EXTENDED REPORT

Genetic characterisation of spontaneous ankylosing arthropathy with unique inheritance from Fas-deficient strains of mice

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Background: The spontaneous onset of macroscopic arthropathy in the ankle of the particular F₁ mice descended from two Fas-deficient strains of mice; a mutant substrain of MRL/Mp.Fas^{lpr} (MRL/rpl) and C3H/He.Fas^{lpr} (C3H/lpr) was recently observed.

Aim: To histopathologically characterise and genetically interpret the unique inheritance mode of disease in this arthropathy model.

Methods: MRL/rpl, C3H/lpr, (MRL/rpl × C3H/lpr; MC) F₁, (C3H/lpr × MRL/rpl; CM) F₁ and MCF₂ mice were bred under specific pathogen-free conditions. Histopathological grade of arthropathy was determined at 6 months by examination under a light microscope. To search for a linkage locus to the arthropathy, the whole genome of selected 48 male MCF₂ mice with 71 polymorphic microsatellite markers was scanned, followed by quantitative trait locus analysis.

Results: The incidence of microscopically defined arthropathy in the male and female MCF₁ groups was 100% and 19.4%, respectively. No incidence was observed in the parental strains, MRL/rpl and C3H/lpr, and in CMF₁ mice. In the MCF₁ mice, the arthropathy mainly affected the ankle joints and was histopathologically characterised by marked enthesal proliferation with chondrocytic differentiation and ossification in the ankle joints, the manifestations similar to ankylosing enthesitis reported previously. An MRL/rpl-derived autosomal dominant susceptibility locus was mapped in the distal of D7Mit68 (60 cM) to the ankylosis onset.

Conclusion: The MCF₁ mice stably develop spontaneous ankylosing disorders in the ankle, with a male predominance. The unique inheritance mode of ankylosis is possibly interpreted by the genetic interaction between the autosomal dominant locus and a Y-linked locus.

Multifactorial diseases develop under a proper genetic condition with a particular environmental condition. Animal models have provided experimental opportunities for understanding what and how genetic conditions contribute to the pathogenesis of human diseases. We introduce a new mouse model with progressive joint disease histopathologically resembling human ankylosing disorders.

Spontaneous joint disorders have been characterised in several mouse models. Previous studies using these models have delineated two distinct types of joint disease. One type is classified as a synovitis-driven joint disease, which represents histopathological and immunological features of human rheumatoid arthritis.^{1–3} The other type is classified as an enthesitis-driven joint disease. Recent studies have suggested a close relationship between enthesitis-driven joint disease and human ankylosing disorders, including spondyloarthritis and psoriatic arthropathy.^{4–7} In contrast with the erosive manifestation of joint cartilage and bone in the former type, proliferation and ossification in the enthesal region are characteristic of the latter type. The pathogenic mechanism in an enthesitis-driven joint disease has been underscored in relation to the regeneration and ageing-related change of joint as well as ankylosing disorders.

The present model spontaneously and stably develops an enthesitis-driven joint disease in the ankle joints of the particular F₁ mice that are descended from a recently established immunodeficient strain and a C3H/He.Fas^{lpr} (C3H/lpr) strain. These two strains are defective in the Fas gene function. The Fas gene encodes a receptor that mediates the death signal in a variety of immune cells. Therefore, a deleterious mutation of Fas causes a congenital

lymphoproliferative disorder in both humans and mice, in which such lupus-like diseases conditionally present as glomerulonephritis, systemic vasculitis, sialoadenitis and arthritis. The immunodeficient strain used in this study was recently isolated as a spontaneous mutant strain of MRL/Mp.Fas^{lpr}, termed as MRL/rpl. We recently identified a loss-of-function mutation on the signalling lymphocyte activation molecule-associated protein (SAP) gene in the MRL/rpl strain by positional cloning.⁸ Of particular interest, the regression of autoimmune traits and lymphoproliferation is a characteristic of MRL/rpl in the presence of the Fas^{lpr} mutation. SAP is a signal adaptor protein for lymphocytic activation.^{9–12} A germline defect of the SAP gene causes a human congenital immune disorder, X-linked lymphoproliferative syndrome (XLP).¹³ In patients with XLP and in SAP-deficient mice, it was shown that the function of T cells^{14–15} and natural killer cells^{16–17} and the development of natural killer T cells^{18–19} was perturbed. As the present F₁ male mice stably developed a high grade of ankylosis under the SAP-deficient condition, we questioned whether SAP regulated ankylosis onset.

