

A Quantitative Trait Locus on Chromosome 8q Influences the Switch From Fetal to Adult
Hemoglobin

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Running Title: Linkage between chr. 8q and fetal hemoglobin

Abstract

The switch from fetal to adult hemoglobin is incomplete; the residual fetal hemoglobin in adults being restricted to a sub-set of erythrocytes called 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-G γ* polymorphism. How the G γ -158 C-T variant influences the expression of the G γ globin gene is unknown but is likely to involve the interaction of a multi-protein transcription complex. In a recent genome-wide linkage study of a large Asian Indian kindred, a genetic interaction between the *XmnI-G γ* 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.

Introduction

Residual amounts of fetal Hb (Hb F) continue to be synthesized throughout adult life and are restricted to a subset of erythrocytes termed F cells (FC) ¹. There is a good correlation between the percentages of Hb F and FC 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. Epidemiological studies have shown FC to be influenced by age ⁴, sex ⁵ and a DNA sequence variant (C to T) at position -158 upstream of the G γ -globin gene, referred to as the *XmnI*-G γ polymorphism ⁶⁻⁸. The *XmnI*-G γ 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*-G γ restriction site is approximately 0.33 in Europeans, and the genotype accounts for 13-32% of the total phenotypic variance ⁸. The G γ -158 T variant predisposes towards increased Hb F production in adult life, particularly in conditions of erythroid stress, such as β thalassemia ⁹⁻¹¹ 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 10-35% in heterozygotes ², the G γ -158 variant does not always raise Hb F levels in otherwise normal individuals. Even within families with heterocellular hereditary persistence of fetal hemoglobin (HPFH) in which increased Hb F was associated with *XmnI*-G γ T/T or T/C genotypes, the association is not complete ¹². Nearly half of the family members with these *XmnI*-G γ genotypes do not have increased Hb F levels, suggesting that the effect of the *XmnI*-G γ site is modulated by the presence of an intermediary factor(s). The most consistent change caused by the 'T' allele at G γ -158 is a high proportion of G γ chains.

Linkage has been reported between three 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 normal individuals¹³. 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 QTL⁸. Most recently, linkage of FC levels to chromosome 8q was found in the Asian Indian kindred¹⁶. The influence of the 8q quantitative trait locus (QTL) was shown to be conditional on the *XmnI*- γ polymorphism genotype, suggesting a genetic interaction. We describe the confirmation of this finding.

Materials and Methods

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 the St. Thomas' UK Adult Twin (TwinsUK) Register^{3,17} and were not selected for Hb F or FC levels. Details of the twin registry, the sample, the determination of the FC levels and *XmnI*- γ genotypes is described elsewhere^{3,17}. The average age was 53 years, ranging from 24 to 77. The average FC level of the sample was 4.04 percent of total erythrocytes (standard deviation = 2.87), ranging from 0.53 to 17.9. FC levels were log transformed to fit the normal distribution. FC levels were adjusted to remove the effect of the *XmnI*- γ genotype where indicated in the results. The additive effect of the T allele was estimated by linear regression. The estimated regression coefficient, β , was subtracted from the FC values; $1 \times \beta$ for those individuals having T/C genotypes and $2 \times \beta$ for those individuals having T/T genotypes. This resulted in the mean FC levels being equal for each *XmnI*- γ genotype group and a reduction in the variance of the overall trait distribution. The Marshfield chromosome 8 sex-averaged linkage map¹⁸ was used to

determine the order and interval distances of the 19 markers (Figure 1). Multipoint quantitative trait linkage analysis was carried out using the MAPMAKER/SIBS program¹⁹. The proportion of identity-by-descent (IBD) information extracted by the markers was 0.78, ranging from 0.68 to 0.86.

Results and Discussion

Multipoint linkage analyses of the *XmnI-G γ* adjusted and unadjusted FC trait were carried out with the results shown in Figure 1. The *XmnI-G γ* adjusted FC trait showed higher multipoint 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 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-G γ* 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 al.¹⁶ 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 Kruglyak²⁰. 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-G γ* genotype. The higher lod scores observed for the *XmnI-G γ* adjusted FC trait suggest that a conditional or epistatic interaction between the two 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 two-locus model in the original genome-wide linkage study of the Asian Indian kindred¹⁶. The maximum single-point lod score was 4.33 at marker D8S1833 (see Table 1 in¹⁶). Marker D8S538 mapped

to the same location as D8S1833 and showed a 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*-G γ genotype was not accounted for; however, statistical significance was not achieved when the FC values were adjusted for the genotype¹⁶, 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 to the European twin sample, and it is conceivable that some alleles of the chromosome 8q QTL act conditionally on the *XmnI*-G γ 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*-G γ site, suggesting a cis-acting mechanism. Although the sequence in the -158 region of the G γ 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 co-activators functioning within multiprotein complexes²¹. Altered expression of the G γ 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*-G γ site. Alternatively, the protein could act as a molecular bridge in a protein-protein interaction. In these situations, changes in expression due to 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*- γ site.

