Postoperative evaluation of patients with OPLL is usually focused on neurological condition; however, many complain of disability, even if their neurological condition is improved. On the other hand, there are very few reports concerning the ADL or quality of life (QOL) of patients with OPLL. Matsunaga et al.[8] evaluated the ability to live without the assistance of an attendant, though they did not use any functional index. To the best of our knowledge, this is the first study to use a functional index, BASFI, for evaluating ADL of patients with OPLL.

AS predominantly affects the spine,[2] and a typical feature is so-called bamboo spine that is a result of marked ectopic bone formation of the spinal ligaments. Similar to AS, patients with OPLL also show marked ectopic bone formation of the posterior longitudinal ligament of the spine. Major complaints of both OPLL and AS patients are disability and stiffness as a result of the common condition, spinal immobility. To evaluate the limits of physical function in patients with AS, some functional indexes have been established.[3-5] One, the Health Assessment Questionnaire (HAQ),[6] was developed primarily for rheumatoid arthritis patients and the questions are weighted toward problems caused by peripheral joint involvement. Daltroy et al. modified the HAQ for spondyloarthropathies by adding some questions concerning spinal involvement, sacroiliitis, and oligoarthritis.[4] Another study[13] noted that the three questions added to form the BASFI improved its content validity and the spectrum of item difficulty, as compared with the Dougados Functional Index.[5] Two of these three additional questions are suitable for evaluating spinal condition in AS; thus, we considered the BASFI to be suitable for evaluating ADL of postoperative patients with OPLL.

The question "looking over your shoulder without turning your body" (number 8) addresses the cervical spine, which was shown by Daltroy et al. to be important in their development of an AS-specific version of the Health Assessment Questionnaire (HAQ).[4] Interestingly, the mean score for this question was lowest among all answers in the present study, which seems to reflect well the spinal immobility of our patients. A previous study found that 70% of total axial rotation occurred between the occipital and the C2 vertebrae.[9] However, in most cases, OPLL is seen in the lower cervical spine. A likely explanation for the low score for question number 8 is a side effect of postoperative treatment. Each patient was fitted with a Philadelphia-type plastic collar for 3 months after surgery; therefore, we considered that this restriction of cervical rotation was partially a result of contracture of the atlantoaxial joint. Patients with cervical spondylotic myelopathy who received the same postoperative therapy in our department also showed a limited rotation of the cervical spine,[14] and OPLL may enhance this tendency.

The item "reaching up to a high shelf without help or aids" (number 3) corresponds to a HAQ item and addresses an important type of functional problem related to impairment of the dorsal spine. The mean result of this question in the present study was lower than all other questions except for two. Approximately 60% of patients with diffuse idiopathic skeletal hyperostosis (DISH) have OPLL; thus, it has come to be recognized as a subtype of DISH.[11,12] Although patients with OPLL do not show marked kyphosis such as those with AS, we considered that spinal immobility of not only the cervical spine but also the thoracic and lumbar spine might have an influence on the BASFI score. Our negative result between BASFI and ROM of the cervical spine may support this idea. The present results suggest that some parts of the BASFI may demonstrate ADL and show conditions independent of the neurological condition of postoperative patients with OPLL; however, it is very difficult to assess ADL that is fully independent of neurological status. Indeed, some questions in BASFI reflected neurological condition. By attaching some conditions, such as "bending forward from the waist," BASFI was able to assess spinal immobility. This point is valuable for establishing a new functional index.

As with any study, there were some problems with our investigation. The first criticism is small sample size. Because we employed the cases that received direct examination after long-term follow-up, only 22 cases could be analyzed in this study. Accordingly, it was difficult to find a significant statistical difference in the present study. Then, to find any positive result, we divided the patients into subgroups according to the ROM in the cervical spine, and obtained a negative result. Second, we were not able to evaluate preoperative BASFI scores; thus, it was impossible to compare BASFI results before and after surgery. In the future, a prospective study should be required to assess the usefulness of BASFI for evaluating ADL in patients with OPLL.

