Quebec Cooperative Study of
Friedreich's Ataxia

HLA and Complement Typing in Olivo-Ponto-Cerebellar Atrophy

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SUMMARY: *HLA antigen typing was carried out in a family with an autosomal dominant form of spinocerebellar degeneration [possibly olivoponto cerebellar atrophy (O.P.C.A.) — Type I]. Eleven ataxic patients, three possibly ataxic subjects, two unrelated spouses and 13 clinically normal at risk siblings were typed for ABO and Rh blood groups, HLA-A and HLA-B antigens, C4 component of the complement and a number of other serum proteins (Cl_q, β -1A, β-1C, C5, β -lipoproteins). No solid evidence for linkage between the ataxia gene and the HLA or C4 loci could be demonstrated in this family. Certain serum proteins, and particularly β-lipoproteins were found to be significantly reduced in some sub-groups of subjects.*

RÉSUMÉ: *Nous avons fait la détermination des antigènes HLA dans une famille de dégénérescence spinocérébelleuse (possiblement O.P.C.A. type I) à transmission génétique autosomale dominante. Nous avons étudié 11 patients ataxiques, 3 possiblement ataxiques, 2 époux non parents, et 13 sujets cliniquement normaux mais génétiquement sous risques. Chez ces sujets nous avons dosé les groupes sanguins ABO, Rh, les antigènes HLA-A et HLA-B ainsi que la composante C4 du complément et un certain nombre d'autres protéines sériques (Cl_q β-1A, β-1C, C5, β-lipoprotéines). Aucune solide évidence de linkage entre le gène ataxique et les loci de HLA ou de C4 ne fut démontrée dans cette famille. Nous avons observé une diminution significative de certaines protéines sériques, en particulier les β-lipoprotéines, chez certains de ces groupes de sujets.*

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INTRODUCTION

It has been known for many years that chromosomes carry in a linear fashion the unities of heredity called genes. The localization of such gene loci on a specific chromosome awaited first the determination of the exact number of chromosomes in man (discovered only in 1956) and the development of new staining methods ("banding techniques"). In man the number of structural genes — those that determine the amino acid sequence of polypeptide chains of proteins — may be of the order of 50,000. These structural genes include those determining the basic structure of the enzymes of intermediary metabolism of the structural proteins such as those of membranes or of collagen, of all the proteins with special functions, such as the hemoglobins and the immunoglobulins, and of all regulator enzymes. The first chromosome to be studied, because of its uniqueness, was the X-chromosome. There are now more than 100 loci identified on the X-chromosome and more than 1,100 on the 22 pairs of autosomes (McKusik, 1975). The relative distance separating two gene loci on a chromosome can be inferred from the frequency with which recombinations of traits determined by genes at these loci occur among the offspring of particular parental pairs. Recently new techniques such as cell hybridization, non recombinational methods, deletion mapping and mapping by homology have added to the tools at our disposal to permit assignment of a particular gene to a given chromosome. McKusik and Ruddle (1977) have presented a complete synopsis of the status of

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the human gene map. The exact position of many gene loci is known.

The major histocompatibility complex in mouse called H-2 and in man called HLA, (for human lymphocyte antigens) has been localized upon the 6th pair of chromosomes (Lamm et al., 1974; Francke and Pellegrine, 1976). The HLA system in man is extremely polymorphic. It has many important and diverse functions. It was first recognized as a leukocyte blood group system and only then as the major histocompatibility system in man, thus permitting better matching in kidney and other organ transplants. It has recently been found that this system contains genes which control the immune response to a variety of antigens and also control some of the components of the complement cascade (Jersild et al., 1976). This has led to greater knowledge of the functions of the immune system (Svejgaard et al., 1975).

