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GENETIC ASPECTS OF PLASMA ALDOSTERONE BINDING GLOBULINS IN FAMILIES OF PATIENTS WITH ESSENTIAL HYPERTENSION, INCLUDING ISOLATION OF NOVEL THERMOSTABLE HOMOLOGUES FROM PLASMA AND URINE

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SUMMARY

Two new thermostable aldosterone binding globulins were partially characterised in plasma (ABG-TS) and urine (ABD-TSu) of normal humans. Analysis comprised ultrafiltration, dialysis, incubation at 37°C preceding or following DEAE cellulose chromatography (pH gradient), and Sephadex G75 chromatography. Both compounds had a molecular weight and isoelectric point similar to that of the already described plasma thermolabile ABG. In studies on the effect of potassium canrenoate, with physiologic range, on aldosterone ABG binding *in vivo* in 4 out of 7 and *in vitro* in 9 hypertensives, a decrease, probably due to a specific competitive displacement of aldosterone, was observed. The transmission of high ABG capacity, as reflecting high plasma ABG concentration, was studied in three generations of 40 French Canadian families from the Montreal area. Samples were taken from 304 relatives (aged 2½–84) of two known essential hypertensive (EH) parents, and also from 290 controls (aged 4–65). Relatives with values similar to controls were designated "normal" in contrast to relatives carrying the high ABG binding trait designated "affected". In "affected" relatives, total mid-afternoon to early evening plasma aldosterone was similar to that of normal relatives or controls but the free fraction was less ($P < 0.001$). Total CBG capacity for other plasma corticosteroids was lower in EH patients and their relatives. In pedigree analysis of the trait, the cumulative frequency distribution was continuous in controls and bimodal in EH families. Affected males or females transmitted the trait, which was expressed in early childhood without skipped generations or sex difference. The distribution of individuals in pedigrees suggests a simple autosomal dominant inheritance of the trait. Because there was no correlation with blood pressure, and because not all hypertensives had increased ABG binding, it is suggested that this high binding could be one of the determining factors of susceptibility or resistance in some EH patients.

INTRODUCTION

Essential hypertension (EH) tends to run in families and is to a large extent genetically controlled. The precise hereditary abnormality and the exact role of genes have not yet been clarified because no precise genetically controlled biochemical defect has been identified.

Recently, it has been postulated that the increased binding of aldosterone to a specific thermolabile plasma globulin fraction (ABG) in EH patients with a normal binding in secondary hypertension may indicate that this quantitative change in a plasma protein represents a genetic marker of EH [1]. Moreover, the increased thermolabile ABG binding has been shown to contribute to significant alterations in aldosterone metabolism, which may enhance its biological activity in EH [1–5], in women on oestrogen-containing oral contraceptives (OC) [6], and in cystic fibrosis patients [7]. Marked increase in thermolabile ABG binding in subjects on OC and its positive correlation with blood pressure [6] also favours this conclusion.

This investigation has been a familial study of thermolabile ABG capacity to bind aldosterone [1, 6, 8, 9] and its transmission among the natural children and grandchildren of two parents with proven EH. To clarify some closely related points, three additional aspects have been included: (1) The effect of the spironolactone on this binding because 42% of treated patients, at the time of the study, received this drug either alone or in association with other antihypertensive agents. (2) Partial characterisation of a novel

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plasma and urinary thermostable ABG homologue has also been included, because the binding of aldosterone to a thermostable plasma protein fraction was increased in some of the relatives. (3) Aldosterone binding to the thermolabile ABG binding has also been compared to that of plasma corticosteroids to the corticosteroid binding globulin (CBG) in order to explore the selectivity in modification in the two binding systems.

MATERIALS AND METHODS

Thermolabile ABG binding capacity and plasma aldosterone

Improvements of the ABG binding capacity procedure. In the procedure initially used for the binding capacity [1, 5, 6] an incubation time of three hours at 37°C of tritiated aldosterone with normal plasma was found most effective. In the course of this study, it was noticed in a few percent of the plasmas from relatives that when this time was prolonged beyond 2 h and 45 min, significantly less of the thermolabile form and proportionally more of the thermostable form was present. Therefore, this incubation time was shortened by 15 min after it had been established that in plasmas from controls and the remaining EH patients, identical results were found with either of the two incubation times. It is not clear what causes this difference, but repeated estimations on different occasions (up to 5 times) on plasma samples from some of the above patients showed this anomaly.

Total plasma aldosterone was determined by radioimmunoassay (10) and free and protein bound by a minor modification of a dextrancoated charcoal method already reported [1, 5, 6] and assessed [1, 6].

Total plasma CBG capacity was measured by adsorption [11] and total plasma corticosteroids (TCS) by competitive protein binding [12]. The intra-assay coefficient of variation for CBG capacity was 7.8% ($n = 8$) and the inter-assay coefficient was 4.8% ($n = 25$).

Spironolactone. The effect of potassium canrenoate (Soldactone,® Searle) on thermolabile ABG binding capacity was studied *in vivo* in 6 EH patients and one control and *in vitro* in 9 patients.

Urinary or plasma proteins. Urine was concentrated by first using Amicon concentrators (capacities 10 ml, 400 ml or 2.5 l) with XM50 and then YM10 Diaflo membranes at 40–50 lb/in^2. Chromatography of urinary and plasma proteins on Sephadex G75, G200 and Sephadex A-50 columns [8, 9] and molecular weight determinations by gel filtration were done as described [6, 8, 9]. In addition, the following ^{14}C-labelled methylated protein molecular weight markers (New England Nuclear) were used: bovine serum albumin (mol. wt 69,000); carbonic anhydride (30,000); cytochrome *c* (12,000); ovalbumin (46,000).

