopes Hospital, Queensland, Australia. The entire pedigree was published previously[13]; part of the family pedigree is shown in fig 1. All patients were screened for hyperaldosteronism with a mid-morning standing plasma ARR,[12 13] collected under random conditions with respect to salt intake. Drugs known to perturb aldosterone (A) or plasma renin activity (R) levels (such as spironolactone, diuretics, β blockers, ACE antagonists, or dihydropyridine calcium antagonists) were withheld for sufficient time to allow their effects to wear off before testing. ARR greater than 25 (A was measured in ng/dl and R in ng/ml/h) was verified at least once, and patients then underwent fludrocortisone/salt suppression testing (fludrocortisone 0.1 mg q6h and oral sodium chloride 1200 mg tid for four days) to show angiotensin-II independent A secretion. Postural testing, adrenal computed tomography scanning, and adrenal venous sampling were performed to determine if there was lateralisation of aldosterone secretion. The diagnostic algorithm used is described in detail elsewhere.[5] No patient identified as affected had greater than 50% suppression of plasma A levels after four days of dexamethasone (0.5 mg q6h), thus excluding GRA. All subjects were screened for signs of multiple endocrine neoplasia type 1 (MEN-1): history of endocrine tumours, urolithiasis, lipomata, and measurements of plasma ionised calcium, serum prolactin, and fasting gastrin levels.

In our previously published study,[13] 34 members in four generations were screened; seven members were found to be affected and the DNA from these subjects and their parents (a total of 12 subjects) was used for a genome wide search. A clinical and biochemical review was undertaken for family members in whom the diagnosis was initially uncertain. An additional member of the family was found to be affected (No 22); subjects 9 and 26 were confirmed to be unaffected, whereas affectation status remained in doubt for subjects 12, 21, and 23 (fig 1). All DNA samples, including the newly collected ones, were then used for examination of linkage between the disease and loci that produced suggestive lod scores in the genome wide search (see below).

DNA STUDIES

Genomic DNA was prepared as previously described.[13] All affected subjects were tested for the presence of the *CYP11B1/CYP11B2* gene by a method developed by our laboratory and described elsewhere.[10] The genome wide screen was performed using 330 polymorphic microsatellite markers that were distributed over the genome at an average genetic distance of 10 cM (Research Genetics, Huntsville, AL). Additional markers around loci of interest were subsequently typed, using all informative members of the family. The sequences of these

Table 1 Two point lod scores between chromosome 7 markers and FH-II at different recombination frequencies

	Lod score at θ =*				
Marker	*0.0*	*0.1*	*0.2*	*0.3*	*0.4*
D7S531	2.97	2.50	1.99	1.40	0.72
D7S616	−0.81	0.09	0.22	0.21	0.13
D7S2521	2.97	2.50	1.99	1.40	0.72
D7S511	3.26	2.70	2.08	1.39	0.63
D7S517	3.26	2.70	2.08	1.39	0.63
CHLC.GATA24F03	0.9	0.76	0.61	0.43	0.23
D7S481	2.15	2.54	2.11	1.49	0.74
D7S2514	2.07	1.75	1.39	0.98	0.50
CHLC.ATA1A10	−1.25	−0.32	−0.13	−0.05	−0.01
AFM074ZG5	−5.20	−1.19	−0.35	−0.04	0.03

*θ values for males were assumed to be equal to those for females.

primers and genomic order of their loci were derived from the publicly available genomic databases.[16 17] In several cases, radiation hybrid mapping was also performed to establish the order of individual STSs (data not shown), as previously described.[14 18]

The reverse primer for each of the markers was end labelled with γ-^{32}P-dATP, using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). Approximately 50 ng of DNA was used in each reaction. The reaction was carried out in a 10 µl volume containing 1 µl of DNA solution, 10 pmol of unlabelled primer, and dNTPs (1 µl total volume dilution of all dNTPs, at 10 µmol/l concentration each), 0.1 pmol of ^{32}P labelled primer, in 1.5 mmol/l MgCl PCR buffer (1 µl of 10 × PCR buffer), and 1 U of *Taq* polymerase (Perkin-Elmer Roche, Branchburg, NJ, USA). Thirty cycles were performed (94°C for one minute, 57°C for one minute, 72°C for 30 seconds), followed by a final seven minute extension at 72°C. Aliquots of amplified DNA were mixed with an equal volume of loading buffer, denatured at 94°C for five minutes, and electrophoresed on a 6% polyacrylamide gel (Promega, Madison, WI). The dried gel was then exposed to Kodak X-OMAT or BIO-MAX autoradiography film for 16-24 hours.