The inheritance mode of ankylosis is a unique characteristic in the present model. It was particularly noted that the ankylosis developed in only (MRL/rpl × C3H/lpr; MC) F₁ mice but not in parental mice. A question arose of how genetic factors controlled the onset of ankylosis in the F₁ generation.

Abbreviations: DBA, dilute brown/non-agouti; FGFR2, fibroblast growth factor receptor 2 gene; IFN, interferon; QTL, quantitative trait locus; SAP, signalling lymphocyte activation molecule-associated protein; XLP, X-linked lymphoproliferative syndrome

In this report, we describe histopathological and genetic characterisations of the new ankylosis model.

MATERIALS AND METHODS

Mice

C3H/lpr mice were purchased from The Jackson Laboratory (Bar Harbor, Maine, USA) and bred under specific pathogen-free conditions in the Animal Research Institute of the Tohoku University Graduate School of Medicine, Sendai, Japan. MRL/rpl, MCF₁, (C3H/lpr×MRL/rpl; CM) F₁ and MCF₂ mice were housed in the same conditions. In all animal experiments, we followed the Tohoku University guidelines for animal experimentation.

Histopathological grading of ankylosis

All mice were killed at 6 months under ether anaesthesia for tissue sampling. Foot joints were fixed with 10% formalin in 0.01 M phosphate buffer (pH 7.2), decalcified in 5% formic acid containing 5% formalin and embedded in paraffin wax. Tissue sections were stained with haematoxylin and eosin for observations by light microscopy. Generally in the F₁, and especially in the F₂ mice, a synovitis-driven lesion was occasionally coincident with an enthesitis-driven lesion to varied extents. To obtain a specific diagnosis for the enthesitis-driven lesion, we adopted new histological criteria as follows: no heterotopic cartilage and bone formation (grade 0), slight heterotopic proliferation of cartilage or fibrocartilage tissue (grade 1), a lesion similar to grade 1, but severe (grade 2), and an advance of grade 2, associated with joint dislocation and macroscopic swelling (grade 3). We defined ankylosis grade of each mouse as the maximal grade in six longitudinal sections prepared from bilateral hind paws. A mouse with grade 1, 2 or 3 was considered to be a positive case for ankylosis.

Mapping of ankylosis susceptibility loci

We bred the 257 MCF₂ mice and determined their ankylosis grades by microscopy examination. As the incidence of ankylosis was evident only in the male group, we selected 48 male MCF₂ mice, of which 24 were most severely affected and 24 were unaffected. The selected 48 male MCF₂ mice were then subjected to a genome-wide scan to estimate ankylosis susceptibility loci with 71 polymorphic microsatellite markers—namely, D1Mit276, D1Mit46, D1Mit494, D1Mit115, D2Mit522, D2Mit37, D2Mit102, D2Mit448, D2Mit286, D2Mit200, D3Mit224, D3Mit137, D3Mit101, D3Mit14, D4Mit89, D4Mit15, D4Mit187, D4Mit147, D4Mit259, D5Mit145, D5Mit149, D5Mit134, D5Mit23, D5Mit68, D5Mit33, D6Mit188, D6Mit15, D7Mit76, D7Mit155, D7Mit230, D7Mit181, D7Mit126, D7Mit68, D7Mit174, D8Mit65, D8Mit107, D9Mit217, D9Mit1001, D9Mit229, D9Mit336, D9Mit182, D9Mit82, D10Mit95, D10Mit164, D11Mit263, D11Mit334, D12Mit136, D12Mit4, D12Mit259, D12Mit141, D13Mit60, D13Mit178, D13Mit202, D13Mit130, D14Mit37, D14Mit39, D14Mit195, D15Mit20, D15Mit28, D16Mit165, D16Mit57, D16Mit185, D17Mit264, D17Mit127, D18Mit20, D18Mit51, D18Mit142, D19Mit60, D19Mit112, DXMit140 and DXMit10. These markers provided coverage of mouse genome with an average of 23.3 cM apart. As the coverage for chromosome X with polymorphic markers was insufficient, we determined genotypes of all male mice at DXMit140 and DXMit10. After determining candidate chromosomes by the genome-wide study ($p < 0.05$), we analysed all the samples to evaluate linkage loci. Polymerase chain reactions were carried out under the following conditions: 94°C for 2 min, 35 cycles of 94°C for 30 s, 55°C for 30 s for 30 s, and a final extension at 72°C for 5 min. The genetic positions of microsatellite markers and genes were based on the Mouse Genome Informatics provided by The Jackson Laboratory.

