# Aggregation of Subclinical Autonomic Nervous System Dysfunction and Autoantibodies in Families With Type I Diabetes

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**The purpose of our study was to evaluate the occurrence of autonomic nervous system autoantibodies (ANS) in the nondiabetic family members of insulin-dependent (type I) diabetic subjects. We studied 24 families, including 45 nondiabetic parents and 53 nondiabetic siblings of a type I diabetic proband. One hundred one nondiabetic population control subjects were also studied. Stored sera from nondiabetic family members and control subjects were evaluated for the presence of complement-fixing (CF) adrenal medullary antibodies (CF-ADM), sympathetic ganglia antibodies (CF-SG), and vagus nerve antibodies (CF-V) by indirect immunofluorescence. HLA-DR3 and -DR4 typing was performed on 42 nondiabetic family members and 104 diabetic subjects. One or more CF-ANS were in 45 of 93 (40%) nondiabetic family members compared to 2 of 70 (2.8%) control subjects. CF-SG were in 28 of 92 (30%) family members compared to 0 of 101 control subjects ($P = 0.0001$). CF-V were in 25 of 95 (26%) family members compared to 0 of 76 control subjects ($P = 0.0001$). CF-ADM were in 10 of 83 (12%) family members compared to 2 of 70 (2.8%) control subjects ($P = 0.056$). There was no HLA-DR3 or HLA-DR4 association with ANS. Subclinical autonomic dysfunction was demonstrated in 3 of 4 family members with autoantibodies compared to 0 of 4 family members without autoantibodies. *Diabetes* 40:1611–14, 1991**

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We have previously described the presence of complement-fixing (CF) autonomic nervous system autoantibodies (CF-ANS) in the sera of subjects with pre-insulin-dependent (type I) and type I diabetes (1–5). Subjects with complement-fixing anti-adrenal medullary (CF-ADM) and anti-sympathetic ganglia (CF-SG) autoantibodies have a greater drop in systolic blood pressure with standing than subjects without autoantibodies (2). These antibodies are associated with diminished norepinephrine and epinephrine levels with orthostasis (4). Vagus nerve antibodies (CF-V) are associated with a lower postural brake index (5).

The purpose of this study was to evaluate the occurrence of CF-ANS in the nondiabetic family members of type I diabetic subjects and to look for subclinical changes in autonomic function among antibody-positive versus antibody-negative subjects.

## RESEARCH DESIGN AND METHODS

We studied 24 families, including 45 nondiabetic parents and 53 nondiabetic siblings (age 1–57 yr) of a type I diabetic proband. All family members were part of the prediabetes screening program of the Joslin Center. They were given a questionnaire asking for symptoms of diabetes. All subjects were screened for islet cell antibodies (ICAs). Nondiabetic family members denied symptoms of diabetes and were $ICA^-$. All subjects >40 yr old were screened with a fasting glucose level <6.72 mM in all subjects. HLA-DR3 and -DR4 typing were performed in 42 nondiabetic family members and 104 diabetic subjects. One hundred one population control subjects (age 10–65 yr) were also studied. Sera were stored at −20°C before use in the assays.

Assays for CF-ADM, CF-SG, and CF-V antibodies were performed as follows. An indirect immunofluorescence

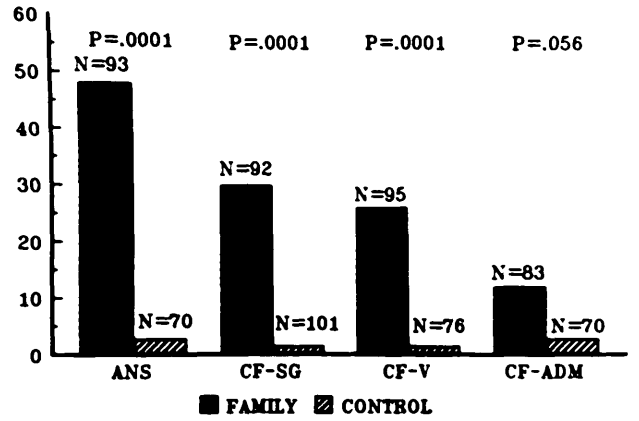


**FIG. 1. Prevalence of autonomic nervous system autoantibodies (ANS) in nondiabetic family members (*solid bars*) versus control subjects (*hatched bars*). CF-SG, complement-fixing sympathetic ganglia autoantibodies; CF-V, complement-fixing vagus nerve autoantibodies; CF-ADM, complement-fixing adrenal medullary autoantibodies.**

technique was used as previously described (1,2,5). Normal human adrenal gland, rabbit sympathetic ganglia and vagus nerve were used as substrates for the adrenal medullary, sympathetic ganglia, and vagus nerve assays, respectively. Cryostat sections of the appropriate tissue were incubated with sera from nondiabetic family members and control subjects. Fresh human serum was used as a complement source. Fluorescein isothiocyanate anti-human C3C (Calbiochem-Behring, La Jolla, CA) was used as the detecting agent. Coded sections were scored for fluorescence intensity in a blinded fashion as previously described (1,2,5).

