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Concise Report

The role of HLA-DRB1 shared epitope alleles in predicting short-term response to leflunomide in rheumatoid arthritis

G. Saruhan-Direskeneli[1], M. Inanc[2], I. Fresko[3], N. Akkoc[4], E. Dalkilic[5], E. Erken[6], Y. Karaaslan[7], G. Kinikli[8], F. Oksel[9], S. Pay[10], E. Yucel[11], S. P. Yentür[1], J. Duymaz-Tozkir[1], V. Yilmaz[1], N. Inanc[12], H. Yazici[1], M. Konice[2] and H. Direskeneli[12]

Objectives. To investigate the role of shared epitope (SE) alleles in the short–term clinical response to leflunomide for the treatment of active RA.

Methods. In an open-label, multi-centre study of 16-weeks duration, 93 patients (82% female) fulfilling ARA 1987 RA criteria were treated with leflunomide (100 mg loading dose for 3 days, then 20 mg/day as the maintenance dose). The primary efficacy criterion was the response status according to the European League Against Rheumatism (EULAR) response criteria using Disease Activity Score-28 (DAS28) activity measure. SE determinations have been undertaken by polymerase chain reaction and sequence–specific oligonucleotide genotyping methods.

Results. The mean (S.D.) Disease Activity Score-28 (DAS28) was 5.1 (1.3) before the treatment, which was significantly decreased after 16 weeks [3.0 (1.1), $P < 0.001$]. According to the EULAR response criteria, 55 patients (59.1%) were classified as good responders. SE was positive in 51 (54.8%) of the patients, with 13 (13.9%) having SE homozygosity or carrying any two SE alleles. Among SE-positive patients, 68.6% (35/51) were good responders, compared with 47.6% (20/42) in SE negatives ($P = 0.04$). No difference was present according to SE hetero- or homozygosity (68.4 *vs* 69.2%). RF was also present significantly more frequently in the SE-positive group compared with negatives (78.4 *vs* 57.1%, $P = 0.03$). However, no significant difference was observed in the prevalence of RF positivity in patients with a good clinical response (72.7 *vs* 63.2%, $P = 0.32$).

Conclusions. The results suggest that HLA–DRB1 SE presence may favourably affect the outcome of leflunomide monotherapy in an unselected group of RA patients with an active disease and naive to leflunomide.

Key words: Leflunomide, Rheumatoid arthritis, Shared epitope.

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by synovitis, joint damage and functional disability. Although the pathogenesis is unknown, a strong genetic association of RA with particular HLA–DRB1 alleles, called shared epitope (SE) (DRB1*0401, *0404, *0405, *0408, *0101, *1001 and *1402), is observed in various populations [1]. Shared epitope alleles are also associated with RA in Turkey [2]. However, whether the determination of SE status is clinically useful in treatment choices is insufficiently investigated. In this study, we aimed to evaluate the effects of SE presence in the short–term clinical response to leflunomide, a novel DMARD for the treatment of active RA.

Materials and methods

In this multi-centre (11 sites), open, non-comparative study of 16 weeks duration, 122 patients fulfilling ARA 1987 criteria with an active disease [Disease Activity Score-28 (DAS28) >3.2] were screened. Patients were unselected for their previous DMARD use including methotrexate, but were naive for leflunomide. One hundred and thirteen patients, eligible for the study, took at least one dose of leflunomide and were analysed once for efficacy. One hundred and three patients completed the 16-week treatment and assessment period. Among this group, 93 with DNA available for analysis were investigated for SE genetic analysis.

Primary efficacy criteria were the good response status according to the European League Against Rheumatism (EULAR) response criteria by using the DAS28 activity measure (week 16 DAS ≤2.4 and decrease >1.2). After a 4-week run-in/wash-out period, when only non-steroidal anti-inflammatory drugs and low-dose corticosteroids (<10 mg/day prednisolone) were allowed, subjects received a 100 mg loading dose of leflunomide for the first 3 days, followed by a maintenance dose of 20 mg once daily. The study drug was stopped in 2.7% (3/113) of the patients because of the side effects.

SE determinations have been undertaken by polymerase chain reaction and sequence-specific oligonucleotide (PCR-SSO) genotyping methods. The second exon of HLA-DQA1 and DQB1 genes was amplified by PCR using the protocols of the XII HLA workshop. PCR products were dot-blotted and hybridized with biotinylated SSO probes, as described previously [3, 4]. In the sequence-specific hybridizations, the reactions with 11 probes for DQA1 and 33 probes for DQB1 locus were screened. The study was approved by the local ethical committees and informed consent was taken according to the Declaration of Helsinki.

Statistical analysis was performed with the chi-squared test, Fisher's exact test and paired *t*-test where appropriate.

