Bartter’s Syndrome and the Atrial Natriuretic Factor Gene

ROBERT M. GRAHAM, KENNETH D. BLOCH, VERA B. DELANEY, EDWARD BOURKE, AND JONATHAN G. SEIDMAN

SUMMARY We investigated whether the gene for atrial natriuretic factor, a recently discovered peptide hormone with potent natriuretic, diuretic, and vasorelaxant properties, was pathogenetically linked to an uncommon but well-defined fluid and electrolyte disorder, Bartter’s syndrome. Restriction fragment length polymorphisms in the atrial natriuretic factor gene were sought in a large kindred with six of 23 family members being affected. A Bgl II polymorphism, identified in two of 40 (5%) apparently normal subjects, was found in one of two first-generation family members. This polymorphism was also present in five of seven unaffected second-generation siblings but in only three of six affected siblings. The failure of the absence or the presence of the polymorphism to cosegregate with the disease clearly indicates that in this kindred, the gene for atrial natriuretic factor is not linked to Bartter’s syndrome. (Hypertension 8: 549-551, 1986)

KEY WORDS • restriction fragment length polymorphism • Bartter’s syndrome • atrial natriuretic factor

In 1962 Bartter and co-workers described two patients with growth retardation, hypertrophy and hyperplasia of the juxtaglomerular apparatus of the kidney, normal or low blood pressure with decreased pressor responsiveness to angiotensin II, increased concentrations of angiotensin II and aldosterone, hypokalemic alkalosis, and impaired urinary concentrating ability that was resistant to vasopressin. This syndrome is well-recognized and has been observed in patients of various racial backgrounds and from different geographical areas, however, it continues to be a relatively rare disorder of unknown prevalence.

Both sporadic and familial cases of Bartter’s syndrome have been reported; the former being more common. The presence of the syndrome at birth or during early life, its occurrence in two or more siblings, and the fact that in all but one of the reported families with Bartter’s syndrome only one generation has been affected, suggest that it is inherited as an autosomal recessive trait. Although numerous theories about the pathogenesis of this disorder have been advanced, including primary vascular hyporesponsiveness to pressor substances, autonomous renin oversecretion, reduced renal sodium or chloride reabsorption, overproduction of renal prostaglandins, and excessive renal potassium loss, the etiology of this syndrome remains unknown.

In 1969 Imai et al., proposed that the primary abnormality in Bartter’s syndrome was the overproduction of a natriuretic factor. This proposal was later extended by Grekin et al., who suggested that Bartter’s syndrome resulted from an excess of a “chloruretic hormone,” which would account for the characteristic renal potassium wasting, hypokalemia, fluid volume depletion, excessive renal prostaglandin E2 production, and most of the other features of the disorder. Atrial natriuretic factor (ANF) is a recently discovered peptide hormone with potent natriuretic, diuretic, and vasorelaxant properties.
humans, ANF produces prompt renal sodium, chloride, and water loss and, to a lesser extent, potassium excretion.10-13 It is thus a prime candidate for the "chloriuretic hormone" posulated to be pathogenetic in Barter's syndrome. Furthermore, preliminary studies of a patient with Barter's syndrome indicate that basal plasma ANF levels are higher than in normal subjects and rise further with saline loading (R. D. Gordon, personal communication, 1985). With the availability of the amino acid sequence of ANF,10-14 and others10-12 isolated an ANF complementary DNA clone that permitted the determination of the structure of the encoded preprohormone, as well as the sequence of the entire ANF gene. In the present study, we used these molecular probes to investigate if the gene for ANF is linked to Barter's syndrome in an Irish family, one of the largest kindreds described thus far, in which six of 23 members of the family are affected.