Familial study. The sample group, studied between 1976–78, consisted of 40 French Canadian families from the Montreal area, in which both grandparents had EH. Blood samples and blood pressure readings were taken in the homes of the assembled families, while on a free diet, first in the parents, then in the children and lastly in the grandchildren. After the subjects had been seated and relaxed for 5 minutes, blood pressure readings were taken in both seated and recumbent postures, usually between mid-afternoon and early evening after normal daily activities, always by the same observer, using a Tycos mercury sphygnomanometer, at 2–3-minute intervals. The results of 2 readings were averaged. Cuff size was adjusted in children. Blood samples were usually drawn while the subjects remained recumbent and plasmas kept frozen until processed. The usual criteria already described [1, 13] have been used for the diagnosis of EH patients. A total of 304 relatives (128 males and 176 females) aged from 2½ to 84 years were studied. 17 women were on OC, two on conjugated oestrogen (Premarin®), one was on cortisone and 32 of the 78 hypertensives being treated were on spironolactone. The drugs were not interrupted during the study. Two women were pregnant.

Blood samples and blood pressures were similarly obtained from apparently healthy untreated volunteers with two normotensive parents. This group consisted of 135 males and 155 females aged between 4 and 65 years who were approximately age and sex matched to the EH families studied.

To eliminate the effects of menstrual cycle [3, 5, 6, 10], which is known to affect total and ABG bound plasma aldosterone, all values were corrected to the first 6 days after the onset of menstruation in females. All subjects studied were white. Statistics, including frequency distributions, mendelian proportions and genetic analysis of ABG bound aldosterone were done as described [14–19]. Student's two-tailed *t*-test for unpaired comparisons was also used. All results were expressed as mean ± SEM and the difference was considered significant when $P < 0.05$.

RESULTS

Plasma aldosterone and ABG binding capacity

Aldosterone binding capacity of thermolabile ABG (relative to total plasma aldosterone present) was 9.7 ± 0.24 (range 2–17) in 290 controls. The value of 17 representing the normal mean plus two standard deviations was taken as the upper limit between those subjects designated as "normal" and those carrying the high ABG binding trait designated as "affected". It is important that 8 out of 297 control subjects initially selected on the basis of parental absence of EH had values exceeding this cut-off at 17. In all but one of them (with a value of 18.3), further investigation into the family history revealed, however, a parent with EH. The above seven subjects were excluded from the control group and to preserve the object of familial aggregation, their values were also not included in the group of relatives.

Aldosterone binding capacity of thermolabile ABG in 118 "normal" relatives (55 males and 63 females) (11.2 ± 0.38) was significantly different ($P < 0.001$) from that of the 98 "affected" relatives (43 males and 55 females) (23.2 ± 0.6). Values in subjects on antihypertensive therapy were omitted since treatment affects both the binding capacity and total plasma aldosterone. (See results further on and Discussion). Similarly, pregnant subjects and those on oestrogen therapy were also excluded.

Total midafternoon to early evening upright plasma aldosterone, uncorrected for age was similar in 290 controls, 118 "normal" relatives and 95 "affected" relatives, but the free fraction was less ($P < 0.001$) in the "affected" relatives (Table 1).

Blood Pressure

No significant correlation between ABG binding and blood pressure was found.

Total plasma corticosteroids and CBG binding capacity

Total CBG capacity in relatives and age-matched control subjects as well as treated and untreated EH

Table 1. Upright mid-afternoon to early evening plasma aldosterone*

	Total (ng/100 ml)	ABG binding capacity	Free (ng/100 ml)
Controls $N = 290$	8.4 ± 0.39	9.7 ± 0.24	7.6 ± 0.35
Relatives designated "normal" $N = 118$	8.7 ± 0.56	11.2 ± 0.38	7.7 ± 0.51
Relatives designated "affected" $N = 95$	6.5 ± 0.5	23.2 ± 0.6†	4.6† ± 0.4

* Mean ± SE.
† Vs controls or "normal" relatives, $P < 0.001$.

patients showed no correlation with age. Significantly lower capacity was found in hypertensive relatives, normotensive relatives and treated or untreated EH patients as compared to age and sex-matched controls (Table 2). TCS were similar in controls and relatives, but were significantly higher in subjects on OC therapy. Markedly increased CBG capacity was also found in those on OC in both groups.

Effect of potassium canrenoate on ABG binding

In vivo. The effect of potassium canrenoate, administered in a 240 min infusion in a dose of 200 mg/250 ml isotonic glucose on plasma thermolabile ABG binding capacity of aldosterone, was evaluated in 6 patients with stable EH and in one control. Blood samples were taken at 0 h (before administration was started) and then at 15, 30, 60, 90, 120, 180, 240 and 300 minutes. In four patients the plasma ABG binding decreased (upper curve) during or immediately after the infusion with no significant change (lower curve) in the remaining three subjects (Fig. 1A).

