

Genetic regulation of plasma von Willebrand factor levels: quantitative trait loci analysis in a mouse model

H. L. LEMMERHIRT,* K. W. BROMAN,† J. A. SHAVIT‡ and D. GINSBURG*§

*Department of Human Genetics, University of Michigan, Ann Arbor, MI; †Department of Biostatistics, Johns Hopkins University, Baltimore, MD;

‡Department of Pediatrics, University of Michigan, Ann Arbor, MI; and §Howard Hughes Medical Institute, Hematology/Oncology Division, and Department of Internal Medicine, Ann Arbor, MI, USA

To cite this article: Lemmerhirt HL, Broman KW, Shavit JA, Ginsburg D. Genetic regulation of plasma von Willebrand factor levels: quantitative trait loci analysis in a mouse model. *J Thromb Haemost* 2007; 5: 329–35.

Summary. *Background:* The genetic factors responsible for the wide variation in plasma von Willebrand factor (VWF) levels observed among individuals are largely unknown, although these genes are also likely to contribute to variability in the severity of von Willebrand disease (VWD) and other bleeding and thrombotic disorders. We have previously mapped two genes contributing to the regulation of plasma VWF levels in mice (*MvWF1* on chromosome 11 and *MvWF2* on chromosome 6). *Objective:* To identify additional quantitative trait loci (QTL) contributing to the genetic regulation of murine plasma VWF levels. *Methods:* To map genetic loci contributing to the > 7-fold difference in plasma VWF levels between two mouse strains (A/J and CASA/RkJ), high-density individual genotyping and R/qtl analyses were applied to a previously generated set of ~200 F2 mice obtained from an intercross of these two inbred lines. *Results:* Genomic loci for two additional candidate VWF modifier genes were identified: *MvWF3* on chromosome 4 and *MvWF4* on chromosome 13. These loci demonstrate primarily epistatic effects when co-inherited with two CASA/RkJ *Vwf* alleles, although *MvWF4* may also exert a small, independent, additive effect. *Conclusions:* *MvWF3* and *MvWF4*, combined with the effect of *MvWF2*, explain ~45% of the genetic variation in plasma VWF level among the A/J and CASA/RkJ strains. *MvWF3* and *MvWF4* exhibit homology of synteny to three human chromosomal segments (on chromosomes 1, 5 and 6) previously reported by the Genetic Analysis of Idiopathic Thrombophilia (GAIT) study, suggesting that orthologs of *MvWF3* and *MvWF4* may also encode important VWF modifier genes in humans.

Keywords: quantitative trait loci, *MvWF* modifier, von Willebrand factor.

Correspondence: David Ginsburg, Howard Hughes Medical Institute, University of Michigan, 210 Washtenaw, Life Sciences Institute, Room 5036, Ann Arbor, MI 48109, USA.

Tel.: +1 734 647 4808; fax: +1 734 936 2888; e-mail: ginsburg@umich.edu

Received 24 July 2006, accepted 10 November 2006

Introduction

In plasma, von Willebrand factor (VWF) mediates platelet adhesion at sites of vascular injury and serves as the carrier protein for factor VIII (FVIII) [1]. Plasma VWF level regulation is critical for the maintenance of hemostatic balance. Deficiencies in this blood coagulation protein can lead to von Willebrand disease (VWD), the most common inherited bleeding disorder in humans [1]. In contrast, elevated levels of VWF and FVIII correlate with an increase in thrombotic risk [2–4].

Although the risk of bleeding or thrombosis segregates with phenotypic extremes, plasma VWF levels are highly variable and present as a broad continuum within the general population, typically ranging between 50% and 200% of the population average among phenotypically normal individuals [1]. The true impact of this variability on disease risk can be difficult to determine, particularly when distinguishing between individuals with VWF levels in the lower range of ‘normal’ and the upper range of those seen in type 1 VWD (20–50% of the mean) [1]. None the less, the VWF level is determined in large part by genetic factors, with heritability ranging from 25–32% from pedigree analysis [5,6] to as high as 66–75% from twin studies [7,8]. A number of environmental factors also contribute to VWF levels, including age, stress and hormonal status [9].