LINKAGE ANALYSIS

Two point lod scores were calculated using the LINKAGE (version 5.1) computer software, using a dominant model of inheritance, 80% penetrance in both sexes, and a gene frequency of 0.001. All marker allele frequencies were calculated using ILINK, also from the LINKAGE suite of programs. Multipoint linkage analysis was performed with the MLINK program,[19] as previously described.[20]

Results

Clinical investigation of the pedigree reported by Torpy *et al*[13] added new genetically informative members for our analysis. Part of this pedigree with the informative breakpoints for chromosome 7 is shown in fig 1. The strength of the linkage data would be enhanced if subjects 12, 21, and 23 (who carry the chromosome with the disease allele) would in fact meet the diagnostic criteria for FH-II. However, they are young (all less than 20 years at the time of their investigation) and despite some indications that they are probably affected (for example, somewhat increased ARR), none of them met the diagnostic criteria for FH-II. Thus, they were included in our analysis as uninformative. These subjects are followed by our department for the development of additional signs of FH-II.

The genome wide screen yielded 19 loci with lod scores greater than 0.8 clustered on 12 chromosomes that included areas 9p, 7p, 16p, Xp, and Xq. Further analysis, with markers derived from the available maps,[16 17] enabled exclusion of loci from chromosome 16 and the X chromosome owing to the presence of

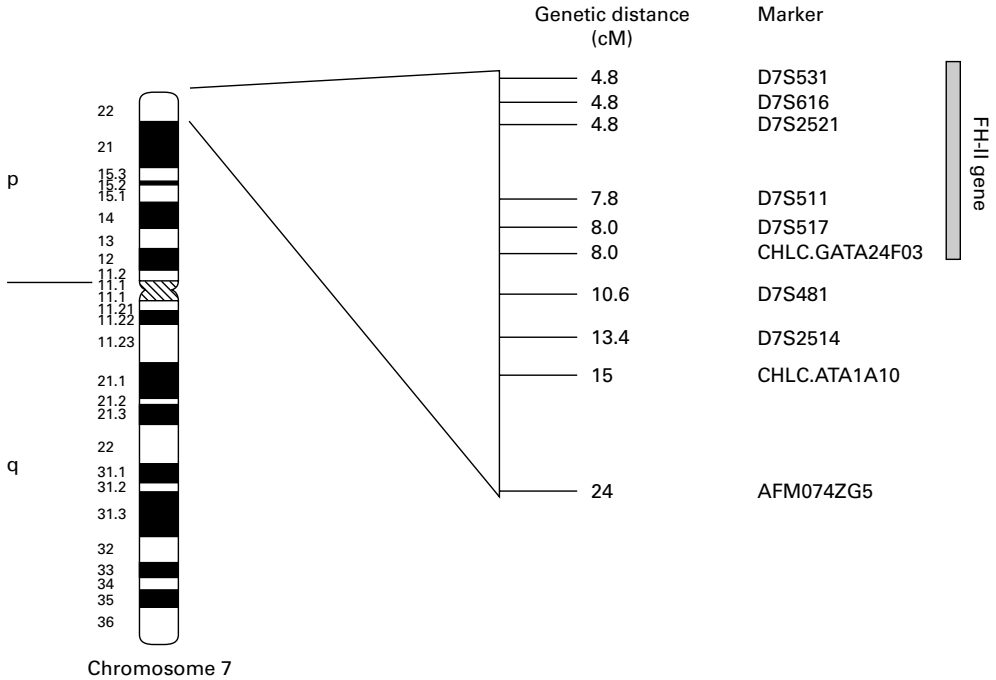

Figure 2 An ideogram of chromosome 7 is shown with the approximate location of the FH-II gene, as determined by the linkage analysis of the indicated markers on 7p22.

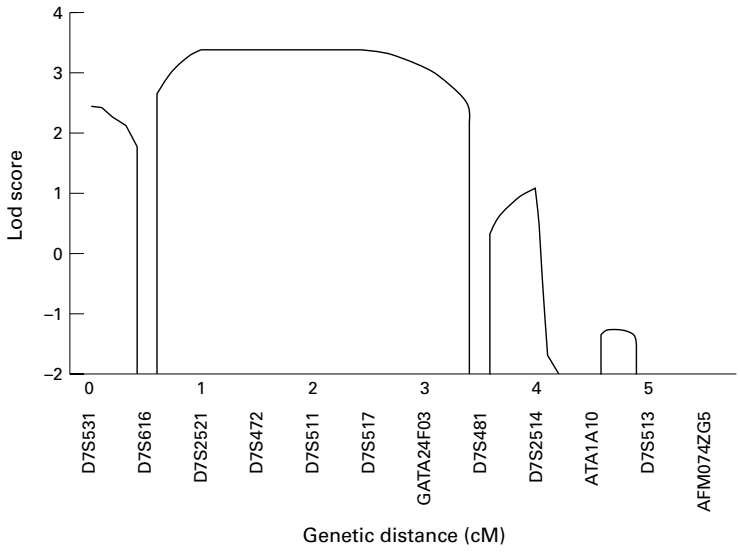

Figure 3 Multipoint analysis: the multipoint lod scores observed between the FH-II phenotype and markers from the 7p22 chromosomal region are presented on the X axis and the positions of marker loci on the Y axis.