In vitro. To aliquots of plasma from 9 EH patients first incubated at 37°C as usual with [^{3}H]-aldosterone for 2 hr 45 min, amounts of potassium canrenoate from 10–200 mg/l plasma were added and the mixture incubated for an additional hour. The percentage of aldosterone bound to thermolabile ABG declined

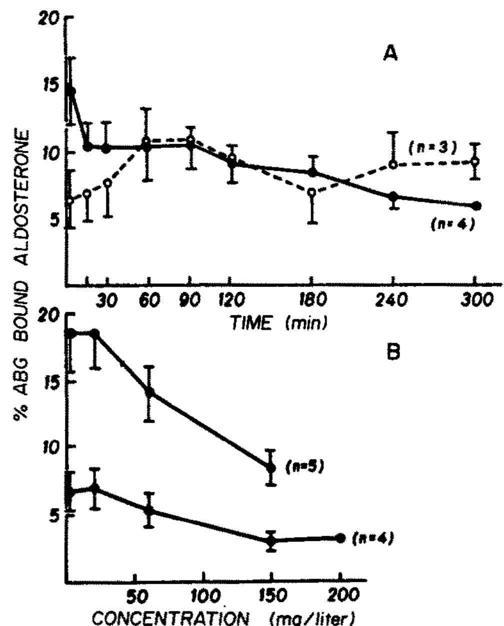

Fig. 1A. Effect of infusions of potassium canrenoate on thermolabile ABG binding capacity in four EH patients with stable EH showing a decline in this binding and three other subjects in whom no significant change occurred. 1B. Similarly, effects of an *in vitro* addition of potassium canrenoate into the plasmas of five patients with high, and four with normal ABG binding capacity of aldosterone.

Table 2.

	Total plasma corticosteroids μg/100 ml; mean ± SEM	CBG Total binding capacity μg/100 ml; mean ± SEM
Controls	(a) 10.7 ± 0.25 (n* = 247)	(e) 20.5 ± 0.2 (n = 157)
Controls on OC	(b) 16.6 ± 0.78 (n = 62)	(f) 37.6 ± 1.0 (n = 64)
Relatives	(c) 10.3 ± 0.29 (n = 274)	(g) 17.5 ± 0.4 (n = 170)
Relatives on OC	(d) 22.6 ± 1.84 (n = 24)	(h) 39.2 ± 1.2 (n = 24)
Treated EH patients		(i) 17.8 ± 0.3 (n = 57)
Untreated EH patients		(j) 17.7 ± 0.2 (n = 130)
Statistics:	(a) vs c, P NS	e vs f, $P < 0.001$
	(a) vs b, $P < 0.01$	e vs g, $P < 0.001$
	(b) vs d, $P < 0.001$	e vs h, $P < 0.001$
	(c) vs d, $P < 0.001$	e vs i, $P < 0.001$
		e vs j, $P < 0.001$
		g vs h, $P < 0.001$

* n = number of subjects.

gradually with a dose of up to 150 mg and then apparently remained constant (Fig. 1B). This decline was more pronounced in 5 subjects with high initial levels.

Novel heat stable plasma ABG-like fraction and aldosterone binding proteins in human plasma and urine

Of those proteins normally present, in small quantities, in urine, one third is albumin and two-thirds are globulins arising mainly from plasma proteins [20]. Investigation of the glycoprotein fraction of normal human urine has led to the isolation and partial characterisation of an apparent homologue of plasma ABG: *i.e. two* apparently related forms of a heat stable molecule. [^{3}H]-aldosterone was first added to urinary ultrafiltrate, extensively dialysed against water (see legend to Fig. 2A), and the completely deionized mixture, which had been incubated for 3 h at 37°C, was then subjected to DEAE-cellulose fractionation using a stepwise pH gradient elution. The pH 8.4 buffer (18–20 ml fraction, peak I) eluted the bulk of protein subsequently shown to bind aldosterone and the pH 5 buffer (158–166 ml, peak II), also eluted a very small amount of an aldosterone binding protein. In other experiments, however, concentrates from the same urine collection obtained by ultrafiltration were first dialysed and then the deionised concentrate was subjected to DEAE-cellulose chromatography, without prior incubation with [^{3}H]-aldosterone. In repeated results, urinary protein migrating close to void volume (Peak I) and transporting aldosterone decreased sharply (Fig. 2B). It was the fraction eluted with the pH 5 buffer (Peak II), previously present in very small amounts (Fig. 2A), which was now the main carrier of [^{3}H]-aldosterone. Binding experiments were performed on aliquots of all fractions of the eluate by incubations with [^{3}H]-aldosterone at 37°C for 3 h, removal of the excess of unbound aldosterone by a treatment with dextran coated charcoal (2:1 w/w) for 10 min at 4°C and counting of aliquots of the supernatant for protein bound radioactivity were carried out. It appears that the incubation causes a change in the structure of this urinary aldosterone binding protein fraction, which completely alters its chromatographic mobility but does not destroy its capacity to bind and transport aldosterone.

When pools of protein eluted in fractions on DEAE-cellulose were rechromatographed on Sephadex G75 gel, a major symmetrical peak binding aldosterone was eluted, with a mobility very close to that of the trypsin inhibitor standard (mol. wt 28,000), and a second small peak emerged with the void volume (Fig. 2C), indicating a molecular weight greater than 70,000. This urinary material contained in the first main peak (mol. wt 27,500) was not affected by heating to 60°C for 25 min.