The majority of VWD cases caused by VWF quantitative deficiency are also associated with mutations in the *VWF* gene [10], especially in severe VWF deficiency (type 3) [1] and the most penetrant forms of mild VWF deficiency (type 1) [11–16]. Several association studies suggest a similar relationship between genotype and phenotype in the general population, where up to 20% of plasma VWF level variability can be traced to the *VWF* gene itself [17–19]. *VWF* alone, however, is not sufficient to explain the heritability of plasma VWF levels. Cases of non-linkage to VWF level in both VWD and non-VWD families [14–16,20–22] as well as the characteristic reduced penetrance and variable expressivity observed among type 1 VWD patients, suggest a significant role for modifier genes in VWF level determination [9].

Aside from *VWF*, the ABO blood group is the most clearly established genetic modifier of circulating VWF levels in

humans, accounting for approximately 30% of heritable VWF level variation [7]. Even with the combined effects of *VWF* and ABO blood group, however, a considerable amount of genetic variation remains to be explained. One effort to dissect this variation by whole-genome linkage and quantitative trait loci (QTL) analysis identified *ABO* and several additional small-effect candidate loci, but failed to identify *VWF* [20], illustrating the difficulty of complex trait dissection and locus validation in human studies.

Plasma VWF levels among inbred mouse strains are also highly variable, making mice a potentially useful model system in which to study and identify candidate *VWF* modifier genes [23,24]. We have previously reported on the identification of two VWF modifier genes from two different intraspecific mouse genetic crosses. Analysis of an (RIIS/J \times CASA/RkJ) F2 population identified *Mvwf1* (for modifier of VWF 1) as a dominant RIIS/J-specific regulatory variant of *B4galnt2* (previously referred to as *Galgt2*) [25]. A recent analysis of an (A/J \times CASA/RkJ) F2 population linked a portion of VWF level variation to a CASA/RkJ-specific coding sequence alteration in *Vwf* (*Mvwf2*) [24]. We now report on a further analysis of this latter F2 population using high-density genotyping and QTL analysis to identify additional genetic modifiers, which may explain some of the previously uncharacterized VWF level variation in the (A/J \times CASA/RkJ) F2 population.

Materials and methods

Plasma sampling and VWF level measurement

A previously described (A/J \times CASA/RkJ) F2 population [24], generated by intercrossing F1 offspring of A/J females and CASA/RkJ males, was used as the test population. Blood samples from parental strains, F1 and F2 mice were obtained as previously described [24]. Briefly, plasma samples from 1–3 retro-orbital bleeds and/or a terminal inferior vena cava bleed were used to determine mean VWF levels for each mouse. Except for two animals in which only three samples were collected, four plasma samples were obtained for each of the F2 animals. A sandwich ELISA method was used to capture and detect the presence of VWF in plasma samples as previously described. ELISA values are represented as a percentage of the pooled CASA/RkJ plasma VWF level (arbitrarily defined as 100%). The VWF levels used here are identical to the data previously reported in Lemmerhirt *et al.* [24].

Purification of liver DNA and F2 genotyping

Purified DNA was obtained by phenol/chloroform extraction of ~ 25 mg of proteinase K digested liver samples. Following precipitation with isopropanol, pellets were washed with 70% EtOH, air dried, and resuspended in 500 μ L TE, pH 8.0. Samples were quantified by spectrophotometer reading at 260/280 (DU530; Beckman Coulter, Fullerton, CA, USA), and diluted to a final concentration of 15 ng μ L⁻¹ before genotyping.

Mice comprising the F2 population were genotyped at 174 polymorphic microsatellite markers and one single nucleotide polymorphism (SNP) across the mouse genome, covering all 19 autosomes and the X chromosome (Fig. S1). The genotyping success rate was approximately 98%, and the average distance between markers was 8.7 cM. Genotyping was conducted at three different locations as follows: 140/175 markers at the Marshfield Clinic (NHLBI genotyping service); 24/175 at the University of Michigan genotyping core using a Model 3730XL DNA Analyzer and primers from Applied Biosystems, Inc. (Foster City, CA, USA); and 11/175 markers were amplified using unlabeled MIT primers (Invitrogen, Carlsbad, CA, USA) and separated by agarose gel electrophoresis or genotyped by a SNP fluorescence polarization assay (primers HR192–194, A/G SNP) [26]. A complete list of the genotyping primers used is provided in Table S1.