HLA-DR3 and -DR4 typing was performed in the laboratory of C. Alper (Center for Blood Research, Harvard Medical School, Boston, MA) with a microlymphocytoxicity assay (6).

Cardiovascular autonomic function was measured in eight family members (4 CF-ANS$^+$ and 4 CF-ANS$^-$) >30 yr old. Mean age for both the antibody-positive and antibody-negative groups was 46 yr. The Valsalva index was calculated after blowing into a sphygmomanometer for 20 s while maintaining a constant pressure of 40 mmHg. The test was repeated twice. The mean circular resultant (MCR) was calculated from a paced breathing maneuver of 25 10-s cycles. The MCR is a vector analysis of the R-R variation with deep breathing. It bears less relationship to the underlying basal heart rate than does the expiration-inspiration (E/I) ratio (7). Valsalva index and MCR results were compared with age-matched normative data. Valsalva index was not obtained in one patient. Subjects were considered to have subclinical autonomic dysfunction if either the Valsalva index or MCR were abnormal.

Statistical analysis was conducted with Pearson's $\chi^2$ test and Student's *t* test.

## RESULTS

One or more CF-ANS were in 45 of 93 (40%) nondiabetic family members compared to 2 of 70 (2.8%) control subjects ($P = 0.0001$) (Fig. 1). CF-SG were in 28 of 92 (30%) family members compared to 0 of 101 control subjects ($P = 0.0001$). CF-V were in 25 of 95 (26%) family members compared to 0 of 76 control subjects ($P = 0.0001$). CF-ADM were in 10 of 83 (12%) family members compared to 2 of 70 (2.8%) control subjects ($P = 0.056$).

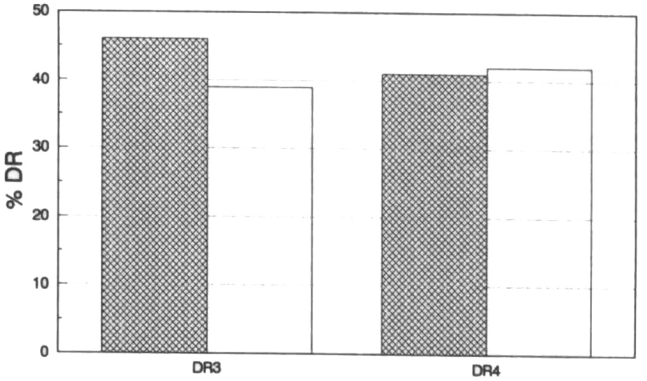


**FIG. 2. Autonomic autoantibodies versus DR type. *Crosshatched bars*, ANS$^+$; *open bars*, ANS$^-$.**

HLA-DR3 and -DR4 typing were performed in 42 nondiabetic family members and 104 diabetic subjects. There was no HLA-DR3 or -DR4 association with CF-ANS (Fig. 2). We were unable to demonstrate an HLA-DR3 or -DR4 association for CF-SG, CF-V, or CF-ADM when analyzed separately. There was a trend toward a negative HLA-DR3 association with CF-ADM.

Cardiovascular autonomic function was evaluated in nondiabetic family members >30 yr old. Four subjects were positive for one or more CF-ANS, and four subjects were CF-ANS$^-$. The mean Valsalva index was 1.21 for the antibody-positive group and 1.84 for the antibody-negative group ($P < 0.04$). Two antibody-positive subjects had frankly abnormal values for their ages (Fig. 3). The mean MCR for the antibody-negative group was 50. The mean MCR for the antibody-positive group was 39. The difference between groups did not achieve statistical significance with a few subjects. However, one antibody-positive subject had an abnormal MCR (Fig. 4). An abnormality in cardiovascular autonomic function as de-

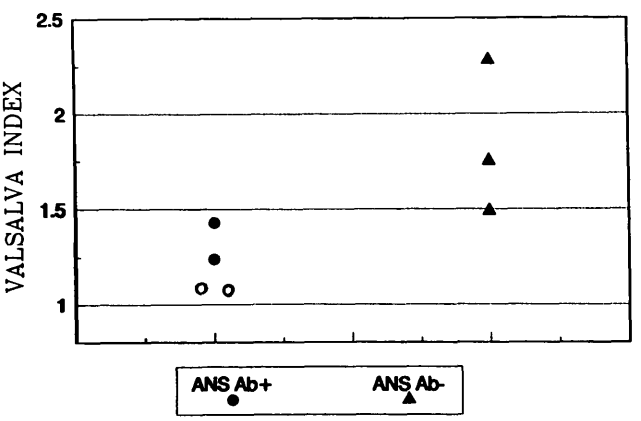


**FIG. 3. Valsalva index in nondiabetic family members >30 yr old. ●, Subjects with autonomic nervous system autoantibodies (ANS Ab); ▲, subjects without ANS Ab. Two Ab$^+$ subjects had frankly abnormal values for their age (○).**