The above observation promoted a search for a similar protein in normal human plasma. It has been subsequently established that chromatography of human plasma on the same DEAE-cellulose column indicated the presence of peaks I and II behaving similarly with either non-incubated plasma or with plasma incubated with [^{3}H]-aldosterone (37°C for 3 h). Peak II from normal intact plasma contained

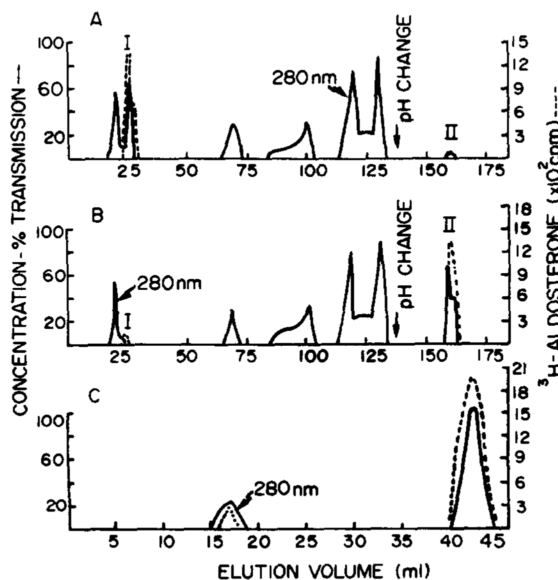

Fig. 2. Runs A and B: Typical fractionation of urinary proteins on DEAE-cellulose. Sample, human urine ultrafiltrate obtained by first forcing it through an Amicon diaflo membrane XM50 (mol. wt 50,000) and then again through a YM10 (mol. wt 10,000) membrane and retaining the concentrate. Pharmacia column K16/40, bed height 20 cm, eluent, veronal (0.04 M, pH 8.4) with stepwise gradient to acetate (0.2 M, pH 5.0); flow rate 1.6 ml/min. (Pharmacia peristaltic pump p-3). The compositions of the peaks indicated were further examined by gel filtration on Sephadex G75 gel. Run C: Typical elution profile of fraction II from the DEAE-cellulose rechromatographed on a column (0.9 cm × 70 cm) of Sephadex G75 gel. See materials, methods and results sections for more details on Runs A-C.

Table 3. Aldosterone-binding globulin (ABG) complex in human plasma

α_1-Glycoprotein—heat labile (60°C for 35 min)
Amount in normal plasma approx. 3 μg/l
Mol. wt 26,500–27,500 (Sephadex G75 and G200)
Apparent association constants at 37°C: $K_1 = 10^7\ M^{-1}$ $K_2 = 10^6\ M^{-1}$
Isoelectric point (Sephadex G75 S) pI—4.75
Electrophoretic mobility at pH 7.95 Rf—0.82
Bromophenol blue—negative
Sudan black—negative
Periodic acid Schiff's stain—positive
$(NH_4)_2SO_4$ Precipitation at 1.4 M

about 90% thermolabile protein identical to ABG (characterised before, Table 3) and about 10% of the novel thermostable homologue. Comparison of this urinary material with the plasma thermostable aldosterone binding fraction indicated that it might be identical with the plasma thermostable (TS) fraction previously isolated (but not characterised) by DEAE Sephadex A-50 (ionic gradient) chromatography [8]. Both the plasma and urinary thermostable materials were eluted from this column in the same fraction (0.1 M NaCl) and were identical in both their molecular weights (27,500 on Sephadex G75 and G200 gel) and isoelectric points. They are abbreviated hereafter as ABG-TS for the plasma and ABG-TSu for the urinary fractions.

Heating normal plasma to 60°C for 25 min invariably resulted in a strong diminution of the binding to the molecule with mol. wt 27,500 as estimated by both DEAE-cellulose and Sephadex gel filtration, but never in its complete disappearance. That there is only a relatively small amount of this protein in normal plasma (about 10% of the total binding) is probably the reason why its presence was overlooked in our initial study [8] performed on a pool of normal human plasma. The presence of this protein is, however, of utmost importance in studies of EH samples and especially for the construction of pedigrees of relatives in this study, since it has been found that in some EH patients the thermostable fraction, measured by our method, is also increased.

In order to better understand the nature of this thermostable binding, solutions of increasing concentrations of human purified albumin fraction have been tested by our method for the percentage of thermolabile and thermostable binding of aldosterone. The thermostable binding increased gradually to reach a stable value of 11% at a physiological concentration of approximately 70 mg/dl of albumin, while the thermolabile portion invariably remained unmeasurable. A further increase in albumin greatly exceeding the physiological concentrations resulted in only slight increases of thermostable binding. It may probably be concluded that any thermostable binding significantly exceeding the 11% value can be safely attributed, not to albumin, but to ABG-TS.

At present, the only indication that aldosterone binding to ABG-TS is of high affinity stems from the fact that the [^{3}H]-aldosterone bound to this fraction can be carried through four different chromatographic steps without any appreciable loss of radioactivity. Besides, exhaustive dialysis has demonstrated the reversibility of the binding in both thermostable fractions by the complete liberation of [^{3}H]-aldosterone from the bound portion.

Genetic aspects of ABG

What has been investigated is the mechanism of transmission of high ABG binding capacity among families of EH patients. The significance of this parameter is discussed later. Relatives with high thermolabile ABG binding capacity, *i.e.* ≥ 17 were designated as "affected" and are considered as carriers of the trait and those with binding < 17 as normal. Consequently, it appeared from a previous study [1] that 52% of 109 untreated hypertensive patients with either labile or stable EH with either "normal" or low plasma renin activity carried the trait.

Frequency distribution of ABG binding in 40 families. The data for all relatives from the second (GII) and third (GIII) generations were examined for indications of tri- or bi-modality by comparing their frequency distribution with a normal distribution of approximate age-matched controls with both normotensive parents. On the histogram (Fig. 3), the distribution appears unimodal in controls and bimodal in relatives. Moreover, the difference in means between the "normal" relatives and those carrying the high ABG trait was more than the sum of the two respective standard deviations.