Statistical analysis

In the association study, we used male MCF₂ mice. An association between ankylosis onset and genotype was evaluated using the χ^2 test with standard 2×3 contingency matrices. Estimation of significant or suggestive association was based on $p = 0.000052$ or $p = 0.0016$, respectively, as recommended by Lander and Kruglyak.²⁰ Quantitative trait locus (QTL) was determined with the 123 male mice. Histopathological grades of ankylosis were used as the indicators of phenotype. Logarithmic odd scores were calculated by the interval mapping and composite interval mapping methods in the Windows QTL Cartographer (V.2.5) software developed by Zeng.²¹ We adopted the Model 6 with forward and backward regression method for composite interval mapping. The number of control markers, the window size and the walk speed were set at 5, 10 and 2 cM, respectively. The statistically suggestive level ($p = 0.05$) was determined with the permutation test (1000 times) developed by Churchill and Doerge.²²

RESULTS

Histopathological characterisation of the arthropathy in the MCF₁ mice

By 6 months, arthropathy was observed macroscopically as swelling and rigidity of ankle in the male MCF₁ group, although not in the female MCF₁ group (fig 1A,B). The disease readily progressed from initial onset of maximal joint swelling followed by irreversible ankle rigidity. The maximal joint swelling was an acute incidence, although the swelling continued to a lesser extent. Microscopically, pathological changes were observed mainly in the ankle joints (fig 1C). The arthropathy was characterised by fibroblast-like cell proliferation with chondrocytic differentiation and ossification at the region where joint capsule and ligaments attached to bone (fig 1D,E). Periarticular enthesophytes and marginal ankylosis were also evident (fig 1F). However, enthesal tissue associated with Achilles tendon was barely affected. Erosive change in cartilage and lymphocytic inflammation were not common features. In a few cases, subcutaneous acute inflammation with neutrophilic infiltration was observed. Macroscopically, there was no evidence for the involvement of interphalangeal joints. These findings suggest that the arthropathy in MCF₁ mice was similar to ankylosing enthesitis as previously reported in some mouse strains.^{23–24}

Incidence of the arthropathy

Table 1 summarises the incidence of microscopically defined arthropathy. A mouse with ankylosis grade 1, 2 or 3 was considered to be a positive case for ankylosis. It was found that all male MCF₁ mice developed ankylosis. In contrast, female MCF₁ mice scarcely developed it (19.4%, $p < 0.001$ v male). None of CMF₁ mice developed ankylosis. It should be particularly noted that ankylosis was not observed in the parental strains, MRL/rpl and C3H/lpr. In the male MCF₂ group, the incidence decreased (61.8%) as compared with the male MCF₁ group, but the histopathological feature of ankylosis was similar to that in MCF₁ mice. Male predominance of ankylosis onset was also evident in the MCF₂ group ($p < 0.001$). During this study, we maintained every mouse in a caged group of 3–7 mice. The caged group of mice was not rearranged after weaning, as some previous studies have done to facilitate ankylosis onset.^{5–6} In the MCF₁ mice, we found no significant difference in incidence of ankylosis between the caged groups of ≤ 3 mice and ≥ 4 mice ($\chi^2 = 2.06$, $p = 0.15$).