recombinations in affected subjects for the regions with the peak lod scores (data not shown). Analysis of candidate loci on chromosomes 9 and 7 indicated that both areas could possibly be harbouring the FH-II locus in this family with lod scores of 2.1 (D9S925) and 1.2 (D7S513), respectively (at θ=0). Examination of recombination breakpoints with several other markers from the candidate regions on 9p and 7p enabled exclusion of the 9p locus, whereas there was linkage with markers at 7p (D7S511, D7S517) with a peak lod score of 3.26 (at θ=0) (table 1). This area corresponds to the top of the short arm of chromosome 7 (cytogenetic band 7p22), covering at least 5 cM of genetic distance (fig 2).[21]

For multipoint analysis the markers used were (order and distance as in the on line databases) 7pter - D7S531 (4.8 cM) - D7S616 - D7S2521 - D7S472 (7.8 cM) - D7S511 - D7S517 (8 cM) - CHLC.GATA24F03 - D7S481 (10.6 cM) - D7S2514 (13.4 cM) - CHLC.ATA1A10 (15 cM) - D7S513 - AFM074ZG5 (24 cM). This analysis showed a maximum multipoint lod score of 3.5 between markers D7S2521 and CHLC.GATA24F03 (fig 3).

Discussion

FH-II is an inherited, non-glucocorticoid remediable form of hyperaldosteronism that was relatively recently recognised as a distinct entity.[5 12] The present study reports the identification of a locus for this condition on the short arm of chromosome 7 corresponding to the band 7p22 to which a large kindred maps with a peak two point lod score of 3.26 at a recombination fraction of 0. Multipoint linkage analysis yielded a peak lod score of 3.5. Unfortunately, this area of chromosome 7 is not well represented in existing maps (perhaps because of its telomeric location) and, at this point, the only available polymorphic markers from 7p22 are the ones used in this study.

Examination of known genes in the region 7p22[22] showed several possible candidates. Those are either involved in tumorigenesis or have a possible role in the central or peripheral regulation of the renin-aldosterone system. They include the genes coding for G protein coupled receptor 30 (*GPR30*), the human homologue of postmeiotic segregation increased *S cerevisiae* (*PMS2*), cyclic AMP dependent protein kinase regulatory protein type-1β or R1β (*PRKAR1B*), and the centaurin-alpha1 gene.[22] *GPR30* codes for a novel G protein coupled receptor that is ubiquitously expressed in human tissues although it is overexpressed in oestrogen receptor positive breast cancer.[23] It has been suggested that *GPR30* may be involved in various physiological responses of hormonally regulated tissues.[23 24] *PMS2* encodes a protein involved in DNA mismatch repair that is associated with hereditary non-polyposis colorectal cancer type 4, and Turcot syndrome with glioblastoma, and has been localised to 7p22 by FISH.[25] This is an unlikely candidate for FH-II. *PRKAR1B* is also found on 7p22.[22] It binds with the catalytic subunits of protein kinase A (PKA), forming a heterotetramer. Its homologue is found on mouse chromosome 5 and has been shown to be neural specific.[26] It is noteworthy that *PRKAR1A*, the gene encoding the regulatory subunit type IA (R1α) of PKA, a functional partner of R1β, is mutated in Carney complex (MIM 160980), another syndrome associated with autosomal dominant inheritance of adrenocortical, glucocorticoid producing tumours.[27] A final candidate is centaurin-alpha1, which, however, is predominantly expressed in the brain; it appears to function as an inositol triphosphate receptor[28] and may have an involvement in the central control of the renin-aldosterone system. FH-II is the fourth genetically determined form of hypermineralocorticism to be described,[13] the others being glucocorticoid remediable hypertension (GRA, FH-I), 11-β-hydroxylase deficiency, and 17α-hydroxylase deficiency. In addition, three inherited forms of apparent mineralocorticoid excess are known to exist: activating mutations of the mineralocorticoid receptor,[29] Liddle syndrome (MIM 177200),[30] and the syndrome of apparent mineralocorticoid excess (MIM 218030).[31] Although several possible candidate genes have been localised to the 7p22 region, none of them stands out as the obvious gene for FH-II. We could not find any gene(s) with functional relationship to aldosterone synthase, a gene that, if upregulated, may lead to primary aldosteronism.[8 32 33] Similar to the other genetically determined causes of hypertension, identification of the gene(s) responsible for FH-II may provide important insight into the regulation of blood pressure in the general population.

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