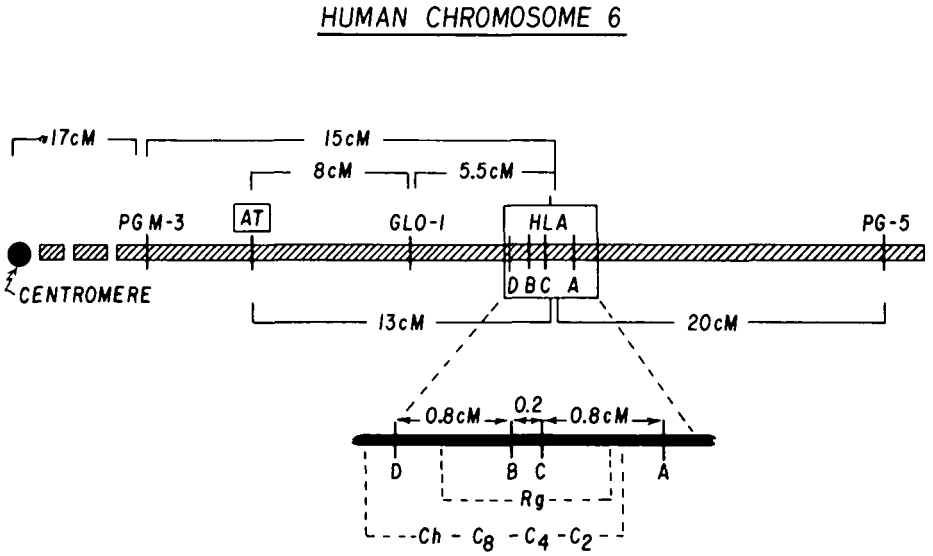

Figure 1—Mapping of the human 6th chromosome (1977) as modified from McKusick and Ruddle (1977) and Jackson et al. (1977). HLA loci are indicated as A, B, C, D: AT: presumed ataxia locus according to Jackson et al. (1977). GLO: Glyoxylase: PGM3: Phosphoglucomutase — 3; Pg: Pepsinogen; C2, C4, C8: components of the complement; Ch: chido blood group; Rg: Rogers blood group.

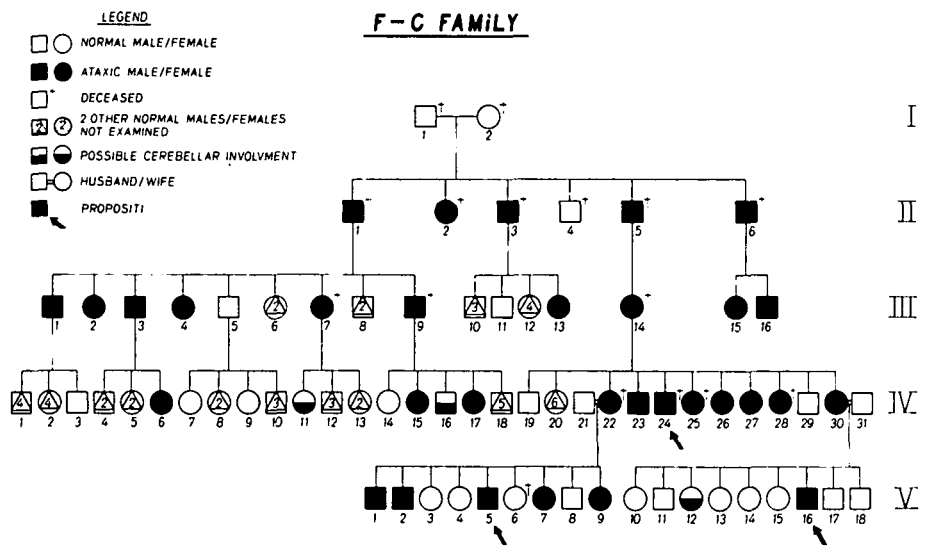

Figure 2—Abbreviated family tree of a kinship with autosomal dominant spinocerebellar degeneration (OPCA — type I) from the Gaspé Peninsula, Quebec.

The importance of the HLA system was emphasized when it was demonstrated that certain diseases occur preferentially in individuals possessing given HLA factors. These associations can be very strong — as in ankylosing spondylitis — and serve in diagnosis. For other diseases, such as multiple sclerosis and juvenile diabetes mellitus (Cudworth and Woodrow, 1975; Rubinstein et al., 1977), the demonstration of this association has opened new leads in the study of pathogenesis and etiology (Bach and van Rood, 1976; Neel, 1977). For many of these diseases the association with a given HLA antigen could be explained by linkage disequilibrium between the genes determining the antigen with which the association has been found, and the immune-response (Ir) genes. It is possible that such antigens could function as receptors for viruses and so influence the pathologic process. In *association,* a specific gene or genes are found in the same persons with a disease more often than in the general population. Such is the case for the antigen HLA-B27 which is present in more than 90% of patients with ankylosing spondylitis (Calin and Fries, 1975). On the other hand *linkage* means that two or more loci on the same chromosome are sufficiently close that they tend to segregate together. *Linkage disequilibrium* is the tendency in a population for some alleles at closely linked loci to occur together in the same haplotype (haploid genetic composition of a chromosomal region usually transmitted as a unit) more often than expected by chance (for example, in Caucasians, the HLA haplotype A1, B8 occurs considerably more often than the product of the individual frequencies of these alleles).