Graphical analysis of polymodal frequency distributions on logarithmic probability paper [18, 19]: The cumulative frequency distribution of controls was unimodal (straight segment) while a sigmoid curve in relatives appeared to be the result of two or even three distinct straight segments with different slopes leading to the assumption that the distribution was bimodal or even trimodal (Fig. 4).

Pedigree analysis. In Figures 5 and 6, there are 31 pedigrees of nearly complete families with 118 males and 145 females. Figure 7 comprises 5 incomplete, similar pedigrees with 7 males and 23 females. Four additional incomplete families not shown were also considered. Subjects with high thermolabile ABG binding were considered as "affected". They carry the high ABG binding trait. All three figures and Tables 4 and 5 usually contain three and sometimes two-generational, family subgroups with two grandparents or untreated parents which can be subdivided into three categories: In the first (a) (Table 4) both parents had normal aldosterone binding plasma ABG; in the second (b) both parents had elevated binding; in the third category (c) (Table 5) one parent exhibited increased and one normal ABG binding. Shaded symbols in Fig. 5 represent four individuals (F7 II-4, F15 II-4, F19 II-2, and F30 II-2) with thermolabile ABG binding, usually close to the upper limit of normal

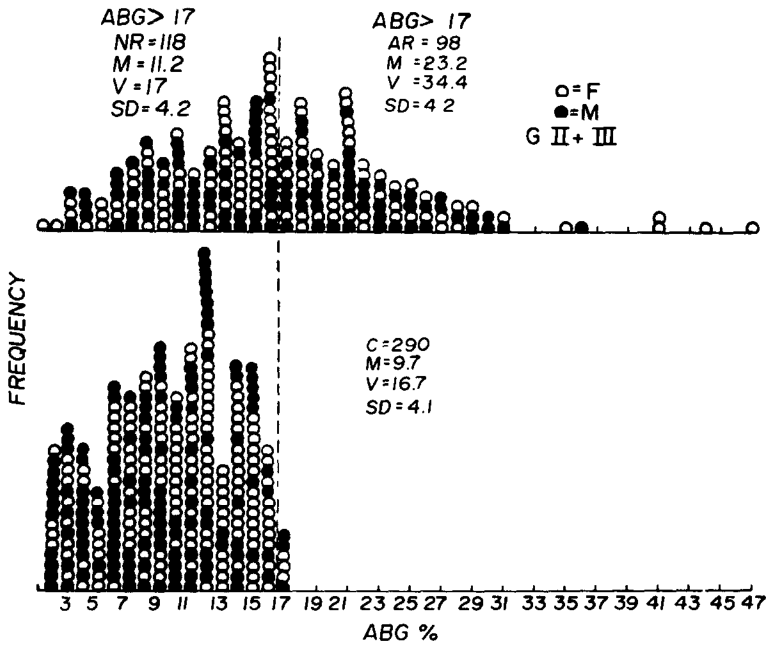

Fig. 3. Frequency distribution in 118 untreated designated "normal" relatives (NR), 98 untreated designated "affected" relatives (AR) and 290 controls.

range, but whose binding to ABG-TS as well as TCS were significantly increased (Table 4). These individuals were also considered as carrying the trait.

Sex repartition. The number of females 176 was higher than the number of males 128 but the ratio of males and females among "affected" relatives is not different from that in all relatives in GII $\chi^2 = 1.22$ (NS) and in GIII $\chi^2 = 1.33$ (NS) indicating no sex linkage of the trait.

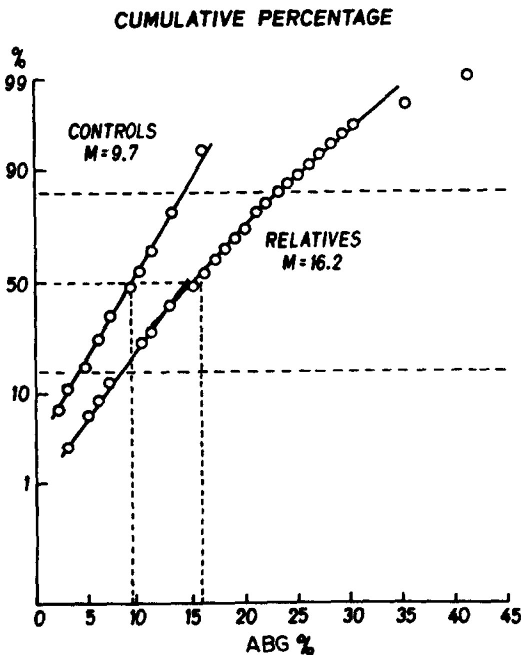

Fig. 4. Cumulative frequency distribution (percentage) of plasma ABG binding capacity in 290 controls and 216 untreated relatives on logarithmic probability paper.

Vertical transmission of the trait. The transmission through three generations, of the actually measured, elevated thermolabile ABG binding occurred 12 times (Figs 5–7; families F20, F9, F19, F8).

Family subgroups with both untreated "affected" parents of GII had 62 "affected" and 36 "normal" offspring.

Similarly, a vertical transmission of "normal" binding in families with both untreated parents in the GII exhibiting "normal" binding and all resulting children being "normal" occurred 20 times (Figs. 5–7).

Thus, the trait appears to be transmitted vertically from grandparents to parents and grandchildren with no skipping of generations.

