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Report

Genomewide Scan for Gout in Taiwanese Aborigines Reveals Linkage to Chromosome 4q25

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Gout is a disorder of uric-acid metabolism. The Pacific Austronesian population, including Taiwanese aborigines, has a remarkably high prevalence of hyperuricemia and gout, which suggests a founder effect across the Pacific region. We report here a genomewide linkage study of 21 multiplex pedigrees with gout from an aboriginal tribe in Taiwan. From observations of familial clustering, early onset of gout, and clinically severe manifestations, we hypothesized that a major gene plays a role in this trait. Using 382 random polymorphic markers spread across 22 autosomes, we demonstrated a highly significant linkage for gout at marker D4S2623 on chromosome 4q25 ($P = .0002$ by nonparametric linkage [the NPL_{all} statistic]; empirical $P = .0006$; LOD = 4.3, $P = 4.4 \times 10^{-6}$ by logistic regression). When alcohol consumption was included as a covariate in the model, the LOD score increased to 5.66 ($P = 1.3 \times 10^{-6}$). Quantitative traits, including serum uric acid and creatinine, also showed a moderate linkage to this region. To our knowledge, this is the first genome-scan report to identify a genetic locus harboring a gout-susceptibility gene.

Gout (MIM 138900) is characterized by elevated serum urate and recurrent attacks of intra-articular crystal deposition of monosodium urate monohydrate. Clinical manifestations include recurrent painful attacks of acute inflammatory arthritis, tophi, uric-acid urolithiasis, renal impairment, and, eventually, renal failure. Uric acid is produced within all mammalian cells as the product of purine degradation. Homeostasis of uric acid depends on the balance between cellular production and renal clearance. Hyperuricemia develops as the result of overproduction or decreased renal excretion of uric acid. The incidence of acute gout is ~5% each year among patients with hyperuricemia and a serum-urate concentration of 9.0 mg/dl (Campion et al. 1987). Gout has genetic components and is complicated by environmental factors, such as diet and alcohol intake, and by age and sex. Most genetic findings are derived from rare forms of Mendelian disorders—for example, hypoxanthine guanine phosphoribosyltransferase (HPRT) related to X-linked gout, autosomal dominant medullary cystic kidney disease (ADMCKD) on chromosome 1q21, familial juvenile hyperuricemic nephropathy on chromosome 16, uric-acid nephrolithiasis on chromosome 10, or genes for uric-acid transport in the kidney (see entry for MIM 138900).

A series of studies on indigenous groups from Polynesia (Jackson et al. 1981; Prior et al. 1987; Prigent et al. 1992), Melanesia (Prior 1981), Micronesia (Zimmet et al. 1978), Indonesia (Darmawan et al. 1992), and Taiwan (Chang et al. 1997) show uric-acid levels significantly higher than those found in white populations. Linguistic, archaeological, and genetic evidence suggests that the insular populations across the Pacific region, including Polynesians, Micronesians, Melanesians, and Taiwan aborigines, are part of an Austronesian population (Bellwood 1991; Diamond 2000; Gray and Jordan 2000; Chang et al. 2002; Diamond and Bellwood 2003). This genetic relationship and the high morbidity of gout

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Table 1

Characteristics of Subjects with Gout and Unaffected Subjects from 21 Multiplex Aboriginal Families in Taiwan

Characteristic	Finding in		
	Subjects with Gout (n = 91)	Unaffected Subjects (n = 63)	P
Age (years)	47.3 ± 14.9	46.9 ± 15.0	.90
No. who consume alcohol:			
Regularly	56	27	.02
Rarely	35	36	…
Age (years) at start of alcohol consumption	21.87 ± 6.5	23.2 ± 7.3	.57
No. of years of alcohol consumption	20.2 ± 10.2	23.3 ± 17.0	.46
BMI (kg/m^2)	25.4 ± 4.1	25.5 ± 4.2	.99
Uric acid (mg/dL)	9.5 ± 2.4	7.6 ± 1.8	<.001
Blood pressure (mmHg):			
Systolic	136.4 ± 23.2	127.7 ± 24.2	.028
Diastolic	87.9 ± 15.5	82.3 ± 15.1	.029
Triglyceride (mg/dL)	265.8 ± 240.7	207.8 ± 120.3	.053
Cholesterol (mg/dL)	190.3 ± 45.3	176.6 ± 27.1	.022
Liver function (IU/L):			
GOT	30.9 ± 27.2	27.7 ± 15.4	.40
GPT	29.6 ± 20.4	27.4 ± 24.9	.54