The first indication that an ataxia-gene locus could be on the sixth human chromosome near the HLA loci came from the studies of Yakura et al. (1974) who investigated a single family of dominant Marie's ataxia. Three of five children with this disease had received the same HLA haplotype (HLA-A9, B5) from their affected father. In contrast,

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two children who had received the other haplotype (HLA A_{11} BW_{10}) were healthy. Pursuing this lead Jackson et al. (1977) recently studied a kindred with dominantly inherited olivo-ponto-cerebellar ataxia (OPCA) type I, and demonstrated in that family the ataxia locus was situated on chromosome 6 at 12-cM (later recalculated to 13-cM) distance from the HLA-complex. From this data and that of many other recent studies, a tentative map of the human 6th chromosome can now be drawn, particularly the region near the HLA complex (Fig. 1).

Recently we had the opportunity to test this hypothesis by the identification, during our survey of Friedreich's ataxia in Quebec, of another large family (Fig. 2) with a form of autosomal dominant spinocerebellar degeneration of the olivo-ponto-cerebellar form (probably type I). In addition to the HLA antigens we also studied other serum proteins including some components of the complement.

SUBJECTS, MATERIALS AND METHODS

The French-Canadian family illustrated in figure 2 was first identified in 1965 (through patient IV-24) by Drs. Raymond Robillard and A. Roch Lecours of Montreal, who traced it back four generations. At about the same time one of us (AB) first saw independently two patients of this group (V-5 and V-16). In the summer of 1977 a field expedition was organized to Madeleine Centre, in the Gaspé Peninsula of Quebec, where most of the subjects to be studied lived. The FC family has had 48 known and definite cases of ataxia, 32 of which are illustrated in the abbreviated pedigree of Figure 2. (The others are found mainly in descendants of II-6 living elsewhere and in another branch from another Gaspé village related to the FC family three generations earlier than I-2). The field expedition permitted the examination of 44 persons (including young children) directly linked to the FC family. HLA and complement typing could be done on 29 individuals above the age of 12 (11 definitely ataxic patients; 3 with possible ataxia; 2 unrelated normal spouses of ataxia victims; 11 at risk but normal siblings and two at risk siblings too young for the clinical manifestations of the disease to have appeared).

TABLE 1

AGE OF ONSET OF DISEASE

(Difficulties in writing and staggering)

GENERATION	CASE NO.	AGE AT EXAMINATION	AGE OF ONSET	
			ONSET	MEAN
III	III-1	61	40	45.0
	III-3	63	50	
IV	IV-6	28	25	
	IV-15	43	28	28.3
	IV-30	47	32	
V	V-1	37	30	
	V-2	36	24	
	V-5	30	17	18.8
	V-7	17	14	
	V-9	21	16	
	V-16	22	12	

In this kinship the neurological disease is manifested by signs of imbalance, clumsiness, frequent falls and staggering. Difficulties in writing are usually the first symptom and have appeared at ages ranging from 12 to 55. As can be seen in Table 1, there is evidence of anticipation. There is no evidence that this is due to greater awareness of the disease or earlier examinations. Cases in the 4th and 5th generations started about the same year because of the large family-related imbalance in the ages of the parents. Once the disease is established the main symptom is a severe truncal ataxia, progressive inability to walk leading to use of canes then of a wheel-chair. Cranial nerves are normal except for occasional strabismus and a frequent bilateral horizontal nystagmus of moderate intensity. Optic atrophy is a rare finding. There is eventually severe dysrathria in most patients, but this symptom starts relatively late. The arms are strong with dysmetria, past pointing and finger-to-nose ataxia in some patients. Alter-