Mode of inheritance

Segregation ratios in familial subgroups. Several conclusions can be drawn from the segregation pattern shown in Figs 5–7 and Tables 4 and 5. From eight "normal" × "normal" matings among "normal" untreated parents in the GII, none of the 20 progeny had high ABG binding (Table 4 group (a)). Approximately only 52% of the treated parents would be expected to have elevated ABG binding. (1) Because many of them had been treated with antihypertensive drugs, some of which apparently tend to lower this binding (*i.e.* spironolactone), this figure is lower. It is impossible, therefore, to identify all families with both grandparents (GI) or parents (GII) with high binding. Consequently, in order to find out if theoretical ratio 1:3 is present, only those families with two parents or children, exhibiting high ABG levels were considered (Table 3 group (b)). In the children of five families with both untreated parents, the ratio of "normal" to high binding was 3:11, very close to the

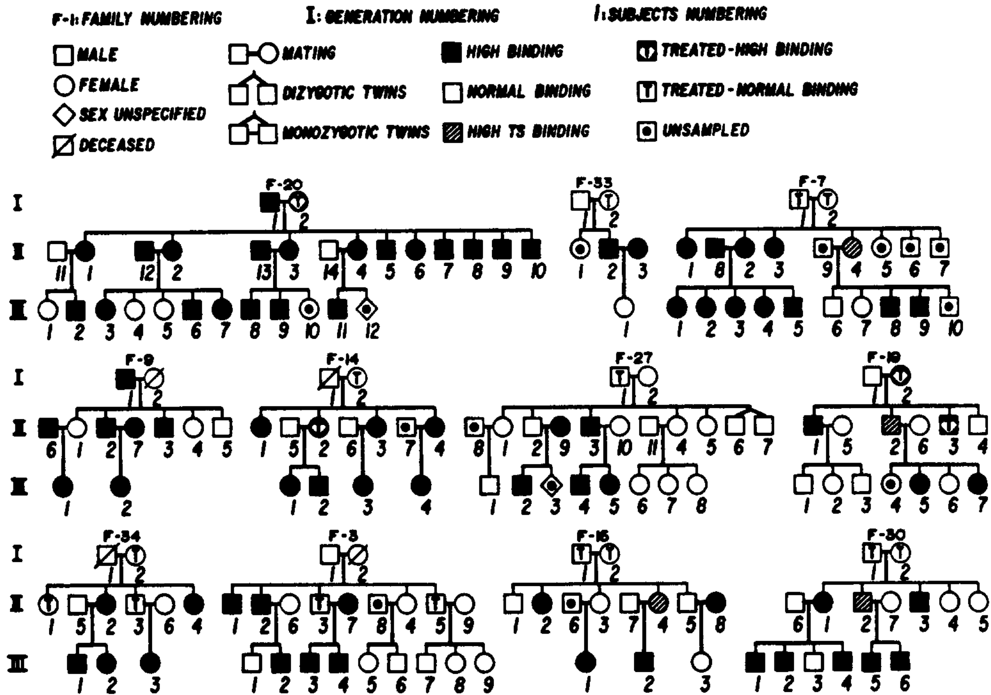

Fig. 5. Pedigrees of 11 families.

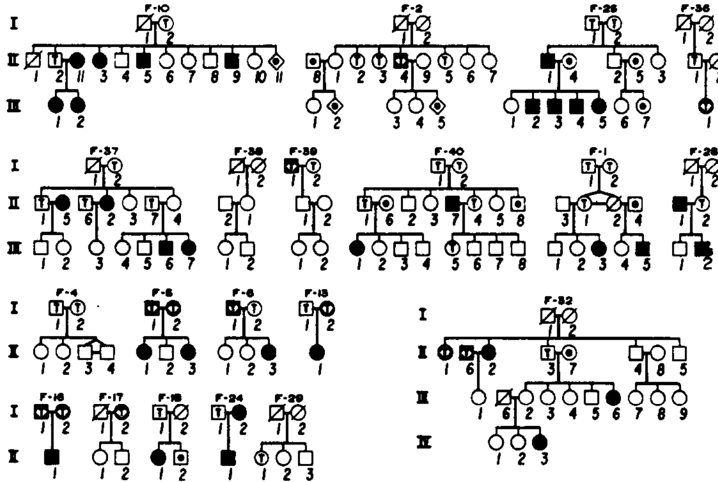

Fig. 6. Pedigrees of 20 families.

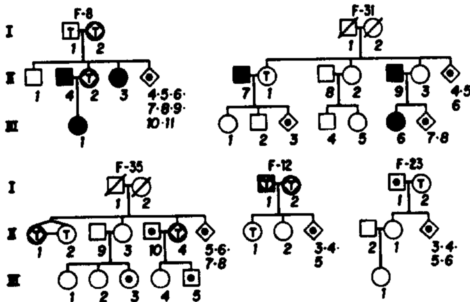

Fig. 7. Pedigrees of 5 incomplete families.

expected figure. When treated parents (five families) with an elevated, actually measured binding were also included, this ratio became 5:25. Since no antihypertensive treatment seems to elevate this binding there would not appear to be any reason to exclude these values.

The ratio of "normal" to "affected" relatives resulting from "affected" × "normal" matings among relatives with two or more children is 14:16, or not very different from the expected 1:1 ratio (Table 5 group (c)) from 7 additional similar matings, a single "affected" child resulted.

