Brief report

Quantitative trait locus on chromosome 8q influences the switch from fetal to adult hemoglobin

Chad Garner, Nicholas Silver, Steve Best, Stephan Menzel, Charlotte Martin, Tim D. Spector, and Swee Lay Thein

The switch from fetal to adult hemoglobin is incomplete; the residual fetal hemoglobin in adults is restricted to a subset of erythrocytes termed F cells. F-cell levels are influenced by a sequence variant (C → T) at position –158 upstream of the γ-globin gene, termed the XmnI-Gγ polymorphism. How the Gγ–158 C → T variant influences the expression of the Gγ-globin gene is unknown but is likely to involve the interaction of a multiprotein transcription complex. In a recent genome-wide linkage study of a large Asian Indian kindred, a genetic interaction between the XmnI-Gγ site and a locus on chromosome 8q was reported to influence adult F-cell levels. We report the replication of linkage to chromosome 8q in a sample of European twin pairs. This result provides strong evidence that a quantitative trait locus exists on chromosome 8q that influences the developmental switch from fetal to adult hemoglobin. (Blood. 2004;104:2184-2186)

Introduction

Residual amounts of fetal hemoglobin (Hb F) continue to be synthesized throughout adult life and are restricted to a subset of erythrocytes termed F cells (FCs).1 There is a good correlation between the percentages of Hb F and FCs, indicating that increased Hb F can be ascribed to the higher number of F cells produced.2 The heritability of FC levels was estimated to be 0.89 in the European population.3 Epidemiologic studies have shown FCs to be influenced by age,4 sex,5 and a DNA sequence variant (C → T) at position –158 upstream of the Gγ-globin gene, referred to as the XmnI-Gγ polymorphism.6-8 The XmnI-Gγ polymorphism is common in several populations; the “T” variant, which creates a cleavage site for the XmnI restriction enzyme, has been shown to be associated with increased FC levels.7 The frequency of the XmnI-Gγ restriction site is approximately 0.33 in Europeans, and the genotype accounts for 13% to 32% of the total phenotypic variance.8 The Gγ–158 T variant predisposes toward increased Hb F production in adult life, particularly in conditions of erythroid stress, such as β-thalassemia9-11 and sickle cell anemia.9 However, unlike rare mutations in the γ-globin promoter that are associated with clearly defined phenotypes of elevated Hb F levels of 10% to 35% in heterozygotes,2 the Gγ–158 variant does not always raise Hb F levels in otherwise healthy individuals. Even within families with heterocellular hereditary persistence of fetal hemoglobin (HPFH) in which increased Hb F was associated with XmnI-Gγ T/T or T/C genotypes, the association is not complete.12 Nearly half of the family members with these XmnI-Gγ genotypes do not have increased Hb F levels, suggesting that the effect of the XmnI-Gγ site is modulated by the presence of an intermediary factor(s). The most consistent change caused by the T allele at Gγ–158 is a high proportion of Gy chains.

Linkage has been reported between 3 regions of the genome and adult Hb F or FC levels. Chromosome Xp22.2-p22.3 has been linked to FC levels in sickle cell disease and healthy individuals.13 A locus has been mapped to chromosome 6q23 in a large Asian Indian kindred with β-thalassemia and high FC levels (in the range defined as heterocellular HPFH).14,15 An analysis of 316 European twin pairs showed no evidence of linkage to the chromosome 6q23 quantitative trait locus (QTL).6 Most recently, linkage of FC levels to chromosome 8q was found in the Asian Indian kindred.16 The influence of the 8q QTL was shown to be conditional on the XmnI-Gγ polymorphism genotype, suggesting a genetic interaction. We describe the confirmation of this finding.