Mapping of the ankylosis susceptibility loci

We first determined a candidate linkage locus based on selective genome wide screening using the 48 male MCF₂

Figure 1 Ankylosis in the ankle joints spontaneously developed in the MCF₁ mice. (A) Unaffected ankle of the 6-month-old female MCF₁ mouse. (B) Affected ankle with remarkable swelling in the 6-month-old male MCF₁ mouse. (C) Microscopic features of the advanced ankylosis lesion. The joint fusion with ossified fibroblastic tissues. Haematoxylin and eosin (H&E) staining. Bar indicates 1 mm. (D) Fibroblast-like cell proliferation with chondrocytic differentiation in the ligament (arrow). H&E staining. Bar indicates 100 μ m. (E,F) Fibroblast-like cell proliferation with chondrocytic differentiation (arrow in E) and ossification (arrowhead in E) in the ligaments. Peritarticular enthesophyte (asterisk) and marginal ankylosis (double asterisks) were commonly observed. H&E staining. Bars indicate 200 μ m and 1 mm in (E) and (F), respectively. Cal, calcaneus; Cun, cuneiform bone; Met, metatarsal bone; Nav, navicular bone; Tal, talus.

Table 1 Incidence and histopathological grades of ankylosis in MRL/rpl, C3H/lpr, F₁ and F₂ mice

Strain, sex	Grades of ankylosis*				Incidence† (%)
	0	1	2	3	
MRL/rpl					
Female	12	0	0	0	0
Male	16	0	0	0	0
Total	28	0	0	0	0
C3H/lpr					
Female	6	0	0	0	0
Male	18	0	0	0	0
Total	24	0	0	0	0
MCF ₁					
Female	25	6	0	0	19.4‡
Male	0	5	15	19	100
Total	25	11	15	19	64.3
CMF ₁					
Female	4	0	0	0	0
Male	9	0	0	0	0
Total	13	0	0	0	0
MCF ₂					
Female	112	19	3	0	16.4‡
Male	47	24	44	8	61.8‡
Total	159	43	47	8	38.1

*Values represent the number of mice with the indicated ankylosis grade.

$$\dagger \text{Incidence (\%)} = 100 \times (\text{the number of total mice with grades 1, 2 and 3}) / (\text{the number of total mice}).$$

±Significant difference between male and female, $p < 0.01$, χ^2 test.

mice, which consisted of 24 mice with grade 0 and 24 mice with grade 2 or 3. The results suggested associations at a few marker positions on chromosome 7, but not on any other chromosome. We next determined genotypes of all male MCF₂ mice at *D7Mit76*, *D7Mit155*, *D7Mit230*, *D7Mit181*, *D7Mit126*, *D7Mit68* and *D7Mit174*, and evaluated the association between the genotype of every marker position and onset of ankylosis. The results confirmed linkages at *D7Mit68* and *D7Mit174* (table 2). As markers' coverage for chromosome X was unsatisfactory, we determined genotypes of all the 122 male MCF₂ mice at *DXMit140* and *DXMit10*. There was no association at these markers (table 2). Comparison of the ankylosis grades between three genotype groups at *D7Mit174* suggest that the linkage locus on chromosome 7 governed ankylosis severity in a dominant susceptibility mode of inheritance of the *MRL* allele (table 3).

In QTL analysis, interval mapping and composite interval mapping showed linkage between *D7Mit68* and *D7Mit174*, where the threshold level ($p = 0.05$) of logarithmic odd scores was determined by the permutation test (fig 2). A suggestive interval was defined between 62 cM and the telomeric end of chromosome 7, where fibroblast growth factor receptor 2 gene (*FGFR2*) and clustered interferon-inducible genes could be highlighted as currently possible candidates for the ankylosis susceptibility in the present model (reason discussed later).