**Subjects and Methods**

The pedigree of the kindred investigated is shown in Figure 1. The characteristics of the family and the evaluations documenting the diagnosis of Barter's syndrome in the six affected family members have been reported previously.2-4 The pedigree shown in Figure 1 differs from that depicted in Reference 2 because in the present investigation all 13 family members of the second generation were studied and are listed in chronological order. There was no consanguinity in the family nor in the ancestral tree traced for three and two generations on the paternal and maternal sides, respectively. Neither the mother, the father (first generation, see Figure 1, I) nor the eight children of five of the affected second-generation siblings has the disorder, which underscores the apparent autosomal recessive nature of its inheritance.

Blood was collected from the parents, all 13 members of the second generation, and from some of the children of the affected siblings as well as 40 apparently normal, unrelated medical students, and DNA was isolated from leukocyte nuclei as described by Bell et al.16 Restriction fragments were prepared by digestion of the DNA with a variety of restriction enzymes, using standard techniques.17 Restriction fragment length polymorphisms in the ANF gene were then sought by Southern blot analysis and hybridization of the DNA fragments17 with a nick-translated Pvu II restriction fragment of the human ANF gene, which includes the first exon, the first intervening sequence, and a portion of the second exon.15 The study was performed in accordance with institutional guidelines.

**Results**

Two different restriction fragment length patterns were detected with the restriction enzyme Bgl II (Figure 2). One pattern consists of a single fragment of about 9.7 kb (1 kb = 1 kilobase, or 1000 base pairs). This pattern was identified in 38 (95%) of 40 normal subjects. In two (allele frequency, 2.5%), a second pattern (polymorphism) consisting of the common 9.7 kb fragment as well as a 10.8 kb fragment was identified (see Figure 2). This polymorphism was identified in one of the first-generation (see Figure 1, I) family members and in five of the seven unaffected second-generation (see Figure 1, II) siblings. However, neither the single 9.7 kb fragment pattern nor the 10.8 kb fragment cosegregated with the disease, since three of the six affected subjects had the 10.8 kb fragment and three did not (see Figure 1).

**Discussion**

The use of restriction fragment length polymorphisms, which reflect differences in the DNA nucleotide sequence within or surrounding the region defined by a particular DNA probe, to investigate genetic linkage with disease is a powerful tool that is being applied increasingly to evaluate the genetic basis of inherited disorders. For example, in Huntington's disease, a dominantly inherited central nervous system disorder, identification of polymorphisms has permitted the early diagnosis of affected persons.18 Similar investigatory methods have also been applied to studies of disorders of lipoprotein metabolism, in which linkage with the gene for human apoprotein A-II19 and the low density lipoprotein receptor20 has recently been identified.

In the present investigations, linkage between the ANF gene and Barter's syndrome was evaluated.
restriction fragment length polymorphism in the ANF gene, identified with an allele frequency of 2.5% in 40 apparently normal subjects, was also present in one of the two alleles of the male member of the first generation (see Figure 1, I). If complete linkage existed between the ANF gene and Bartter’s syndrome, and given the likely autosomal recessive nature of its inheritance, then one of this first-generation male’s ANF alleles should have carried the genotype for the disorder. Since each child of this individual inherits one of his two alleles, linkage of the ANF gene to Bartter’s syndrome, and the affected progeny being heterozygous for the ANF polymorphism. The failure to find cosegregation of the polymorphism with the disease clearly indicates that, at least in this kindred, the ANF gene is not genetically linked to Bartter’s syndrome. This finding does not exclude the possibility that the same phenotype, in this case Bartter’s syndrome, could be due to different genotypes, which might involve the ANF gene. To investigate this possibility, we plan to perform similar studies in genetically unrelated kindreds.

The failure to find a linkage with the ANF gene in Bartter’s syndrome also does not exclude the possibility that ANF is pathogenetically linked to the disease. Thus, genetic abnormalities at other sites of control of ANF, for example, its release, metabolism, and target-organ responsiveness, may be involved. Elucidation of such abnormalities will require detailed studies to further define the physiology of ANF and its role, if any, in Bartter’s syndrome and other circulatory, fluid, and electrolyte disorders, such as essential hypertension, Gordon’s syndrome, and hyporeninemic hypoaldosteronism.

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References

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