in these Austronesian populations suggest a possible founder effect of gout susceptibility. Without a priori knowledge of the predisposing genes for the complex primary gout disease, we conducted a genomewide linkage study on 21 multiplex pedigrees with gout from an isolated highland aboriginal tribe in Taiwan, with the rationale that this population is presumably more homogeneous than other populations and, thus, provides a better power to illustrate genetic effects (Wright et al. 1999).

The ascertainment of the gout probands was initially done in a community public-health survey conducted in the local health stations in Taiwan, with the aim of health education and prevention. The general health history and specific conditions, including gout and hypertension, of middle-aged and elderly people were recorded. The diagnosis of the proband with gout was confirmed by a rheumatologist on the basis of criteria from Wallace et al. (1977). Family structure and the family history of each proband were obtained in the survey. All affected members reported by the probands were confirmed by the rheumatologist. Blood was drawn by trained medical technologists. Informed consent from each study subject was obtained before the study. In total, we recruited 154 individuals from 21 pedigrees.

Genomic DNA was prepared from blood with the PureGene DNA isolation kit (Gentra Systems). Genotyping was provided by the Mammalian Genotyping Service of the Marshfield Clinic Research Foundation. The samples were genotyped using the newer screening set, Weber Screening Set 13 (see the Center for Medical Genetics Web site), which has more-accurate allele calling and less error or mistyping as a consequence of its use of higher-quality tri- and tetranucleotide STRPs with more accurate mapping and spacing (Ghebranious et al. 2003). In this study, 382 autosomal markers were used, with an average of 9.3-cM spacing (range 0.3–18 cM) and 0.69 heterozygosity.

We applied PedCheck (O'Connell and Weeks 1998) and MERLIN (Abecasis et al. 2002) software to check for any inconsistent Mendelian inheritance, nonpaternity, or other genotyping errors. Any inconsistency was zeroed out to avoid bias. We removed three individuals from analysis because of many genotyping errors found in the samples. Gene frequencies were estimated by allele counting in all genotyped individuals and were automatically calculated by MERLIN. The results were not different when we used other strategies to calculate allele frequency, such as the maximum-likelihood estimate.

For discrete traits, we first performed nonparametric linkage (NPL) (Whittemore and Halpern 1994) with the use of MERLIN. This method calculates inheritance distribution for sets of affected pairs and then uses a score function to determine the significance of linkage. We used the NPL_{all} statistic, which estimates identical-by-descent (IBD) allele sharing among all affected members and is averaged over all possible inheritance patterns, normalized, and weighted across pedigrees. Because some of our pedigrees are complicated and their information may not be fully utilized by the NPL statistics, we applied another multipoint analysis using the conditional-logistic model (Olson 1999) implemented in the S.A.G.E. program package. This method estimates β parameters on the basis of λ_i, the relative risk for a pair of relatives that shares i alleles IBD, where $\lambda_i = e^{\beta_i}$. Multipoint IBD estimates were obtained using the GENIBD