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TABLE 2 HLA TYPING, ABO/Rh BLOOD GROUPS AND SERUM PROTEINS

	Identification							C1q	BlA	BlA/BlC	BlC	C4	C5	β-lipo
A- Ataxic Patients	Code	Init.	Sex	Age	ABO	Rh	HLA Antigens	mg %	mg %	mg %	mg %	mg %	Mg %	mg %
(1)	III-1	W.F.	M	61	A	+	$A_2,B_{12}/A_3B_8$	34	56	81	25	23	9	660
(2)	III-3	L.F.	M	63	A	+	$A_2,B_{12}/A_3B_8$	30	68	96	28	28	11	490
(3)	IV-6	L.F.C.	F	28	A	+	$A_2,B_8/A_2,B_{12}$	37	60	86	26	34	10	330
(4)	IV-15	M.F.A.	F	28	A	+	$AW_{24},BW_{39}/AW_{29},-$	32	68	100	32	28	11	490
(5)	IV-30	G.B.	F	47	O	+	$AW_{26},B_8/A_2,B_{18}$	28	83	115	32	31	15	620
(6)	V-1	P.A.G.	M	37	O	+	$A_2,-,B_8,BW_{15}$	30	90	120	30	23	11	1160
(7)	V-2	A.M.C.	F	36	A	+	$A_9,AW_{26},B_{14},BW_{15}$	22	58	81	23	20	9	280
(8)	V-5	F.G.	M	30	ND	ND	$A_9,A_{11},BW_{15},BW_{40}$	27	58	96	28	33	10	690
(9)	V-7	L.G.	F	17	AB	+	$A_{11},B_5/AW_{29},B_{12}$	30	68	86	18	29	10	460
(10)	V-9	F.S.	F	21	O	+	$A_{11},B_5/AW_{29},B_{12}$	37	86	130	44	36	11	580
(11)	V-16	G.B.	M	22	O	+	$A_1,B_8/A_2,B_{18}$	28	49	72	23	23	10	660
B- Possible Ataxics														
(1)	IV-11	D.S.L.	F	28	A	-	$A_1,A_3,B_8,-$	21	110	165	55	23	11	820
(2)	IV-16	B.F.	M	26	O	+	A_2,A_3,B_7,B_8	52	86	120	34	24	10	490
(3)	V-12	R.B.F.	F	26	O	+	$A_2,B_5/AW_{26},B_8$	25	63	86	23	20	11	490
C- Too Young to Know														
(1)	IV-3	D.F.	M	13	A	+	$A_2B_{12}/AW_{31}BW_{40}$	42	71	100	29	31	9	350
(2)	V-15	G.B.	F	18	O	+	$A_1,B_8/A_2,B_{18}$	27	56	86	30	23	12	460
D- Unrelated Normal Husbands														
(1)	IV-21	C.G.	M	42	B	+	$AW_{24},BW_{15}/A_{29},B_{12}$	42	71	100	29	36	11	580
(2)	IV-31	A.B.	M	56	O	+	$A_1B_8/A_2,B_5$	37	63	91	28	34	14	660
E- At-Risk Normals														
(1)	III-5	P.E.F.	M	57	A	+	$A_2,B_{12}/A_3,B_8$	30	60	96	36	29	12	700
(2)	III-11	R.F.	M	51	A	+	$A_2,B_{12}/AW_{24},B_7$	54	86	130	44	66	13	1400
(3)	IV-7	D.P.	F	31	A	+	$A_2,B_{12}/A_1,B_8$	37	74	115	41	41	12	880
(4)	IV-9	S.P.	F	29	A	+	$A_2,B_{12}/A_2,BW_{15}$	27	84	260	170	44	12	580
(5)	IV-19	H.G.	M	43	A	+	$A_2,B_{18}/AW_{26},B_8$	48	105	145	40	44	13	700
(6)	V-4	D.G.	F	33	A	+	$A_{11},B_5/A_{24},B_{12}$	34	80	115	35	41	12	440
(7)	V-8	G.G.	M	23	ND	ND	$A_2,B_8/A_{29},B_{12}$	22	52	72	20	21	10	360
(8)	V-11	B.B.	M	28	O	+	$A_1,B_8/AW_{26},B_8$	27	74	100	26	23	12	620
(9)	V-13	L.B.G.	F	24	O	+	$A_1,B_8/A_2,B_{18}$	27	71	110	39	26	11	700
(10)	V-14	P.L.	F	20	O	+	$A_1,B_8/AW_{26},B_8$	21	60	81	21	16	10	520
(11)	V-17	B.B.	M	27	O	+	$A_2,B_5/A_2,B_{18}$	25	51	130	79	18	10	390

Legend

a) $A_1,B_8/A_2,B_{18}$: individual haplotypes known

b) A_2,A_3,B_7,B_8: individual haplotypes not known, genotypes only are listed.