DISCUSSION

It was shown that DBA (dilute brown/non-agouti)/1 mice spontaneously developed ankylosing arthropathy in the foot joints, resembling human spondylarthropathy or psoriatic arthropathy.⁴⁻⁷ On the other hand, the incidences of another class of ankylosing arthropathy, currently termed as ankylosing enthesitis, was shown in some inbred strains of mice.²³⁻²⁴ Although these two classes were shown to be distinct in preferentially affected sites and distribution of mice strains, they were commonly characterised by ageing-dependent and male-predominant ankylosis. Ankylosing enthesitis affected ankle joints, whereas arthropathy in DBA/1 mice affected more distal foot joints, including phalangeal joints and the toe region.²⁵⁻²⁶ Enteseal proliferation has been thought to be a preceding feature of ankylosis in those models. Bone erosion and lymphocytic inflammation were not their main representation, which is in contrast to the models established as rheumatoid arthritis. The present MCF₁ model shares pathological characteristic with the previously defined

ankylosing models, including male predominance and enteseal involvement without bone erosion.

It was of particular interest how the unique inheritance mode of disease was genetically interpreted in the present model. The high incidence at the F₁ generation posed an insight into a strong genetic contribution in ankylosis onset. On completing the present genetic study, we identified only a single autosomal dominant susceptibility locus on chromosome 7. Our findings from reciprocal breeding of MRL/rpl and C3H/lpr suggested an implication of the Y chromosome of the C3H/lpr mice in ankylosis onset. Although we presently cannot rule out sum effects of minor loci, the present findings may allow us to interpret the F₁ onset mode and male predominance with the additive effect of the autosomal dominant locus and a Y-linked locus. This inheritance mechanism may in part interpret a sporadic mode of disease transmission in human.

Oishi *et al*²⁷ reported the spontaneous occurrence of ankylosis in (MRL × DBA/1; MD) F₁ mice. Similar to the present model, their study on ankylosis was characterised by enteseal involvement in male-predominant manner. However, the incidence of ankylosis in male MDF₁ mice (29.4%) was much lower than that in the present model, suggesting a role for *lpr* mutation or particular C3H-derived factors in acceleration of ankylosis onset. The genetic study using MDF₂ mice suggested four autosomal susceptibility loci to the severity or onset of ankylosis, none of which overlapped with our locus. In addition, the incidences of ankylosis were seen equally in two reciprocally mating groups, MDF₁ and (DBA/1 × MRL) F₁, indicating no association of the Y chromosome with ankylosis onset in MDF₁ mice. This example is different from our case. Male predominance in the present model may not simply be interpreted by a Y-linked locus.

The locus identified in this study, the telomeric region of chromosome 7, includes many positional candidates for the susceptibility gene to ankylosis onset. Of them, we first evaluate a candidacy of the *FGFR2* gene. The *FGFR2* protein comprises three immunoglobulin-like domains in the extracellular region, a single membrane-spanning segment and a cytoplasmic tyrosine kinase domain. Currently accumulating evidence has shown that a mutation on *FGFR2* causes several human congenital disorders, including Crouzon syndrome,²⁸ Jackson-Weiss syndrome,²⁹ Apert syndrome³⁰ and Pfeiffer syndrome.³¹⁻³² These disorders are characterised by pathological bone fusion of craniofacial region or limbs.

Table 2 Association of microsatellite genotype and the incidence of ankylosis in the MCF₂ mice

Marker	Position (cM)	Incidence of ankylosis						χ^2_{*}	p
		Negative			Positive				
		MM	MC	CC	MM	MC	CC		
Chromosome 7									
D7Mit76	3.4	10	23	7	14	49	19	1.29	0.53
D7Mit155	15	10	22	8	17	47	18	0.29	0.86
D7Mit230	24.5	7	25	8	15	46	21	0.55	0.76
D7Mit181	37	6	19	15	23	36	23	2.77	0.25
D7Mit126	50	6	15	19	27	36	19	8.57	0.014
D7Mit68	60	6	15	19	25	43	14	13.00	0.002
D7Mit174	69	8	15	17	23	49	10	14.38	0.008
Chromosome X									
DXMit140	19	23	0	17	52	0	30	0.40	0.53
DXMit10	63.2	14	1	22	45	4	28	5.44	0.066

MM, MC and CC genotypes indicate MRL/MRL homozygote, MRL/C3H heterozygote and C3H/C3H homozygote, respectively.