PCR amplification of a previously characterized marker linked to *Mvwf2* (*D6Mit12*) detected two genotyping discrepancies between the original characterization of the F2 population at *D6Mit12* and the replicate DNA samples prepared for high-density genotyping. These two samples were eliminated from subsequent analyses, resulting in an F2 population where $n = 198$ (108 females, 90 males).

Statistical analyses

Statistical analyses were performed with R/qtl version 1.02–2 [27], an add-on package to the general statistical software R [28]. We considered \log_{10} transformed VWF levels, as a result of a slight skew in the F2 phenotype distribution. The influence of sex on the phenotype was established via a *t*-test.

QTL analysis was initially performed by interval mapping [29], whereby each locus is considered, one at a time, as a putative quantitative trait locus, and LOD scores are calculated to measure the evidence for linkage at each position. Because of the clear sex difference in the VWF level phenotype, sex was included as an additive covariate (and this adjustment for the sex-specific effect was used in all subsequent analyses). In this model, the effect of a quantitative trait locus is assumed to be the same in males and females. Separate analyses of the males and females, and combined analyses with sex included as a covariate interacting with the putative quantitative trait locus, indicated no evidence for a sex-specific difference in the QTL effects.

The strong effect of the *Vwf* locus on the phenotype led us to include the marker *D6Mit12* (near *Vwf*) as an additive covariate. In this model, we assume that the effect of a putative quantitative trait locus is not dependent on the *D6Mit12* genotype. In order to identify loci exhibiting possible interactions with *Vwf*, interval mapping was also performed with *D6Mit12* included as an interactive covariate, in which case the effect of a quantitative trait locus is allowed to be dependent on the genotype at *D6Mit12*. As the flexibility of this model weakens our ability to detect QTL, and as no evidence of linkage was found by this approach, we then split the F2 population into three groups, according to the individual

genotype at *D6Mit12*, and performed interval mapping separately within each group. Two-dimensional genome scans, with two-QTL models, were also performed, but no evidence for linkage to additional loci was obtained.

The statistical significance of the results was evaluated by permutation tests [30]; 100 000 permutation replicates were used. Approximate confidence intervals for the locations of the identified QTL were obtained via 1.5-logarithm of odds (LOD) support intervals: the intervals in which the LOD score did not fall below 1.5 of its maximum on the chromosome [29]. Estimates of the percentage of the phenotypic variance explained by sex and *D6Mit12* genotype were obtained via the change in the residual sum of squares in the fit of a linear model with and without each factor. The percentage of the phenotypic variance explained by all identified QTL was estimated by the change in the residual sum of squares in the fit of a linear model including sex and all QTL.

Results and discussion

Plasma VWF levels in the inbred mouse strains A/J and CASA/RkJ differ by approximately 8-fold [24]. When plasma VWF levels were examined in a population of (A/J × CASA/RkJ) F2 progeny ($n = 198$), a broad range of VWF levels were observed (11–83% of CASA/RkJ; Fig. 1). This distribution is reminiscent of the wide range of plasma VWF levels observed in humans and suggests the involvement of multiple genetic and/or environmental factors. Previous heritability estimates suggest that the majority (approximately 65%) of VWF level variability between the A/J and CASA/RkJ strains is genetically regulated [24]. We noted that approximately 5% of the total F2 phenotypic variance was attributable to sex differences; the average (\pm SE) of VWF in males and females was 42.5% (± 1.5) and 36.3% (± 1.2), respectively. While we have not reported significant sex-specific differences in VWF levels among mouse populations previously, sex-specific effects are well documented in humans [1,31] and sex-specific variants