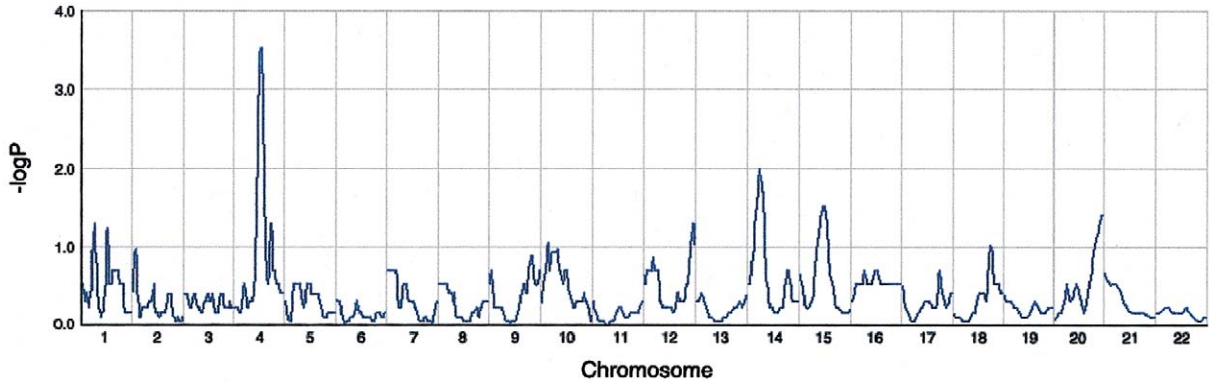

Figure 1 Multipoint linkage analysis using NPL in the genomewide scan on multiplex Taiwanese aboriginal families with gout. The *X*-axis represents the chromosome location for the 22 autosomes, and the *Y*-axis represents the $-\log P$, where P is derived from the NPL_{all} statistic. The best peak is on chromosome 4, with $-\log P = 3.70$ (NPL $Z = 3.58$).

program in S.A.G.E. We used a one-parameter model and the default value that constrains the relative risks, $\lambda_2 = 3.634\lambda_1 - 2.634$, without assuming any mode of inheritance. The likelihood-ratio statistic (LRS) is computed by multiplying the LOD score by 4.6. The *P* value for the one-parameter model is derived from the LRS distribution with 50:50 mixture of a point mass at 0 and a 1-df χ^2 distribution. When alcohol consumption was included in the model, the LRS distribution was a 50:50 mixture of a χ^2 with 1 df and a χ^2 with 2 df (Goddard et al. 2001). The significance of the covariate is determined by the difference between the LRS with the covariate and the LRS without the covariate.

For quantitative-trait linkage analysis, we applied the Deviate method in MERLIN to test for excess sharing among individuals in the same tail of trait distribution, without making the normality assumption of the trait distribution. This method is based on the frameworks of Whittemore and Halpern (1994) and Kong and Cox (1997) to define a score function and to test the significance of linkage. The quantitative phenotype was subtracted from the population mean, which was based on an epidemiological survey of the same aboriginal tribe for the biochemical measurements.

To examine the false-positive rate in the genome scan, we performed 10,000 multipoint simulations under the null hypothesis of no linkage or association to the phenotype, by use of MERLIN. The simulation generates random marker data sets that use the original data—including family structure, phenotypes, marker informativeness, map distance, and missing-data patterns—without simulating their value, through gene-dropping procedures (Abecasis et al. 2002). Empirical *P* is determined by the number of replicates that exceed the observed *Z* score, divided by the total number of replicates (10,000).

Among 21 families, 1 extended pedigree was too big to fit the software we used; therefore, we divided this pedigree into 3 families for the conditional-logistic model (LODPAL in S.A.G.E.) or into 5 families for NPL (MERLIN). Among the 23 pedigrees that were analyzed by the logistic model, there was a total of 66 affected sib pairs, 30 affected parent-child pairs, 4 affected half-sib pairs, 14 affected grandparent-grandchild pairs, 61 affected avuncular pairs, and 29 affected cousin pairs.

We found no significant difference in age between the group with gout and the unaffected group (table 1). More subjects with gout consumed alcohol on a regular basis (at least twice a week) than did unaffected subjects. However, starting age and duration of alcohol consumption and liver function measurements (glutamic-oxaloacetic transaminase [GOT] and glutamic-pyruvic transaminase [GPT]) did not differ from those of the unaffected subjects. The affected group also had higher blood pressure and higher levels of serum uric acid and cholesterol than those of the unaffected group. No significant difference was found in BMI between the affected and unaffected groups.

With error-checked genotype data on 382 autosomal markers, we first applied multipoint NPL analysis based on IBD sharing among affected relative pairs, using all marker information in each chromosome to screen all 22 chromosomes for linkage results. We chose NPL_{all} to estimate allele sharing among all affected members together. Figure 1 shows the NPL *Z* score obtained from MERLIN for all 22 chromosomes. The highest NPL *Z* score (3.58) among the 22 chromosomes scanned was located at 114 cM on chromosome 4, with a *P* value of .0002 (fig. 1). The empirical *P* value was .0006.