Results

Eighty-two percent of our patient group were female. The mean age (S.D.) of the patients was 45.8 (11.5) yrs and the mean disease duration was 5.3 (2.8) yrs. RF was positive in 68.8% (64/93).

[1]Department of Physiology, [2]Division of Rheumatology, Istanbul Faculty of Medicine, [3]Division of Rheumatology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, [4]Division of Rheumatology, 9 Eylul Faculty of Medicine, Izmir, [5]Division of Rheumatology, Bursa State Hospital, Bursa, [6]Division of Rheumatology, Cukurova University Medical School, Adana, [7]Division of Rheumatology, Ankara Numune Hospital, [8]Division of Rheumatology, Ankara University Medical School, Ankara, [9]Division of Rheumatology, Ege University Medical School, Izmir, [10]Division of Rheumatology, GATA Medical School, [11]Division of Rheumatology, Baskent University Medical School, Ankara and [12]Division of Rheumatology, Marmara University Medical School, Istanbul, Turkey.

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Correspondence to: H. Direskeneli, Division of Rheumatology, Marmara University Hospital, Tophanelioglu Cad. 13/15, Altunizade, Istanbul, Turkey. E-mail: direskeneli@superonline.com

TABLE 1. Baseline clinical analysis according to SE presence

	SE+ ($n=51$)	SE− ($n=42$)
PJC, mean (S.D.)	18.2 (6.6)	19.7 (7.1)
SJC, mean (S.D.)	10.9 (5.7)	11.0 (5.3)
DAS28, mean (S.D.)	5.1 (0.7)	5.2 (1.8)
Morning stiffness (h)	2.2 (2.1)	2.2 (3.5)
ESR (mm/h)	40.1 (21.2)	53.2 (35.4)
Rheumatoid factor (%)	78.4	57.1

PJC, total painful joint count; SJC, total swollen joint count; ESR, erythrocyte sedimentation rate.

TABLE 2. EULAR clinical outcome according to SE alleles

	Good-response (%) (n)	Moderate/non-response (%) (n)
SE+ ($n=51$)	68.6 (35)	31.4 (16)
SE− ($n=42$)	47.6 (20)	52.4 (22)
SE+/+ ($n=38$)	68.4 (26)	31.6 (12)
SE+/− ($n=13$)	69.2 (9)	30.8 (4)
*0101 ($n=16$)	81.3 (13)	18.8 (3)
*0404 ($n=12$)	66.7 (8)	33.3 (4)
*0405 ($n=10$)	60 (6)	40 (4)
*0401 ($n=8$)	75 (6)	25 (2)

The mean (S.D.) DAS28 score was 5.1 (1.3) before the treatment, which was significantly decreased after 16 weeks [3.0 (1.1), $P<0.001$]. Similarly, the painful joint count and swollen joint count also decreased significantly [18.9 (6.9) and 10.9 (5.5) to 6.7 (6.9) and 3.9 (3.7), respectively, both $P<0.001$]. Erythrocyte sedimentation rate fell from 46.5 (29.0) to 35.3 (28.1) mm/h ($P<0.001$) and morning stiffness from 2.2 (3.0) to 0.3 (0.4) h ($P=0.007$). According to the EULAR response criteria, 55 patients (59.1%) were classified as good responders, 28 patients (30.1%) as moderate responders and 10 patients (10.8%) as non-responders.

SE was positive in 51 (54.8%) of the patients and 13 (13.9%) had SE homozygosity or carried any two SE alleles. When patients were stratified according to SE presence at baseline, RF was observed to be significantly more frequent in the SE-positive group compared with the negatives (78.4 *vs* 57.1%, $P=0.03$) (Table 1). However, no significant difference was observed in the prevalence of RF positivity in patients with a good clinical response (72.7 *vs* 63.2%, $P=0.32$).

Among SE-positive patients, 68.6% (35/51) were good responders, compared with 47.6% (20/42) in SE negatives ($P=0.04$) (Table 2). No difference was present according to SE hetero- or homozygosity (68.4 *vs* 69.2%) (Table 2). In another perspective, SE positivity was present in 63.6% of good responders, compared with 42.1% in moderate/non-responders. Clinical response was then analysed according to specific SE alleles. In DRB1*0101- and *0401-positive groups, a good response was more commonly observed compared with other alleles, however, without reaching statistical significance (81.3 and 75%, respectively).

Discussion

Our results suggest that SE-positive patients may respond more favourably to leflunomide monotherapy, when analysed according to their SE status. This observation might have been influenced by the baseline differences between the groups and, indeed, RF positivity was higher among SE-positive patients. However, in contrast to SE presence, RF positivity did not influence a good EULAR response to leflunomide, suggesting that SE is an independent factor in our study.