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nate movements are performed slowly and with difficulty. The reflexes are generally hyperactive except the ankle jerk. With age and progression of the illness the reflexes tend to diminish. Clonus was seen only once. Babinski signs are present in ⅓ of the patients, occasionally only on one side. Fine tremor may be noted in both hands. Progression of the disease is slow, compatible with partial employment. Death occurs usually after the age of 60. This is only a few years before the mean life span observed in the region. However, the last 10-15 years may have been spent in a wheel-chair. Clinically, this family appears to best fit the description of Marie's ataxia or of olivo-ponto-cerebellar atrophy of the dominant type. We do not have autopsy material to confirm the clinical impression. Only one patient, previously studied by Dr. Robillard (Case IV-24/4), provided autopsy material. Macroscopic photographs (Fig. 3) showed atrophy of the cerebellum and the pons. Histopathology was not available.

As control groups, other patients with various forms of ataxia were studied. Thus, 19 patients with the spastic ataxia of Charlevoix-Saguenay (see this issue) as well as 19 of their normal at-risk siblings were studied for proteins. Sixteen patients with typical (group Ia) Friedreich's ataxia from the Montreal area were also used in these studies. Normal controls, mainly laboratory workers, were used for each of the protein determinations.

HLA typing was performed using peripheral blood lymphocyte microtoxicity techniques testing for 20 HLA-A antigens and 20 HLA-B antigens (Terasaki and McClelland, 1964; modified by Mittal et al., 1968). Antisera was obtained from Dr. Terasaki.

Serum Cl_q esterase inactivator and β-lipoprotein levels were determined by radial immunodiffusion according to Mancini et al., (1965). Antisera and standards were from Behring Worke. Serum β-1A, β-1C, C4 and C5 were determined by single radial immunodiffusion, according to the technique modified by Fahey and McKelvey (1965). Antisera and standards were from Hyland Laboratory (β-1A, β-1C) and Melloy (C4 and C5). β-1C was the difference between β-1A/β-1C and β-1A serum levels.

Linkage estimation, calculated as lod scores (logarithm of relative odds at several recombination frequencies) are being calculated by computer, but were not available in time for this issue.

RESULTS AND DISCUSSION

Inheritance in this family (Fig. 2) was of the autosomal dominant type although both subjects from generation I did not have symptoms. However, I-2 died at age 39 and could have carried the gene for two reasons: first, the age of onset in the following generation was a mean of 45.0 years (Table I); secondly, the disease is frequent in another branch of the family related to the direct ancestors of I-2. Over the 5 generations studied by us, 35 individuals of a possible 91 developed the disease.

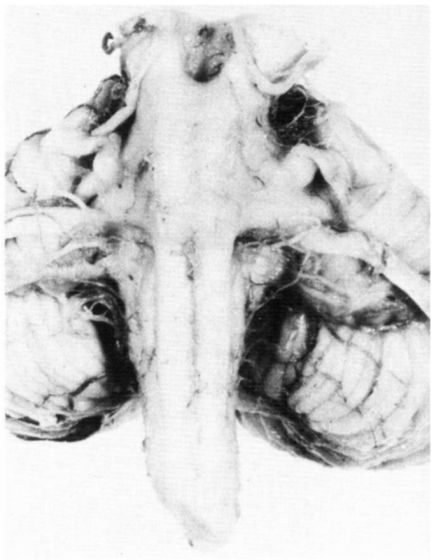

Figure 3—Photograph of the brain stem and pons of case IV-24 from the FC family (Fig. 2).

The results of HLA and serum protein typing are given in Table 2. Each individual results is given in this table, with reference to the code identification from Figure 2, so that this data can be used subsequently by other investigators who wish to pool data from many families. While awaiting analysis with the computer program before drawing final conclusions, a preliminary analysis fails to uncover strong evidence for either association, or linkage as reported by Jackson et al., (1977). As seen in Table 3 the occurrence of individual HLA antigens and HLA-haplotypes was essentially the same between known ataxic patients in the FC kinship and their at-risk but clinically normal siblings. Since unaffected individuals below the age of 20 were eliminated from compilation in this table, it is unlikely that many carriers would be included. No HLA antigen or haplotype was consistently found in the majority of ataxics in any one branch of the family, which was not found in normal at-risk siblings. This contrasts to the haplotype A_9B_5 in all affected cases of Yakura et al., (1974) and the haplotype A_3B_{14} in 13/16 ataxics in the paper by Jackson et al. (1977). The only significant difference in our series is the reduced occurrence of the A_1B_8 haplotype in affected patients. It is not known, because of the small numbers involved, if this particular haplotype (A_1B_8) could have conferred immunity against the disease (or decreased susceptibility to the disease).