* χ^2 values for the chromosome 7 or X markers were determined by the χ^2 test with 2×3 or 2×2 contingency matrices, respectively.

Table 3 Association of ankylosis grade and genotypes on chromosome 7 in the MCF₂ mice

Marker	Position (cM)	Ankylosis grade			p*
		MM	MC	CC	
D7Mit126	50	1.0 (1.0 to 1.0)	2.0 (1.5 to 2.0)	2.0 (1.5 to 2.5)	0.037
D7Mit68	60	1.0 (1.0 to 1.0)	2.0 (1.5 to 2.0)	1.5 (1.5 to 2.5)	0.002
D7Mit174	69	1.0 (1.0 to 1.5)	1.5 (1.0 to 1.5)	0.5 (0.0 to 1.0)	0.002

MM, MC and CC genotypes indicate MRL/MRL homozygote, MRL/C3H heterozygote and C3H/C3H homozygote, respectively.

Values are median (95% CI) of ankylosing grade.

*Determined by Kruskal–Wallis test.

Interestingly, the transgenic mice with a gain-of-function mutation on *FGFR2* showed skeletal abnormalities associated with a premature fusion of cranial sutures and multiple joint fusions, which were considered to be similar to the phenotypes of some cases of Apert or Pfeiffer syndromes.^{33–34} They confirmed not only the link between a gain-of-function mutation on *FGFR2* and the human congenital disorders but also the role of *FGFR2* in endochondral bone formation. It may be possible that an unidentified *FGFR2* polymorphism leads to chondrocyte proliferation in the enthesal region of the present model. In addition, a gene family consisting of interferon (IFN)-inducible transmembrane proteins could be considered to be a reasonable candidate, because the previous study showed the mandatory role of IFN γ in the spontaneous occurrence of ankylosis in DBA/1 mice.³⁵ It would be particularly interesting to study whether those candidate genes are polymorphic between the two strains used in this study.

We initially observed ankylosis onset in the MCF₁ mice, which were descended from the SAP-deficient female MRL/rpl mice and male C3H/lpr mice. All the male MCF₁ mice became SAP deficient, because the *SAP* is an X-linked gene. Therefore, we hypothesised a suppressive role of *SAP* in ankylosis onset. But no linkage was proved between ankylosis onset and *DXMit140*, which is located very close to the *SAP* gene. This fact clearly shows that *SAP* deficiency is not associated with ankylosis onset. Previous studies have shown that *SAP* deficiency in mice and humans causes severe perturbation of the development of natural killer T cells. Taken together with the findings were, the ankylosis in the present model seems to occur independently of natural killer

T cell contribution. Previous studies have shown that T cells are dispensable⁷ but IFN γ is indispensable for the development of enthesal ossification in DBA/1 mice.³⁵ Now T cell-independent and IFN γ -dependent mechanisms may be underscored in mouse ankylosis models.

To date, careful observations have shown increased evidence of ankylosis-related arthropathy in the rodent species. Most cases are evident without any specified mutation in male-specific manner. This fact may pose an insight that incidence of ankylosis is dominated by both common genetic factors and male-specific condition. Our high-incidence model will provide an opportunity for further understanding the genetic mechanisms of ankylosing joint disorders.

