Association Between Osteoprotegerin G1181C and T245G Polymorphisms and Diabetic Charcot Neuroarthropathy

A case-control study

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OBJECTIVE — Charcot neuroarthropathy is a disabling complication of diabetes. Although its pathogenesis remains unknown, we suppose that genetics may play a relevant role.

RESEARCH DESIGN AND METHODS — We performed a case-control study with 59 subjects with diabetic Charcot neuroarthropathy (Ch group), 41 with diabetic neuropathy without Charcot neuroarthropathy (ND group), and 103 healthy control subjects (H group) to evaluate the impact of two single nucleotide polymorphisms (SNPs) of the osteoprotegerin gene (G1181C and T245G) on the risk of Charcot neuroarthropathy.

RESULTS — Regarding the SNPs of G1181C, we found a significant linkage between the G allele and Charcot neuroarthropathy (Ch vs. ND, odds ratio [OR] 2.32 [95% CI 1.3–4.1], P = 0.006; Ch vs. H, 2.10 [1.3–3.3], P = 0.002; and ND vs. H, 0.80 [0.7–1.9], P = 0.452); similarly, we found a linkage with the G allele of T245G (Ch vs. ND, 6.25 [2.2–19.7], P < 0.001; Ch vs. H, 3.56 [1.9–6.7], P = 0.001; and ND vs. H, 0.54 [0.6–5.7], P = 0.304), supporting a protective role for the allele C and T, respectively. For this reason we investigated the frequency of the protective double homozygosis CC + TT (7% in Ch) that was significantly lower in Ch compared with H (0.18 [0.06–0.5], P = 0.002) and with ND (0.17 [0.05–0.58], P = 0.006), whereas there was no difference between H and ND (1.05 [0.43–2.0], P = 0.468). In a multivariate logistic backward regression model, only weight and the lack of CC and TT genotypes were independently associated with the presence of Charcot neuroarthropathy.

CONCLUSIONS — This is the first study that shows an association between genetic regulation of bone remodeling and Charcot neuroarthropathy.

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Charcot neuroarthropathy is a chronic and progressive disease of bone and joints, defined by painful or relatively painless bone and joint destruction, in limbs that have lost sensory innervation; it is characterized by pathological fractures, joint dislocation, and deformity (1). With the decline in numbers of cases of tertiary syphilis, the primary etiology today is diabetes. The incidence is ~0.1–5% in diabetic patients with peripheral neuropathy, but it is likely that many cases are undiagnosed (2). The majority of patients with Charcot neuroarthropathy are from 50 to 60 years old, and most will have had diabetes for at least 10 years (3,4).

The pathogenesis of Charcot neuroarthropathy is still unknown, but it is undoubtedly multifactorial (1,5); probably this is one of the reasons that there is no pharmacological treatment available to stop the progress of the disease. The difference between the higher prevalence of diabetic neuropathy and the lower prevalence of Charcot neuroarthropathy (neuropathy seems to be necessary but not sufficient for its presence) and the different clinical features of the two conditions support the hypothesis of the probable involvement of other factors in its pathogenesis.

A common feature of Charcot neuroarthropathy is bone reabsorption, and the association between diabetes and osteoporosis could contribute to the presence of Charcot neuroarthropathy (6–8). Indeed, the study of bone turnover markers in acute Charcot neuroarthropathy shows that there is an increase in osteoclast activity compared with osteoblast activity (9); this can lead to osteopenia, which could predispose to fracture, even as a consequence of minimal trauma.

New insights into the regulation of osteostalagogenes have resulted from the discovery of three members of the tumor necrosis factor (TNF) and TNF receptor superfamily; one of these receptors, osteoprotegerin (OPG), is an important regulator of bone remodeling (10). OPG gene single nucleotide polymorphisms (SNPs) have been associated with osteoporosis (11,12) and are considered early predictors of cardiovascular disease (13). Two of the most studied polymorphisms are G1181C (located in exon I) and T245G (located in the promoter region); the latter is in complete linkage with A163G and G209A polymorphisms (14). Because of their regulatory function in bone remodeling and for their involvement in the pathogenesis of osteoporosis, we focused our investigation on these two...
Neuropathy Disability Score (15). All diabetic subjects had a definite diagnosis of peripheral neuropathy with a biothesiometer, according to Young et al. The vibration perception threshold was performed with a DIABETES CARE, VOLUME 32, NUMBER 9, SEPTEMBER 2009