Table 4. Families with untreated parents

(a) Both parents exhibiting normal ABG binding	Pedigrees Fig:	(b) Both parents exhibiting high ABG binding	Pedigrees Fig:
F23 II-1,2; III-1	7	F7 II-8,2; III-1,2,3,4,5	5
F27 II-1,8; III-1 and		F9 II-2,7; III-2 and	5
II-4,11; III-5,6,7	5	F20 II-2,12; III-3,4,5,6,7	
F31 II-2,8; III-4,5	7	and II-3,13; III-8,9	5
F32 II-4,8; III-7,8,9	6	F33 II-2,3; III-1	5
F35 II-3,9; III-1,2	7	F34 II-2,5; III-1,2	5
F38 II-1,2; III-1,2	6	same as (b) but with one or both parents treated.	
F39 II-1,2; III-1,2	6	F5 I-1,2; II-1,2,3	6
		F8 II-2,4; III-1	7
		F12 I-1,2; II-1,2	7
		F16 I-1,2; II-1	6
		F20 I-1,2; II-1,2,3,4,5,6,7,8,9,10	5
		F32 II-2,6; III-1	6

Table 5. Families with untreated parents one exhibiting normal and one high ABG binding who had more than one child (c)

	Pedigrees Fig:	Comments:
F3 II-2,6; III-1,2 and		
II-9,5; III-7,8,9	5	II-5 Cortisone treated
F19 II-1,5; III-1,2,3 and		
II-2,6; III-4,5,6,7	5	II-2*
F20 II-1,11; III-1,2 and		
II-4,14; III-10	5	II-4*
F27 II-3,10; III-3,4	5	
F30 II-1,6; III-1,2,3,4 and		
II-2,7; III-5,6	5	II-2*
Same as (c) but with one treated parent exhibiting high ABG binding		
F2 II-4,9; III-3,4	6	
F14 II-2,5; III-1,2	5	
F19 I-1,2; II-1,2,3,4	5	

* Increased thermostable homologue ABG-TS and high TCS.

Frequency of "affected" relatives in GII. When all first and second generation relatives were considered together, and after having made assumptions that approx. 52% of treated grandparents carry the trait, that those with high ABG-TS are similarly "affected" (Table 5 group (c)) and that approx. 52% of treated parents (GII) also carry the trait, it is possible to test if the expected frequency of "affected" subjects in GII is in agreement with the hypothesis of a genetic transmission.

Expected proportions. The proportion of 52% of "affected" grandparents allows for the assumption of the following frequency of three different types of matings among the GI where all subjects are hypertensive:

Both parents "affected"—$(0.52)^2$
Only one parent "affected"—$2 \times 0.52 \times 0.48$
None "affected"—$(0.48)^2$

On the basis of the Hardy-Weinberg's law for random-mating pairs [15–17] one can estimate that if the frequency of the dominant allele (M) in grandparents (GI) is p, and the frequency of the recessive allele (m) is q:

$$p^2 + 2pq = 0.52 \quad \text{and} \quad q^2 = 0.48,$$

where p^2 represents the homozygotes (MM) for the allele and $2pq$ the heterozygotes (Mm). From this, it can be deduced that for an "affected" GII subject taken at random the probability to be homozygote (MM) or heterozygote (Mm) is:

$$p(\text{MM})\frac{p^2}{0.52} \quad \text{and} \quad p(\text{Mm})\frac{2pq}{0.52}$$

Therefore, the expected frequency of "affected" relatives out of the 136 in the GII is 0.5193.

Observed proportions. There were 50 "affected" untreated; 29 treated of whom 52% were assumed "affected"; *i.e.* 15; giving a total of 65 "affected" subjects.

Testing the divergence between expected and observed values is.

$$\chi^2 = 0.43; \alpha = 0.50 \text{ NS}.$$

As expected the repartition of p^2, $2pq$ and q^2 is verified among the second generation and is not significantly modified in relation to the first generation because of no new contribution of genes. The above is compatible with the hypothesis of a genetic inheritance of the trait.

The same computation cannot unfortunately, for the time being, be applied to the GIII since the frequency of the trait among the spouses in the GII remains unknown.

DISCUSSION

Familial study of aldosterone ABG binding led to additional inquiries of some closely related subjects such as: the improvement of the ABG binding capacity procedure; study of the effects of spironolactone on ABG binding and the detection of a novel plasma and urinary homologue. As decribed under Methods, the procedure for plasma aldosterone binding capacity had to be slightly modified because of the as yet unelucidated differences in this binding in some EH patients. In all of them and in some others, there was an increase in a novel thermostable fraction ABG-TS which appears to be similar to a urinary counterpart ABG-TSu.

By applying the technique of two successive ultrafiltrations to normal urines it was found that the 10,000–50,000 urinary ultrafiltrate contained two protein fractions similar to plasma. The component ABG-TS isolated by ion exchange chromatography from the plasma appeared to be identical to urine ABG-TSu and possibly also to plasma TS previously isolated by DEAE Sephadex A-50 chromatography [8]. Investigations and partial characterisation of these protein fractions were necessary for the pedigree analysis in familial studies.

Also, measured TCS and plasma CBG capacity in the majority of relatives and controls as well as in some EH patients, indicated a decrease in corticosteroids bound to CBG in presence of normal levels of circulating TCS in EH patients and their relatives. This is in contrast to an increase in thermolabile ABG binding of aldosterone and points to selective modifications in both binding systems. CBG capacity unlike ABG binding, was also unaffected by antihypertensive treatment. Decreased CBG capacity has already been occasionally reported in hypertension [21, 22] as well as no change in this parameter with age [23]. These latter conclusions further indicate that aldosterone and other corticosteroids are bound to different and distinct plasma protein fractions.