BASFI may not sufficiently assess the ADL of postoperative patients with OPLL in its present form; however, it is one of the candidate functional indexes to evaluate the ADL of patients with OPLL. Establishment of an ideal functional index to evaluate such patients is needed.

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Association of *CYP17* with HLA-B27-Negative Seronegative Spondyloarthropathy in Japanese Males

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Susceptibility genes for seronegative spondyloarthropathy (SNSA) other than HLA-B27 remain unclarified. Sex hormones are implicated in the pathogenesis of SNSA. Cytochrome P450c17a (CYP17) is a key regulator of androgen biosynthesis, and a single nucleotide polymorphism (SNP) in the 5′-untranslated region of the CYP17 gene (*CYP17*), −34C > T, is associated with variety of diseases. We have investigated the association between the *CYP17* SNP and SNSA in Japanese males. Genomic DNA was extracted from 149 Japanese male SNSA patients and 380 controls. The *CYP17* SNP was genotyped using polymerase chain reaction-restriction fragment length polymorphism analysis. Allelic and genotypic frequencies of the SNP were compared between SNSA patients and controls, and within SNSA patients. We also computed haplotype frequencies using an expectation-maximization algorithm, analyzed the difference between SNSA and control groups, and examined the potential association of other known SNPs in the *CYP17* gene. The frequency of the −34T allele was significantly increased in HLA-B27-negative SNSA, but not in total or HLA-B27-positive SNSA when compared to controls. The T allele was more prevalent in HLA-B27-negative SNSA than in HLA-B27-positive SNSA, and the T/T genotype was over-represented in HLA-B27-negative SNSA. Haplotype analysis did not demonstrate more significant association. The *CYP17* SNP is associated with SNSA in HLA-B27-negative Japanese males. © 2004 Wiley-Liss, Inc.

KEY WORDS: *CYP17* gene; SNP; SNSA; AS; sex hormone; androgen

INTRODUCTION

Seronegative spondyloarthropathy (SNSA) is a group of chronic inflammatory diseases characterized by inflammation of the enthesis, sacroiliac joint and spine, including ankylosing spondylitis (AS), reactive arthritis (ReA), psoriatic arthritis (PsA), pustulosis palmaris et plantaris (PPP), inflammatory bowel disease (IBD), and undifferentiated spondyloarthropathy (SpA). A crucial role for HLA-B27 in the development of SNSA, especially in AS, was found through epidemiological studies [Rubin et al., 1994]. However, twin studies have indicated the presence of other genetic factors than HLA-B27 [Jarvinen, 1995]. Our understanding of the etiology and pathogenesis of SNSA is far from complete.

Sex hormones are important in the human immunological response [Cutolo and Accardo, 1991], and a role for sex hormones in the pathogenesis of rheumatic diseases has been reported [Lockshin, 1998]. While rheumatoid arthritis (RA) is more prevalent in females, a male preponderance has been noted in SNSA, particularly in AS and ReA. Androgens have also been reported to play a role in the pathogenesis of AS [Lockshin, 1998]. The P450c17a (17a-hydroxylase; 17/20-lylase) enzyme, encoded by *CYP17*, catalyzes a rate-limiting step in androgen biosynthesis [Brentano et al., 1990]. The 5′-untranslated region of *CYP17* contains a single nucleotide polymorphism (SNP), −34C > T [Carey et al., 1994]. The two alleles of this SNP, A1 (−34T) and A2 (−34C), are distinguished by the absence or presence of *MspA1* restriction sites [Feigelson et al., 1997]. The A2 allele, which contains an additional Sp-1 binding site (CCACC box), has shown positive association with polycystic ovaries/premature male pattern baldness (PCO/MPB) [Carey et al., 1994], breast cancer [Feigelson et al., 1997], and age at onset of RA [Huang et al., 1999]. Interestingly, *CYP17* is located in chromosome 10q24.3 between *D10S192* and *D10S190*, where significant linkage has been identified through whole genome screening in British AS patients [Brown et al., 1998]. Thus, *CYP17* is a good candidate gene for SNSA.