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REFERENCES

- 1 **Hang L**, Theofilopoulos AN, Dixon FJ. A spontaneous rheumatoid arthritis-like disease in MRL/l mice. *J Exp Med* 1982;155:1690–701.
- 2 **Bouvet JP**, Couderc J, Bouthiller Y, Franc B, Ducaillat A, Mouton D. Spontaneous rheumatoid-like arthritis in a line of mice sensitive to collagen-induced arthritis. *Arthritis Rheum* 1990;33:1716–22.
- 3 **Nakamura K**, Kashiwazaki S, Takagishi K, Tsukamoto Y, Morohoshi Y, Nakano T, et al. Spontaneous degenerative polyarthritis in male New Zealand black/KN mice. *Arthritis Rheum* 1991;34:171–9.
- 4 **Nordling C**, Karlsson-Parra A, Jansson L, Holmdahl R, Klareskog L. Characterization of a spontaneously occurring arthritis in male DBA/1 mice. *Arthritis Rheum* 1992;35:717–22.
- 5 **Holmdahl R**, Jansson L, Andersson M, Jansson R. Genetic, hormonal and behavioural influence on spontaneously developing arthritis in normal mice. *Clin Exp Immunol* 1992;88:467–72.
- 6 **Weinreich S**, Capkova J, Hoebe-Hewryk B, Boog C, Ivanyi P. Grouped caging predisposes male mice to ankylosing enthesopathy. *Ann Rheum Dis* 1996;55:645–7.
- 7 **Corthay A**, Hansson AS, Holmdahl R. T lymphocytes are not required for the spontaneous development of enthesal ossification leading to marginal ankylosis in the DBA/1 mouse. *Arthritis Rheum* 2000;43:844–51.
- 8 **Komori H**, Furukawa H, Mori S, Ito MR, Terada M, Zhang MC, et al. A signal adaptor SLAM-associated protein regulates spontaneous autoimmunity and Fas-dependent lymphoproliferation in MRL-Fas^{lpr} lupus mice. *J Immunol* 2006;176:395–400.

Figure 2 Logarithmic odd (LOD) scores for quantitative trait loci that control the severity of ankylosis of male MCF₂ mice. The results from both interval mapping (IM, break curve) and composite interval mapping (CIM, grey solid curve) are shown. Horizontal straight lines indicate the threshold levels for significant linkage determined by the permutation test (1000 times permutations, $p = 0.05$), 2.258 (break line) and 2.106 (solid line) in the IM and CIM analyses, respectively. Genetic positions are indicated with the markers used in this study.