Some of our data show that approximately therapeutic doses of potassium canrenoate decrease the plasma thermolabile ABG binding capacity of aldosterone in some EH patients. This may support the findings that only about 30% of EH patients, 42% of which received aldactazide (at the time of the study, between 1976–78), usually in association with other drugs, exhibit high ABG binding, in contrast to an incidence of about 52% in untreated patients previously studied [1]. It is suggested that the observed decrease in plasma thermolabile ABG binding in treated patients may be due to a competitive displacement of aldosterone by spironolactone leading to a decline in percentage binding. Because the *in vivo* effects of spironolactone were only suggestive, they were further supported by a significant *in vitro* displacement of [^{3}H]-aldosterone from ABG binding by potassium canrenoate added to plasma samples. It may be that one of the possible mechanisms of action of spironolactones is an increase in the turnover rate of aldosterone due to this displacement.

Total plasma aldosterone, with blood samples obtained between midafternoon and early evening after normal daily activities, was corrected for the levels in the early follicular phase of the menstrual cycle in females, but not for age or the circadian rhythm. For this reason, these values cannot be compared with previously reported levels measured under very different sampling conditions [1]. In "affected" relatives, total plasma aldosterone was similar to that of "normal" relatives or controls but the free fraction was significantly lower.

Genetic aspects

The frequency of observed "affected" children in GII is no different from the frequency of "affected" relatives in GI when the assumption that any treated hypertensive is a potential carrier of the trait, is considered. The results are consistent, according to the Hardy-Weinberg's law, with a genetic transmission of the trait, but they do not indicate either a dominant or recessive hypothesis.

Further genetic analysis had to be carried out, however, on familial subgroups comprising untreated subjects only, due to the fact that it is not possible at present to identify all of the treated subjects carrying high ABG binding trait. Consequently, there are some additional findings which do support a possibility of a dominant transmission of high ABG binding: (1) The bimodality in the untreated relatives, as shown by the frequency distribution either on the histogram or on the logarithmic probability paper; (2) the transmission through three generations of high binding actually measured without any skipping of generations and the evidence of "affected" relatives in early childhood (the trait appearing as early as at age 4 and probably before). It should be pointed out here that a direct transmission through three generations of an inherited defect is practically diagnostic of dominant inheritance. (3) The proportion of "affected" children, when one or two parents are "affected" in clear-cut situations where treated relatives are not considered. These proportions suggest again a dominant rather than a recessive hypothesis if the proportion of heterozygosity is kept in mind.

If the inheritance of high ABG is of this type, we could expect two or more polymorphic forms of the protein since a monogenic defect is more frequently qualitative than quantitative. Our electrophoretic patterns and isoelectrofocusing revealing the apparent presence of a single homogeneous protein do not exclude the presence of microheterogeneity [6, 9]. However, both qualitative and quantitative variations may be associated with identifiable gene differ-

ences [16]. The variation in ABG binding capacity in our study can be clearly subdivided into two non-overlapping or only slightly overlapping distributions and then its inheritance can be studied using standard techniques of Mendelian analysis [16]. If individuals fall into one or more essentially non-overlapping ranges of measurement, indicated by bimodal familial distribution in this study, a quantitative character can be subdivided into qualitatively different categories.

It must be pointed out that because the affinity of the binding might be altered by certain drugs (spironolactones) and a variety of physicochemical changes, including an increase in ACTH, circulating cortisol [24], oestrogens [6] and pregnancy [5], factors other than molecular alterations or absolute concentrations could play a role in the altered binding capacity seen in EH subjects. However, it seems reasonable to assume that in untreated and relatively unstressed subjects, the increased binding capacity of ABG, measured following a 2:45 h equilibration of the sample with the [^{3}H]-aldosterone, was due rather to a proportionately increased ABG concentration in the plasma.

An association between a familial increase in ABG and the blood pressure has not been clearly established in this study. This may be at least in part explained by the proportion of treated hypertensives that could not be considered.

The amounts of aldosterone bound to thermolabile ABG, thermostable homologue (ABG-TS) or albumin will be determined by the concentration of each protein, the respective association constants of each of the binding protein fractions, the total concentrations of aldosterone and probably also cortisol [24] in the system. Increase in cortisol which is also bound to albumin will change not only the free aldosterone concentration but also the partition of aldosterone among all three binding protein fractions probably by displacing it from the albumin binding. An increase in thermolabile ABG in the system will result in its increased binding of aldosterone and a decline in its binding to the two other protein fractions. The free aldosterone will also decline similar to OC subjects [6]. Conversely, a decrease in the thermolabile ABG in the system will result in an increase in free aldosterone and in concentration of aldosterone associated with albumin or ABG-TS. Increase or addition of free aldosterone to the system will result in proportional increases in aldosterone bound to all protein fractions and a rise in the free aldosterone with no change in the percentage of bound fraction until the total binding capacity of ABG will be reached.

It is impossible from our data to make any approximation of the frequency of high ABG binding in the general population because the control group has been selected by taking age matched subjects, with relatives neither of whose parents had essential hypertension. But this frequency is probably higher than the approximate 15% incidence of EH among the white population at large [25]. This would be in agreement with the reasoning that the elevated ABG binding represents part of the genetic susceptibility to EH and that the only predisposed individuals who may develop EH are those who live long enough and are under sufficient pressure from a multitude of environmental factors which may cause a rise in blood pressure.

In conclusion, it is proposed that high ABG binding could be inherited as part of a predisposition which given time and environmental factors may lead to EH. Since it is present only in some EH patients, this observation further indicates that EH is heterogenous in nature. Moreover, high bindings in many young children suggest that it is expressed in very early childhood. Because the pedigrees are relatively small, however, and because the antihypertensive treatment, as well as many physiological variables tend to affect this binding capacity, the interpretation of these results must be made with caution.

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