RESULTS — Table 1 shows the clinical and laboratory characteristics of the Ch, H, and ND groups. Comparison of OPG genotypes showed significant differences in the frequencies of alleles between Ch versus ND and Ch versus H, whereas ND and H were not different (Table 2). We found a positive association with the G allele of G1181C in Ch compared with ND (OR 2.32 [95% CI 1.3–4.1], \(P = 0.006\) and 2.10 [1.3–3.3], \(P = 0.002\), respectively), whereas H and ND were overapped (0.90 [0.7–1.9], \(P = 0.452\); regarding T245G, we showed a strong positive association with the G allele in Ch compared with ND (0.25 [2.2–19.7], \(P < 0.001\) and 3.56 [1.9–6.7], \(P = 0.001\), respectively), whereas there were no differences between H and ND (0.54 [0.6–5.7], \(P = 0.304\). Because the frequencies of C (G1181C) and T (T245G) alleles were lower in Ch, we analyzed the distribution of the protective double homozygosis CC + TT, which was significantly lower in Ch (frequency 7%) compared with that in H (0.18 [0.06–0.5], \(P = 0.002\) and ND (0.17 [0.05–0.58], \(P = 0.006\), whereas there was no difference between H and ND (1.05 [0.43–2.0], \(P = 0.468\). Thus, the risk to have Charcot neuroarthropathy in diabetic and neuropathic subjects with CC/TT homozygosis is approximately six-fold lower (1/OR CC + TT). In a multivariate logistic backward regression model built using Charcot disease as a dependent variable and SNPs and clinical/laboratory values as independent variables (Table 1), only weight and the lack of CC and TT genotypes were independently associated with the presence of Charcot neuroarthropathy (1.07 [1.03–1.12], \(P = 0.001\); 0.17 [0.04–0.71], \(P = 0.013\); and 0.06 [0.01–0.36], \(P = 0.002\), respectively). For example, in our population, subjects without TT polymorph-
phisms have a 16-fold higher risk of Charcot neuroarthropathy (1/OR TT [0.06]), indicating the protective role played by the alleles C and T, respectively.

Conduction velocity and amplitude, Neuropathy Disability Score, and Autonomic Neuropathy Score were similar between Ch and ND (Table 1); moreover, no significant difference was found in a comparison of these four variables in relation to OPG SNPs (data not shown).

Genetic distribution of both SNPs were in Hardy-Weinberg equilibrium. There was a weak linkage disequilibrium between the two SNPs ($D' = 0.330$) analyzed.

**CONCLUSIONS** — The difference between the high prevalence of diabetic neuropathy compared with the low prevalence of Charcot neuroarthropathy is a disease in which genetics plays an essential role.

**Table 1—Clinical and laboratory characteristics of Charcot, neuropathic + diabetic, and healthy subjects**

|                       | Ch group | ND group | H group | $P^*$  |
|-----------------------|----------|----------|---------|-------|
| A1C (%)               | 8.2 ± 2.4$^1$ | 8.0 ± 1.8$^2$ | 5.1 ± 0.4$^1,2$ | <0.001 |
| Disease duration (years) | 20 ± 11  | 21 ± 10  |         |       |
| Weight (kg)           | 97 ± 16$^1,2$ | 84 ± 17$^1,3$ | 74 ± 42$^3$ | <0.001 |
| Waist circumference (cm) | 115 ± 14$^1,2$ | 106 ± 12$^1,3$ | 97 ± 52$^3$ | <0.001 |
| Age (years)           | 59 ± 9$^1$ | 64 ± 10$^3$ | 62 ± 6  | 0.013 |
| Sex (male/female)     | 39/19    | 26/13    | 63/40   | NS    |
| Total cholesterol (mg/dl) | 178 ± 47 | 182 ± 38 | 182 ± 38 | NS    |
| LDL cholesterol (mg/dl) | 105 ± 35 | 103 ± 35 | 103 ± 35 | NS    |
| HDL cholesterol (mg/dl) | 43 ± 0   | 45 ± 12  | 45 ± 12  | NS    |
| Triglycerides (mg/dl) | 161 ± 98$^1$ | 144 ± 67$^1$ | 144 ± 67$^1,2$ | <0.001 |
| Conduction velocity (m/s) | 32.2 ± 4.5 | 33.2 ± 4.2 |         | NS    |
| Conduction amplitude (µV) | 1.2 ± 0.4 | 1.3 ± 0.4 |         | NS    |
| NDS: 6-7-8-9-10$^T$  | 10-13-17-13-6 | 7-8-12-11-3 |         | NS    |
| ANS: 3-4-5-6-7-8$^T$ | 8-16-14-14-5-2 | 6-12-10-9-3-1 |         | NS    |

Data are means ± SD. *Significance of difference for the correspondent row. If the $P$ value is significant ($<0.05$), apex numbers locate the difference between corresponding row subgroups. $^T$Data regarding Neuropathy Disability Score (NDS) (16) and Autonomic Neuropathy Score (ANS) (17) are expressed as frequencies of patients belonging to each score (e.g., Charcot: NDS 7, 3 patients; ND: ANS 4, 12 patients).

**Table 2—Frequencies of G1181C and T245G genotypes**

|         | ND group | Ch group | H group |
|---------|----------|----------|---------|
| G1181C  |          |          |         |
| CC      | 15 (36.5) | 6 (10.1) | 34 (33) |
| GC      | 19 (46.3) | 34 (57.6) | 50 (48.5) |
| GG      | 7 (17.2)  | 19 (32.3) | 19 (18.5) |
| T245G   |          |          |         |
| GG      | 0 (0)    | 7 (11.9) | 2 (1.9) |
| GT      | 4 (9.75) | 16 (27.1) | 14 (13.6) |
| TT      | 37 (90.25) | 36 (61) | 87 (84.5) |

Data are absolute number (%). The differences between the groups (Ch vs. ND, $P < 0.001$; ND vs. H, NS; and Ch vs. H, $P < 0.001$) were analyzed with the $X^2$ test.

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