We next applied an alternative approach, to further confirm the findings on chromosome 4. We chose the conditional-logistic model because this approach is par-

ameterized in terms of the allele-sharing–specific relative risks and may capture more information than the NPL method. On the basis of the logistic model, the maximal LOD score for chromosome 4 was located again at 114 cM (LOD = 4.3; $P = .0000044$) (table 2 and fig. 2). Because alcohol consumption plays a role in gout development, we included it as a covariate in the logistic model. The LOD score increased to 5.66, and the overall P value for the linkage was .0000013 on the basis of a mixture of 1-df and 2-df distributions. The alcohol consumption was significant, with $P = .012$ ($\chi^2 = 6.30$, 1 df). The corresponding marker at this peak location was D4S2623. The LOD score from single-point analysis for this marker was 6.37, under a one-parameter model (data not shown). The 1-LOD score support intervals are from 107 cM to 129 cM. The estimate of β_1 from the one-parameter model with the alcohol-consumption covariate was 0.99; thus, $\lambda_1 = 2.68$ and $\lambda_2 = 7.10$. That is, the relative risk for an individual who shares one allele IBD with an affected relative is almost 3, and the relative risk for the person sharing two alleles IBD with an affected relative is >7.

We also analyzed gout-related phenotypes to find consistent mapping with gout. We applied the Deviate method in MERLIN to test for excess sharing among individuals in the same tail of trait distribution for the quantitative trait, because this method is not sensitive to the assumption of trait distribution. The results showed that QTL analysis coincidentally mapped to the same location, at 114 cM on chromosome 4 (table 2). For uric acid, the P value was .012, and for creatinine, the P value was .005. We again performed 10,000 simulations for this analytical method. The empirical P was .0414 for uric acid and .0197 for creatinine. We did not find evidence of linkage for blood pressure, BMI, triglyceride, and alcohol consumption in this region.

The above results demonstrate that, with the use of different analytical approaches and different traits, we have identified a gout susceptibility locus on chromosome 4q25 through a genomewide search on an isolated aboriginal tribe. The probability of type I error is very trivial (<1/1,000 by empirical test), and the significance of linkage increased after we adjusted for the environmental covariate effect. A genetic component of gout has been suggested, but, to our knowledge, there was no other report of significant findings from linkage studies, except of some rare Mendelian syndromes, such as ADMCKD or familial juvenile hyperuricemic nephropathy. Studies failed to identify genes for the complex trait gout from the systematic genome search, which may be attributed to the heterogeneity of the disease and to inadequate power of sample size, sample structure, or

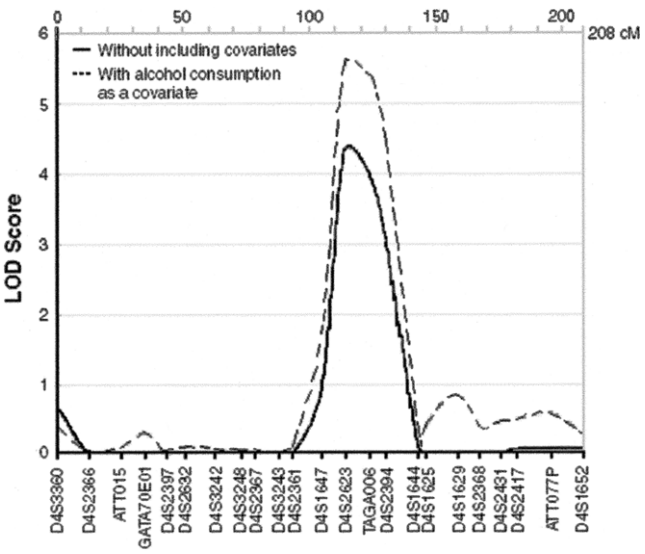

Figure 2 Multipoint linkage analysis on chromosome 4, calculated by the conditional-logistic model. The *X*-axis is the chromosome location (cM), and the *Y*-axis is the LOD score calculated from the logistic model. The peak location is at marker D4S2623.