Analysis of serum proteins in various forms of ataxia (Table 4) is more difficult to interpret and must remain speculative. There are a number of statistically significant differences between the groups investigated. Of the serum proteins studied, only C4 is known to be closely associated with the HLA loci (Rittner et al., 1975). In this study C4 was not deficient in any of the ataxic groups or their normal siblings. This suggests it is unlikely that the gene for the spino-cerebellar degeneration (O.P.C.A. ?) in the FC family is linked to the HLA loci.

Other serum proteins, in particular Cl_q, β-1C and C5, are significantly reduced in all groups of ataxics. The meaning of this is obscured by the fact that the normal at-risk siblings also have low values. This could be related to a general protein deficiency which is necessary in

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TABLE 3 DISTRIBUTION OF HLA ANTIGENS AND HAPLOTYPES IN THE FC KINSHIP

	ANTIGENS	ATAXICS (n = 11)	AT-RISK NORMALS (n = 11)
A Series	A_1	1	5
	A_2	7	10
	A_3	2	1
	A_9	2	0
	A_{11}	3	1
	AW_{24}	1	1
	AW_{26}	2	4
	AW_{29}	3	2
B Series	B_5	2	2
	B_7	0	1
	B_8	6	11
	B_{12}	5	6
	B_{14}	1	0
	BW_{15}	3	2
	B_{18}	2	3
	BW_{39}	1	0
	BW_{40}	1	0

HAPLOTYPES	ATAXICS (n = 11)	AT-RISK NORMALS (n = 11)
A_1 B_8	1	4
A_2 B_8	1 + (1)	1
A_2 B_{12}	3	4
A_2 B_5	0	1
A_2 BW_{15}	(1)	1
A_2 B_{18}	2	3
A_3 B_8	2	1
A_{11} B_5	2	1
AW_{24} BW_{39}	1	0
AW_{29} B_{12}	2 + (1)	2
AW_{26} B_8	1	3
AW_{24} B_7	0	1

(n): haplotype deduced

these families as a background for the appearance of the true genetic defect.

A similar observation is the significant decrease in β-lipoproteins observed in spastic ataxia and Friedreich's ataxia. The decrease also noted in the at-risk normal siblings could be accounted for by the presence of a number of heterozygote carriers. Four of the normal at-risk subjects had values well below one SD from the normal mean. Without these 4 subjects the normal at-risk mean was 797.1 mg%. Low β-lipoproteins are well known in the Bassen-Kornzweig syndrome (1950) and in a dominant form of hypo- β-lipoproteinemia (Mars et al., 1969). Both of these diseases have a clinical picture often similar to Friedreich's ataxia.

Our failure to confirm the findings

TABLE 4 SERUM PROTEINS IN ATAXIA

GROUPS	$C1_q$ mg %	B1A mg %	B1A/B1C mg %	B1C mg %	C4 mg %	C5 mg %	β-LIPO mg %
1. Dominant OPCA - Ataxics	30 ± 4*	68 ± 12	97 ± 17*	28 ± 6*	28 ± 5	11 ± 1*	600 ± 156
(n)	(11)	(11)	(11)	(11)	(11)	(11)	(11)
2. Dominant OPCA - At-Risk Normals (n)	32 ± 10*	72 ± 15	123 ± 48	50 ± 40	33 ± 14	11 ± 1*	663 ± 276
	(11)	(11)	(11)	(11)	(11)	(11)	(11)
3. Recessive Spastic Ataxia Ataxics. (n)	28 ± 6*	74 ± 13	102 ± 21*	28 ± 10*	31 ± 10	11 ± 2*	534 ± 191*
	(19)	(19)	(19)	(19)	(19)	(19)	(19)
4. Recessive Spastic Ataxia At-Risk Normals (n)	30 ± 8*	74 ± 16	102 ± 19*	28 ± 7*	36 ± 10	11 ± 1*	588 ± 178*
	(19)	(19)	(19)	(19)	(19)	(19)	(19)
5. Recessive Friedreich's Ataxia (n)	27 ± 4*	76 ± 23	105 ± 28*	31 ± 7*	30 ± 12	12 ± 2	569 ± 277*
	(16)	(16)	(16)	(16)	(16)	(16)	(16)
6. Normal Controls (n)	43 ± 13	77 ± 20	135 ± 45	44 ± 7	30 ± 10	13 ± 4	713 ± 248
	(29)	(45)	(78)	(45)	(105)	(68)	(44)

* $p < 0.001$

** $p < 0.05$

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of Jackson et al., (1977) is not a negation of the validity of their observation, but only that our FC kinship probably suffers from another genetic form of O.P.C.A. Pooling of our data with theirs in a computer program may reveal where the differences lie.

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