In this study, we have investigated the association between the *CYP17* polymorphism and SNSA in Japanese males.

MATERIALS AND METHODS

Blood samples from male patients with SNSA were collected from several institutions with informed consent. The study protocol was approved by the ethical committees of participating institutions. Female patients were excluded from this study because of their scarcity and matching of sex. All AS patients fulfilled the modified New York criteria, and all other subjects fulfilled the European Spondyloarthropathy Study Group (ESSG) criteria. Male Japanese volunteers undergoing an annual health check were employed as controls as previously described [Huang et al., 1999]. A total of 149 male Japanese SNSA patients with a mean age of 41 (range 18–74) years were included in the study. The mean age of 380 controls, all Japanese males, was 49 (range 17–91) years. We examined

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TABLE I. Genotypic and Allele Frequencies of the −34T > C Polymorphism of *CYP17* in SNSA Patients

	HLA-B27(−)					
	Non-AS	AS	Total	HLA-B27(+)[a]	SNSA (total)	Control
Genotype			Genotype count (%)			
T/T	6 (55)	8 (38)	14 (44)	27 (23)	41 (28)	99 (26)
T/C	3 (27)	10 (48)	13 (41)	53 (45)	66 (44)	176 (46)
C/C	2 (18	3 (14)	5 (16)	37 (32)	42 (28)	105 (28)
Total no.	11	21	32	117	149	380
Allele			Allele count (%)			
T	15 (68)	26 (62)	41 (64)	107 (46)	148 (50)	374 (49)
C	7 (32)	16 (38)	23 (36)	127 (54)	150 (50)	386 (51)
Total no.	22	42	64	234	298	760

SNSA, seronegative spondyloarthropathy; AS, ankylosing spondylitis. *CYP17*, the cytochrome P450c17α gene.
[a]All HLA-B27-positive SNSA fulfilled the criteria for AS.

a total of 138 AS and 11 non-AS patients. Of the AS patients, 117 were HLA-B27-positive and 21 were negative; the frequency of HLA-B27 in this population is comparable to that in the Japanese AS population [Mitsui, 1999]. All of the HLA-B27-positive patients fulfilled the criteria for AS. The non-AS group included one ReA, seven PsA, one PPP, one IBD, and one undifferentiated SpA. Non-AS patients were all HLA-B27-negative. Thus, the HLA-B27-positive group consisted of 117 AS, and the HLA-B27-negative group consisted of 21 AS and 11 non-AS.

Genomic DNA was purified using a DNA Extractor WB kit (Wako Pure Chemical Industries, Japan). Genotyping of the −34C > T SNP was performed using polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis as previously reported, with slight modifications [Carey et al., 1994]. Genotyping of other SNPs in *CYP17* was performed using the Invader assay, as previously reported [Ohnishi et al., 2001].

Allelic and genotypic frequencies were compared between SNSA patients and controls, and within SNSA patients using the χ-square or the Fisher's exact tests. An odds ratio (OR) and 95% confidence interval (CI) were calculated with respect to the presence of the minor allele. A *P*-value less than 0.05 was considered to indicate statistical significance. Maximum-likelihood haplotype frequencies were computed using an expectation-maximization (EM) algorithm [Excoffier and Slatkin, 1995] on both SNSA and control groups. The population difference was calculated using the χ-squared test.

RESULTS

Allelic and genotypic frequencies of the −34C > T SNP in each group are presented in Table I. The controls were in Hardy–Weinberg equilibrium. There was no difference in allelic and genotypic frequencies between overall SNSA and controls, or between AS and controls (Table II).