- 9 **Sayos J**, Wu C, Morra M, Wang N, Zhang X, Allen D, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature* 1998;**395**:462–9.
- 10 **Latour S**, Gish G, Helgason CD, Humphries RK, Pawson T, Veillette A. Regulation of SLAM-mediated signal transduction by SAP, the X-linked lymphoproliferative gene product. *Nat Immunol* 2001;**2**:681–90.
- 11 **Latour S**, Roncagalli R, Chen R, Bakinowski M, Shi X, Schwartzberg PL, et al. Binding of SAP SH2 domain to FynT SH3 domain reveals a novel mechanism of receptor signalling in immune regulation. *Nat Cell Biol* 2003;**5**:149–54.
- 12 **Chan B**, Lanyi A, Song HK, Griesbach J, Simarro-Grande M, Poy F, et al. SAP couples Fyn to SLAM immune receptors. *Nat Cell Biol* 2003;**5**:155–60.
- 13 **Coffey AJ**, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet* 1998;**20**:129–35.
- 14 **Dupre L**, Andolfi G, Tangye SG, Clementi R, Locatelli F, Arico M, et al. SAP controls the cytolytic activity of CD8+ T cells against EBV-infected cells. *Blood* 2005;**105**:4383–9.
- 15 **Wu C**, Nguyen KB, Pien GC, Wang N, Gullo C, Howie D, et al. SAP controls T cell responses to virus and terminal differentiation of TH2 cells. *Nat Immunol* 2001;**2**:410–14.
- 16 **Benoit L**, Wang X, Pabst HF, Dutz J, Tan R. Defective NK cell activation in X-linked lymphoproliferative disease. *J Immunol* 2000;**165**:3549–53.
- 17 **Tangye SG**, Phillips JH, Lanier LL, Nichols KE. Functional requirement for SAP in 2B4-mediated activation of human natural killer cells as revealed by the X-linked lymphoproliferative syndrome. *J Immunol* 2000;**165**:2932–6.
- 18 **Nichols KE**, Hom J, Gong SY, Ganguly A, Ma CS, Cannons JL, et al. Regulation of NKT cell development by SAP, the protein defective in XLP. *Nat Med* 2005;**11**:340–5.
- 19 **Pasquier B**, Yin L, Fondaneche MC, Relouzat F, Bloch-Queyrat C, Lambert N, et al. Defective NKT cell development in mice and humans lacking the adapter SAP, the X-linked lymphoproliferative syndrome gene product. *J Exp Med* 2005;**201**:695–701.
- 20 **Lander E**, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 1995;**11**:241–7.
- 21 **Zeng ZB**. Precision mapping of quantitative trait loci. *Genetics* 1994;**136**:1457–68.
- 22 **Churchill GA**, Doerge RW. Empirical threshold values for quantitative trait mapping. *Genetics* 1994;**138**:963–71.
- 23 **Sweet HO**, Green MC. Progressive ankylosis, a new skeletal mutation in the mouse. *J Hered* 1981;**72**:87–93.
- 24 **Eulderink F**, Ivanyi P, Weinreich S. Histopathology of murine ankylosing enthesopathy. *Pathol Res Pract* 1998;**194**:797–803.
- 25 **Lories RJ**, Matthys P, de Vlam K, Derese I, Luyten FP. Ankylosing enthesitis, dactylitis, and onychoprositis in male DBA/1 mice: a model of psoriatic arthritis. *Ann Rheum Dis* 2004;**63**:595–8.
- 26 **Lories RJ**, Derese I, Luyten FP. Modulation of bone morphogenetic protein signaling inhibits the onset and progression of ankylosing enthesitis. *J Clin Invest* 2005;**115**:1571–9.
- 27 **Oishi H**, Miyazaki T, Mizuki S, Kamogawa J, Lu LM, Tsubaki T, et al. Accelerating effect of an MRL gene locus on the severity and onset of arthropathy in DBA/1 mice. *Arthritis Rheum* 2005;**52**:959–66.
- 28 **Reardon W**, Winter RM, Rutland P, Pulleyn LJ, Jones BM, Malcolm S. Mutations in the fibroblast growth factor receptor 2 gene cause Crouzon syndrome. *Nat Genet* 1994;**8**:98–103.
- 29 **Jabs EW**, Li X, Scott AF, Meyers G, Chen W, Eccles M, et al. Jackson-Weiss and Crouzon syndromes are allelic with mutations in fibroblast growth factor receptor 2 [correction appears in *Nat Genet* 1995;**9**:451]. *Nat Genet* 1994;**8**:275–9.
- 30 **Wilkie AO**, Slaney SF, Oldridge M, Poole MD, Ashworth GJ, Hockley AD, et al. Apert syndrome results from localized mutations of FGFR2 and is allelic with Crouzon syndrome. *Nat Genet* 1995;**9**:165–72.
- 31 **Lajeunie E**, Ma HW, Bonaventure J, Munnich A, Le Merrer M, Renier D. FGFR2 mutations in Pfeiffer syndrome. *Nat Genet* 1995;**9**:108.
- 32 **Rutland P**, Pulleyn LJ, Reardon W, Baraitser M, Hayward R, Jones B, et al. Identical mutations in the FGFR2 gene cause both Pfeiffer and Crouzon syndrome phenotypes. *Nat Genet* 1995;**9**:173–6.
- 33 **Hajihosseini MK**, Wilson S, De Moerloose L, Dickson C. A splicing switch and gain-of-function mutation in Fgfr2-IIIc hemizygotes causes Apert/Pfeiffer-syndrome-like phenotypes. *Proc Natl Acad Sci USA* 2001;**98**:3855–60.
- 34 **Eswarakumar VP**, Horowitz MC, Locklin R, Morris-Kay GM, Lonai P. A gain-of-function mutation of Fgfr2c demonstrates the roles of this receptor variant in osteogenesis. *Proc Natl Acad Sci USA* 2004;**101**:12555–60.
- 35 **Matthys P**, Lories RJ, De Klerck B, Heremans H, Luyten FP, Billiau A. Dependence on interferon-gamma for the spontaneous occurrence of arthritis in DBA/1 mice. *Arthritis Rheum* 2003;**48**:2983–8.