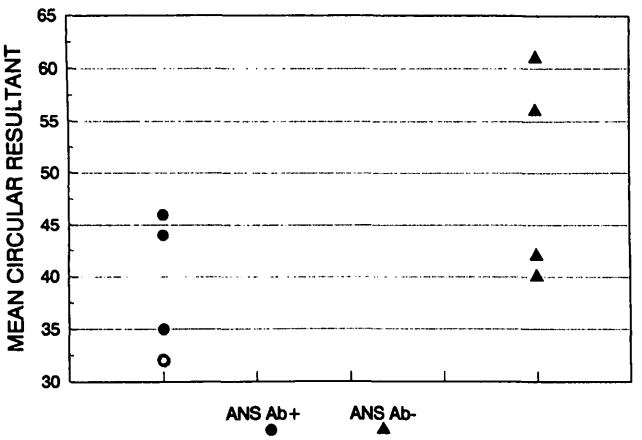


**FIG. 4. Mean circular resultant (MCR) in nondiabetic family members >30 yr old. ●, Subjects with autonomic nervous system autoantibodies (ANS Ab); ▲, subjects without ANS Ab. One Ab$^+$ subject had an abnormal value (○).**

fined by either abnormal Valsalva index or MCR occurred in three of four antibody-positive subjects compared with zero of four antibody-negative subjects ($P = 0.14$ by Fisher's exact test).

## DISCUSSION

We have demonstrated ANS autoantibodies in nondiabetic family members of type I diabetic patients. The antibodies have been described previously in pre–type I diabetes when subjects are still euglycemic (1,2). The data suggest that there is a familial aggregation of ANS. The presence of these antibodies is not associated with HLA-DR3 or -DR4, the haplotypes associated with the development of type I diabetes. Subclinical autonomic nervous system dysfunction is also found in nondiabetic subjects with these antibodies. Our study evaluated two generations of family members including parents and siblings of a type I diabetic proband. Large multigenerational kindreds are needed to determine whether the autonomic defects are inherited in a recognizable pattern.

The concept of inherited neuropathies is not new (8,9). Hereditary motor and sensory neuropathies (HMSN) are heterogeneous disorders (10). HMSN 1 and 2 may display autosomal-dominant and autosomal-recessive inheritance. Within families, phenotypic expression of the dominantly inherited disorders is highly variable, with differences noted in both age of onset and severity. In different families, linkage of HMSN has been demonstrated to the Duffy blood group on chromosome 1 (11,12) and to the pericentromeric region of chromosome 17 (13,14). Chronic relapsing inflammatory polyneuropathy is a steroid-responsive chronic progressive form of Guillian-Barré syndrome that may be linked to HLA-A1, -B8, and -DW3 (15,16). This disease may affect the autonomic, motor, and sensory portions of the nervous system. Dyck et al. (17) reported that some patients with HMSN exhibit a clinical course similar to chronic relapsing inflammatory polyneuropathy. These studies thus suggest a genetic susceptibility to inflammatory demyelination in families with HMSN (17).

The existence of an inherited tendency toward other diabetic complications is debated. Increased capillary basement membrane thickening has been noted in nondiabetic first-degree relatives of diabetic patients (18). An HLA association for capillary basement membrane thickening has been suggested by one study (19). Some studies support an association between HLA and retinopathy, although this is controversial (20,21).

We hypothesize that there is an underlying genetic predisposition toward the development of autonomic neuropathy in the families of subjects with type I diabetes. This may be an immunologic process directed against nerve tissues. Environmental and metabolic factors such as hyperglycemia or neurotoxins might accelerate the pathological process. Prospective follow-up of nondiabetic relatives with subclinical autonomic dysfunction is necessary to determine whether there is progression to overt autonomic dysfunction in a euglycemic setting. Evaluation of large multigenerational kindreds for linkage analysis of autonomic defects to the Duffy blood group on chromosome 1 and to the pericentromeric region of chromosome 17 should also be performed.