Stratification according to HLA-B27 status revealed significant differences in allelic frequency between HLA-B27-negative SNSA patients and controls (Table II). HLA-B27-negative SNSA patients who were homozygous for the A1 allele were significantly increased. We observed no significant difference in allelic and genotypic frequencies between HLA-B27-positive patients and controls. The frequency of the A1 allele was significantly increased in HLA-B27-negative SNSA compared with HLA-B27-positive patients. In the HLA-B27-negative group, there was no difference in allelic and genotypic frequencies between AS and non-AS.

To find SNPs that might show more significant association than the −34C > T SNP, we further analyzed the haplotype structure around the −34C > T using several common SNPs located within *CYP17* in the IMS-JST database (http://snp.ims.u-tokyo.ac.jp/) and in our private SNP database. All

TABLE II. Tests for Association and Odds Ratios Regarding the −34T > C Polymorphism of *CYP17* in SNSA Patients

	Allele			T/T genotype		
Group compared	*P*-value	OR	95% CI	*P*-value	OR	95% CI
Control vs.						
SNSA	0.95	1.0	0.78–1.3	0.74	1.1	0.70–1.7
HLA-B27(−)	0.027	1.8	1.1–3.1	0.039	2.2	1.1–4.6
Non-AS	0.087	2.2	0.89–5.5	0.045	3.4	1.0–11
AS	0.12	1.7	0.89–3.2	0.31	1.7	0.70–4.3
HLA-B27(+)	0.37	0.87	0.65–1.2	0.55	0.85	0.52–1.4
HLA-B27(+) vs.						
HLA-B27(−)	0.011	2.1	1.2–3.7	0.026	2.6	1.1–5.9
Non-AS	0.048	2.5	1.0–6.5	0.033	4.0	1.1–14
AS	0.065	1.9	0.98–3.8	0.17	2.1	0.77–5.5
AS vs.						
Non-AS	0.08	2.3	0.91–5.8	0.072	3.5	1.0–12

SNSA, seronegative spondyloarthropathy. AS, ankylosing spondylitis. CYP17, the Cytochrome P450c17α gene. OR, odds ratio. CI, confidence interval.

SNPs tested were in nearly complete linkage disequilibrium (LD) with each other, and the entire *CYP17* gene is contained in a single LD block; among the common SNPs in *CYP17*, only a SNP, 1400G > A was not in complete LD with the −34C > T. Haplotype association analyses using −34C > T and 1400G > A did not give more significant association than that of the −34C > T alone (data not shown).

DISCUSSION

We have demonstrated that the *CYP17* SNP, −34C>T is associated with SNSA in HLA-B27-negative Japanese males. The A1/A1 genotype is over-represented in HLA-B27-negative SNSA patients. CYP17 is a key determinant of steroidogenesis from cholesterol; changes in expression and/or activity of this enzyme have significant effects on synthesis of sex hormones and glucocorticoid. The A1 allele dose not contain an additional SP-1 binding site [Carey et al., 1994], which could result in altered steroidogenesis though a decrease in *CYP17* promoter activity. Decreased androgen production may lead to immunological disturbance. Roles for sex hormones in immunoresponse and androgens as immunosupressors have been implicated [Cutolo and Accardo, 1991].

In contrast to the crucial role of HLA-B27 in AS [Rubin et al., 1994], the etiology of HLA-B27-negative SNSA is largely unknown. Susceptibility genes for SNSA in addition to HLA-B27 have been reported; however, only the association of *CYP2D6* has been replicated by more than one groups [Beyeler et al., 1996; Brown et al., 2000]. This gene is involved in the metabolism of xenobiotics, which have been shown to promote inflammation via T-cells. Our results that *CYP17* is associated with in HLA-B27-negative SNSA suggest that *CYP17* may be another susceptibility gene for SNSA. It remains to be determined whether this association is present in other populations. The prevalence of SNSA in Japanese is very different from other ethnic groups [Hukuda et al., 2001]. Further association analyses in larger populations and functional analyses are needed to determine the true significance of the polymorphism.

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