Table 2

Linkage Results of Gout and Its Quantitative Traits on Chromosome 4 (at Position 114 cM), by Different Statistical Methods

Trait	Method	LOD Score	*P*
Gout	Conditional-logistic model	4.29	.0000044
Gout with covariate[a]	Conditional-logistic model	5.66	.0000013
Gout	NPL[b]	3.58	.0002[c]
Uric acid	Deviate[d]	1.11	.012[c]
Creatinine	Deviate[d]	1.47	.005[c]

[a] Alcohol consumption was added in binary form as a covariate in the one-parameter logistic model.

[b] NPL score implemented in MERLIN.

[c] Empirical *P* values, based on 10,000 simulations and calculated by MERLIN, were .0006 for gout, .0414 for uric acid, and .0197 for creatinine.

[d] Deviate method developed by Abecasis et al. (2002) and implemented in MERLIN.

statistical methods. The present study has great power because of both the multiplexity of the pedigree samples and the unique nature of an isolated population. The latter, presumably, is more homogeneous for genetic and common environmental effects (Wright et al. 1999). Therefore, we are able to localize the disease-susceptibility locus from the genome search.

Previously, we conducted a genetic study on the same tribe population (<10% of the samples used in the studies overlapped) to test a candidate region that was reportedly linked to an ADMCKD locus on chromosome 1q21 (Wang et al. 2004). From this region, nine markers were tested on 112 sib pairs—including 50 concordantly affected, 14 unaffected, and 48 discordant sib pairs—among 25 pedigrees. A two-point sibpair regression analysis revealed a marginally significant linkage (P = .03–.003) at the region 160–190 cM, and the family-association test detected a significant excess transmission of one allele at marker D1S484 (170 cM). The present study, through a genome search, did not confirm or rule out the significant linkage with this region in chromosome 1 (maximum LOD score is 1.01 by LODPAL and 0.97 by NPL, at 184 cM).

It is interesting that the strongest signal, at marker D4S2623, located at 114 cM on the Marshfield genetic map, is ~1.4 cM from a longevity locus (Puca et al. 2001). The coincidental mapping of a gout candidate gene and a longevity gene implies either that this region harbors two separate susceptibility genes, one for gout and one for longevity, or that a common gene in this region is responsible for both traits. Recently, Geesaman et al. (2003) reported that the SNP markers of the gene encoding microsomal triglyceride transfer protein (MTP), among ~50 putative genes in this 4q25 longevity-locus area, was genetically associated with human lifespan (Geesaman et al. 2003). They hypothesized that the impact of MTP on human lifespan may be mediated through its effects on the lipid profile pathway. On the other hand, uric acid scavenges potentially harmful reactive oxygen species and has long been thought to be a primary antioxidant in humans because of its high levels in plasma, compared with those of other antioxidants (Ames et al. 1981). The positive correlation between lifespan and the concentration of uric acid in serum and in brain, among mammalian species (Cutler 1984), suggests a role of uric acid in longevity. Whether the MTP has a role in uric-acid metabolism or in gout pathogenesis is unknown. MTP plays a role in lipid transfer and the assembly of lipoproteins (Hussain et al. 2003). Dyslipidemia, including abnormalities in triglyceride, low-density lipoprotein, and apoB, is comorbid with gout or hyperuricemia (Chou and Chao 1999; Rott and Agudelo 2003). It would be interesting to study the possible interactions among MTP, lipids, the homeostasis of uric acid, and the development of gout. Alternatively, the hypothesis that a still-unidentified gene within the longevity locus could affect both longevity and gout phenotypes is also remotely possible. The identification of a gout-susceptibility gene in the 4q25 region may shed light on the pathogenesis of gout and possibly on the mechanisms of longevity.

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Electronic-Database Information

The URLs for data presented herein are as follows:

Center for Medical Genetics, Marshfield Clinic Research Foundation, http://research.marshfieldclinic.org/genetics (for Weber Screening Set 13)
S.A.G.E., http://darwin.cwru.edu/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for gout and hyperuricemia)
MERLIN, http://www.sph.umich.edu/csg/abecasis/Merlin/index.html

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