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## REFERENCES

1. Brown FM, Kamalesh M, Sesh Adri MN, Rabinowe SL: Anti-adrenal medullary antibodies in IDDM subjects and subjects at high risk of developing IDDM. *Diabetes Care* 11:30–33, 1988
2. Rabinowe SL, Brown FM, Watts M, Kadrofske MM, Vinik AI: Anti-sympathetic ganglia antibodies and postural blood pressure in IDDM subjects of varying duration and patients at high risk of developing IDDM. *Diabetes Care* 12:1–6, 1989
3. Brown FM, Vinik AI, Ganda OP, Adri MNS, Rabinowe SL: Different effects of prevalence on anti-adrenal medullary and pancreatic islet cell antibodies in type I diabetes mellitus. *Horm Metab Res* 21:434–37, 1989
4. Brown FM, Brink SJ, Freeman R, Rabinowe SL: Anti-sympathetic nervous system autoantibodies: diminished catecholamines with orthostasis. *Diabetes* 38:938–41, 1989
5. Rabinowe SL, Brown FM, Watts M, Smith AM: Complement-fixing antibodies to sympathetic and parasympathetic tissues in IDDM: autonomic brake index and heart-rate variation. *Diabetes Care* 13:1084–88, 1990
6. Bodmer WF, Batchelor JR, Bodmer JG, Festenstein H, Morris PJ, (Eds.): *Histocompatibility Testing 1977*. Copenhagen, Munksgaard, 1978, p. 612
7. Schumer M, Burton G, Burton C, Crum D, Pfeifer MA: Diabetic autonomic neuropathy. I and II. *Am J Med* 85 (Suppl. 5A):137–46, 1988
8. Thomas PK: Inherited neuropathies. *Mayo Clin Proc* 58:476–80, 1983
9. Rabinowe SL: Immunology of diabetic and polyglandular neuropathy. *Diabetes Metab Rev* 6:169–188, 1990
10. Bird TD: Hereditary motor-sensory neuropathies: Charcot-Marie-Tooth syndrome. *Neurol Clin North Am* 7:9–23, 1989
11. Bird TD, Ott J, Giblett ER: Evidence for linkage of Charcot-Marie-Tooth neuropathy to the Duffy locus on chromosome 1. *Am J Hum Genet* 34:388–94, 1982
12. Guiloff RJ, Thomas PK, Contreras M, Armitage S, Schwarz G, Sedgwick

EM: Linkage of autosomal dominant type I hereditary motor and sensory neuropathy to the Duffy locus on chromosome 1. *J Neurol Neurosurg Psychiatry* 45:669–74, 1982
13. Patel PI, Franco B, Garcia C, Slaugenhaupt SA, Nakamura Y, Ledbetter DH, Chakravarti A, Lupski JR: Genetic mapping of autosomal dominant Charcot-Marie-Tooth disease in a large French-Acadian kindred: identification of new linked markers on chromosome 17. *Am J Hum Genet* 46:801–809, 1990
14. McAlpine PJ, Feasby TE, Hahn AF, Komarnicki L, Jame S, Guy C, Dixon M, Qayyum S, Wright J, Coopland G, Lewis M, Kaito H, Philipps S, Wong P, Coopman W, Cox DW, Yee WC: Localization of a locus for a Charcot-Marie-Tooth neuropathy type Ia to chromosome 17. *Genomics* 7:408–15, 1990
15. Adams D, Festenstein H, Gibson JD, Hughes RAC, Jaraquemada J, Papasteriadis C, Sachs J, Thomas PK: HLA antigens in chronic relapsing idiopathic inflammatory polyneuropathy. *J Neurol Neurosurg Psychiatry* 42:184–86, 1979
16. Steward GJ, Pollard JD, McLeod JG, Wolnizer CM: HLA antigens in the Landry-Guillain-Barre syndrome and chronic relapsing polyneuritis. *Ann Neurol* 4:285–89, 1978
17. Dyck PJ, Swanson CJ, Low PA, Bartleson JD, Lambert EH: Prednisone-responsive hereditary motor and sensory neuropathy. *Mayo Clin Proc* 57:239–46, 1982
18. Ganda OP, Williamson JR, Soeldner JS, Gleason RE, Kilo C, Kaldany A, Miller JP, Garavoy MR, Carpenter CB: Muscle capillary basement membrane width and its relationship to diabetes mellitus in monozygotic twins. *Diabetes* 32:549–56, 1983
19. Marks JF, Raskin P, Stastny P: Increase in capillary basement membrane width in parents of children with type I diabetes mellitus: association with HLA-DR4. *Diabetes* 30:475–80, 1981
20. Baker RS, Rand LI, Krolewski AS, Maki T, Warram JH, Aiello LM: Influence of HLA-DR phenotype and myopia on the risk of non-proliferative and proliferative diabetic retinopathy. *Am J Ophthalmol* 102:693–700, 1986
21. Middleton D, Johnston PB, Gillespie EL: HLA-DR antigen association with proliferative retinopathy. *Int Ophthalmol* 8:33–35, 1985