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Figure 1

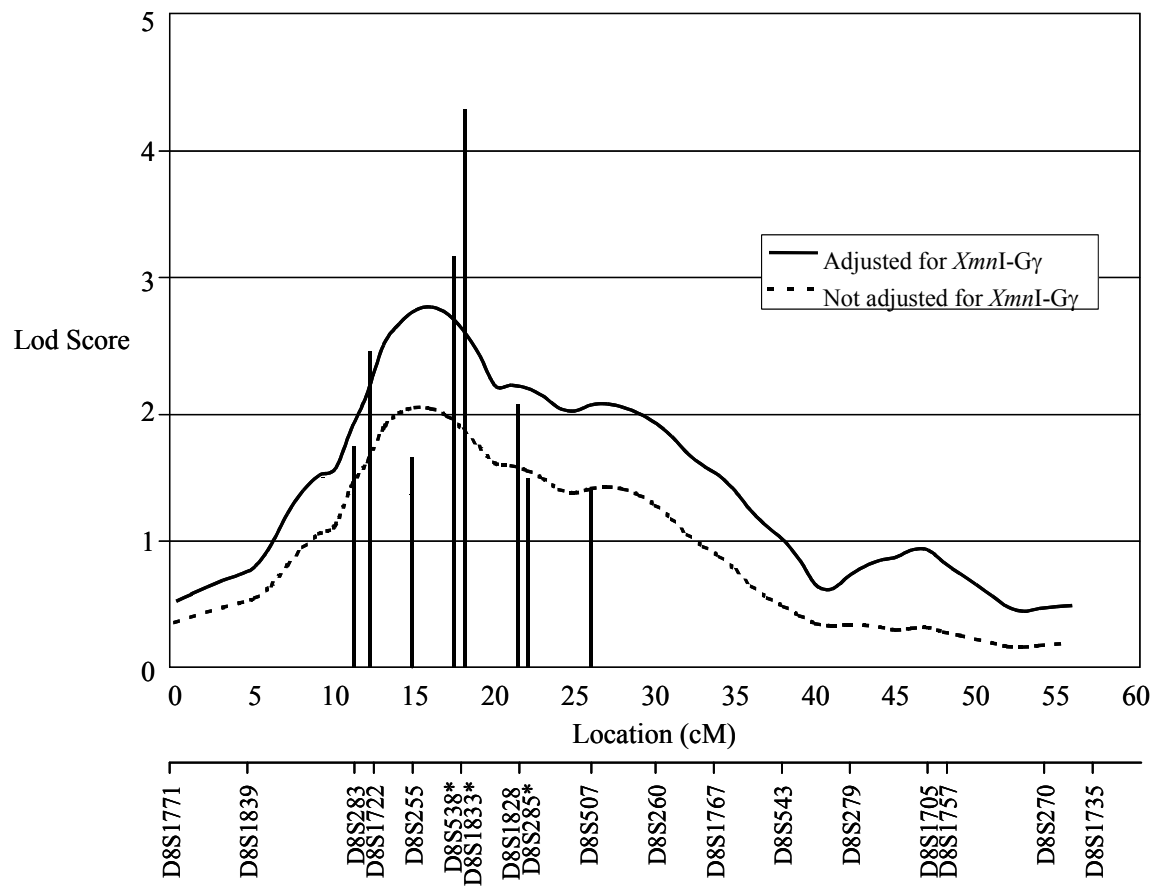


Figure Legends

Figure 1

Multipoint linkage analysis of *XmnI*-G γ adjusted and unadjusted FC traits across 56 cM of chromosome 8q. Markers shown with an asterisks were used in the original linkage study in the Asian Indian kindred but not in the multipoint analysis. Single point lod scores from the original study are shown with bars above the markers tested. Due to missing genotype data, 315 of the 319 total twin pairs were used in the multipoint analysis.