Study Design

A total of 319 same-sex dizygotic (DZ) twin pairs of European descent were phenotyped for FC levels and genotyped for 19 markers spanning 57 cM of chromosome 8. The twin pairs were from St Thomas’s United Kingdom Adult Twin (TwinsUK) Register1,17 and were not selected for Hb F or FC levels. Details of the twin registry, the sample, and the determination of the FC levels and XmnI-Gγ genotypes are described elsewhere.3,17 The average age was 53 years, ranging from 24 to 77 years. The average FC level of the sample was 4.04% of total erythrocytes (standard deviation, 2.87%; range,
Results and discussion

Multipoint linkage analyses of the XmnI-Gy adjusted and unadjusted FC trait were carried out with the results shown in Figure 1. The XmnI-Gy adjusted FC trait showed higher multipoint logarithm of odds (lod) scores across the 56-cM interval than the unadjusted trait. The maximum lod score was 2.76 at the 16-cM position on the map; 0.5 cM was from marker D8S255. A confidence interval of one lod score unit ranges 21 cM from position 10 to 31 cM. Linkage analysis of the unadjusted FC levels showed a multipoint curve that was similar to the XmnI-Gy adjusted data; however, the lod score values were lower across the interval. The maximum lod score for the unadjusted data was 1.99 at the 15-cM position. The results shown in Figure 1 represent a confirmation of the earlier significant linkage reported by Garner et al16 on chromosome 8q in an independent sample from a different human population. The results meet the criteria of a confirmed linkage given by Lander and Kruglyak.20 The sample size in the present study was not large enough to test specific models of genetic interaction between the chromosome 8q QTL and the XmnI-Gy genotype. The higher lod scores observed for the XmnI-Gy adjusted FC trait suggest that a conditional or epistatic interaction between the 2 QTLs may not be the predominant model of FC inheritance in the European sample.

The vertical bars in Figure 1 show the single-point lod scores computed under a 2-locus model in the original genomewide linkage study of the Asian Indian kindred.16 The maximum single-point lod score was 4.33 at marker D8S1833 (see Table 1 in Garner et al16). Marker D8S538 was mapped to the same location as D8S1833 and showed an lod score of 3.14. The best estimate of the location of the chromosome 8q QTL, from the current and from the original linkage studies, was within a 5-cM region between locations 15 and 20 on the map shown in Figure 1. Single-locus analysis of the chromosome 8q region showed significant linkage when the XmnI-Gy genotype was not accounted for; however, statistical significance was not achieved when the FC values were adjusted for the genotype,16 suggestive of a genetic interaction and a pattern opposite to that observed in the European sample. We believe that the difference is related to the considerably reduced genetic heterogeneity in the Asian-Indian kindred compared with the European twin sample, and it is conceivable that some alleles of the chromosome 8q QTL act conditionally on the XmnI-Gy site and others act independently.

The chromosome 8q QTL could be a trans-acting factor, affecting FC levels independent of the β-globin complex; however, in the Asian Indian kindred, the chromosome 8q QTL effect was conditional on the XmnI-Gy site, suggesting a cis-acting mechanism. Although the sequence in the −158 region of the Gy promoter is not a recognized binding motif for any known transcription factors, the mechanism of increased γ-globin expression is likely to involve a network of transcription factors and coactivators functioning within multiprotein complexes.21 Altered expression of the Gγ-globin gene could arise from variation in the cis-binding site itself, or from variation in a transcription factor in the multiprotein network. The chromosome 8q QTL could encode for a regulatory factor, or a subunit thereof, that binds directly to the XmnI-Gy site. Alternatively, the protein could act as a molecular bridge in a protein-protein interaction. In these situations, changes in expression because of the trans-regulatory protein could be conditional on the presence of the cis-binding site. The 8q QTL defines a class of genetically heterogeneous determinants of Hb F levels that are conditional on cis-acting sequences of the β-globin complex, which could explain some of the inconsistent associations of high Hb F and FC levels with the XmnI-Gy site.

Acknowledgment

We thank Claire Steward for help in preparation of the manuscript.

References


Quantitative trait locus on chromosome 8q influences the switch from fetal to adult hemoglobin

Chad Garner, Nicholas Silver, Steve Best, Stephan Menzel, Charlotte Martin, Tim D. Spector and Swee Lay Thein