Dougados *et al.* [5] investigated the potential predictive factors for the treatment response to leflunomide. Only ARA functional class I and disease duration were marginally important factors, whereas age, sex, disease activity, DMARD use and disease severity were not associated with clinical response. No SE analysis was performed in this study. Few studies have investigated the effects of SE presence for specific treatment responses to DMARDs. O'Dell *et al.* [6] demonstrated that SE-positive patients were more likely to achieve an ACR50 response if treated with triple therapy compared with methotrexate (MTX) alone. Lard *et al.* [7] have also shown that early and aggressive combination DMARD treatment decreases the negative influence of SE presence on radiological damage in early RA patients.

In routine practice, Gonzales-Gay *et al.* [8] have shown that patients who require a combination of MTX and cyclosporin carry SE alleles more frequently compared with patients continuing without cyclosporin. Response to TNF-α-antagonists is also investigated in relationship to SE presence with controversial results [9, 10]. Criswell *et al.* [9] have demonstrated that *0404- and *0101-containing SE haplotypes are significantly associated with response to etanercept treatment in early RA. However, in another study, although patient selection for infliximab treatment is associated with SE presence, clinical response cannot be predicted by HLA typing [10].

A major difference of our study compared with the recent literature is the evaluation of responses to a single DMARD according to SE status, enabling us to show the effects of SE presence on the outcome of monotherapy in RA. Our results suggest that SE presence favours a good outcome in patients using leflunomide monotherapy; whether this effect would diminish with an aggressive approach is currently unclear. Möttönen *et al.* [11] have previously shown that delay of therapy is the only factor affecting disease remission in early RA patients in a study comparing single *vs* combination DMARD use. However, SE was present in a larger group (78%) of the patients in this study, which might have influenced the results.

Major limitations of our study are its open-label design, short duration and small sample size. The lack of a control group also limits us from arriving at a definite conclusion of a clinical response related to leflunomide. However, as the first study investigating the role of SE alleles in clinical response to leflunomide monotherapy, we have shown that SE alleles might influence the clinical outcome of RA patients in the short term. Further studies with larger sample sizes are required to confirm whether long term clinical efficacy and structural changes are also affected by the genetic status of RA patients with leflunomide monotherapy.

Rheumatology key message

- In an unselected group of RA patients who are active and naive to leflunomide, HLA-DRB1-SE allele presence may affect the outcome of leflunomide monotherapy favourably.

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References

1 Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987;30:1205–13.
2 Istanbul Rheumatology Study Group, Saruhan-Direskeneli G. Shared epitope homozygosity is strongly associated with rheumatoid arthritis in Turkey. Br J Rheumatol 1998;37:1126–28.

3 Bignon JD, Fernandez-Vina MA. Protocols of the 12th International Histocompatibility Workshop for typing of HLA class II alleles by DNA amplification by the polymerase chain reaction (PCR) and hybridization with sequence specific oligonucleotide probes. In: Charron D, ed. HLA 1996. Paris, France: EDK, 1997;584.
4 Saruhan–Direskeneli G, Uyar FA, Bakar S, Eraksoy M. Molecular analysis of HLA-DRB1, -DQA1 and -DQB1 polymorphism in Turkey. Tissue Antigens 2000;55: 171–4.
5 Dougados M, Emery P, Lemmel EM *et al.* Efficacy and safety of leflunomide and predisposing factors for treatment response in patients with rheumatoid arthritis: RELIEF 6-month data. J Rheumatol 2003;30:2572–9.
6 O'Dell JR, Nepom BS, Haire C *et al.* HLA-DRB1 typing in rheumatoid arthritis: predicting response to specific treatments. Ann Rheum Dis 1998;57:209–13.
7 Lard LR, Boers M, Verhoeven A *et al.* Early and aggressive treatment of rheumatoid arthritis patients affects the association of HLA class II antigens with progression of joint damage. Arthritis Rheum 2002;46:899–905.
8 Gonzales-Gay MA, Hajeer AH, Garcia-Porrua C *et al.* Patients chosen for treatment with cyclosporine because of severe rheumatoid arthritis are more likely to carry HLA-DRB1 shared epitope alleles and have earlier disease onset. J Rheumatol 2002;29:271–5.
9 Criswell LA, Lum RF, Turner KN *et al.* The influence of genetic variation in the HLA-DRB1 and LTA-TNF regions on the response to treatment of early rheumatoid arthritis with methotrexate or etanercept. Arthritis Rheum 2004;50:2750–6.
10 Marotte H, Pallot-Prades B, Grange L *et al.* The shared epitope is a marker of severity associated with selection for, but not with response to, infliximab in a large rheumatoid arthritis population. Ann Rheum Dis 2006;65:342–7.
11 Möttönen T, Hannonen P, Korpela M *et al.* Delay to institution of therapy and induction of remission using single-drug or combination-disease modifying anti-rheumatic drug therapy in early rheumatoid arthritis. Arthritis Rheum 2002;46:894–8.