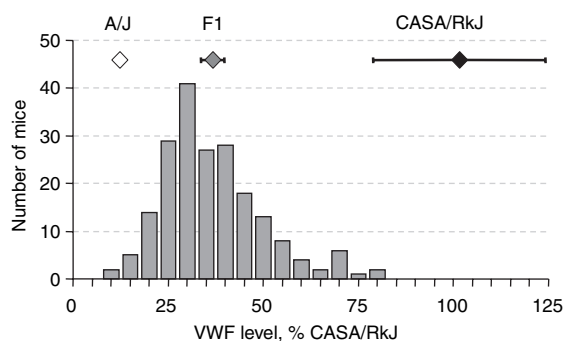


Fig. 1. Original distribution of plasma von Willebrand factor (VWF) levels in 200 (A/J × CASA/RkJ) F2 mice. VWF levels were calculated as a percentage of the CASA/RkJ parental strain. The latter is arbitrarily defined as 100%. F2 values are summarized by the bar graph. Averages for the parental and F1 populations (\pm SD) are shown: A/J (open diamond), CASA/RkJ (black diamond) and F1 (gray diamond). This figure is adapted from Figure 1 of Lemmerhirt *et al.* (*Blood* 2006; 108: 3061).

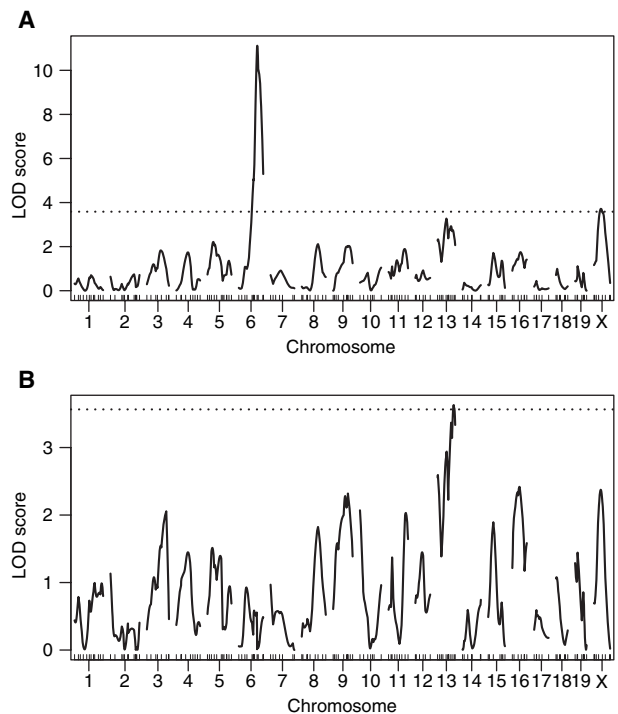


Fig. 2. Logarithm of odds (LOD) curves from a single quantitative trait locus analysis. (A) Inclusion of sex as an additive covariate. (B) Inclusion of both sex and the genotype at *D6Mit12* as additive covariates. The dashed horizontal lines indicate the 95% LOD thresholds.

in measures of partial thromboplastin time (potentially related to VWF and/or FVIII) have also been observed in mice [32].

Following genotyping with 175 polymorphic markers (Fig. S1), QTL analysis employing a single-locus model of inheritance (with adjustment for the effect of sex on VWF phenotype) identified two markers with significant linkage: *D6Mit12* and *DXMit19* with LOD scores of 11.12 and 3.72, respectively (Fig. 2A, Table 1). A third marker, *D13Mit24*, was considered strongly suggestive (LOD = 3.27), but fell below the 95% LOD threshold of 3.59. *D6Mit12* was by far the strongest genetic influence identified, accounting for approximately 18% of the total phenotypic variance, or about 28% of the genetic variance, confirming our previous estimate of

Table 1 Summary of suggestive and significant LOD scores from R/qlt analysis

Covariates	Position		LOD	P-value
	Chromosome	(cM) Marker		
Sex	6	63.7 <i>D6Mit12</i>	11.12	< 0.0001
	13	40.0 <i>D13Mit24</i>	3.27	0.094
	X	33.3 <i>DXMit19</i>	3.72	0.038
Sex and <i>D6Mit12</i>	13	65.0 <i>D13Mit196</i>	3.63	0.049
	X	32.3 <i>DXMit19</i>	2.37	0.500
Sex, in group CC at <i>D6Mit12</i>	4	40.2 <i>D4Mit132</i>	4.45	0.016
	13	26.0 <i>D13Mit248</i>	4.23	0.024

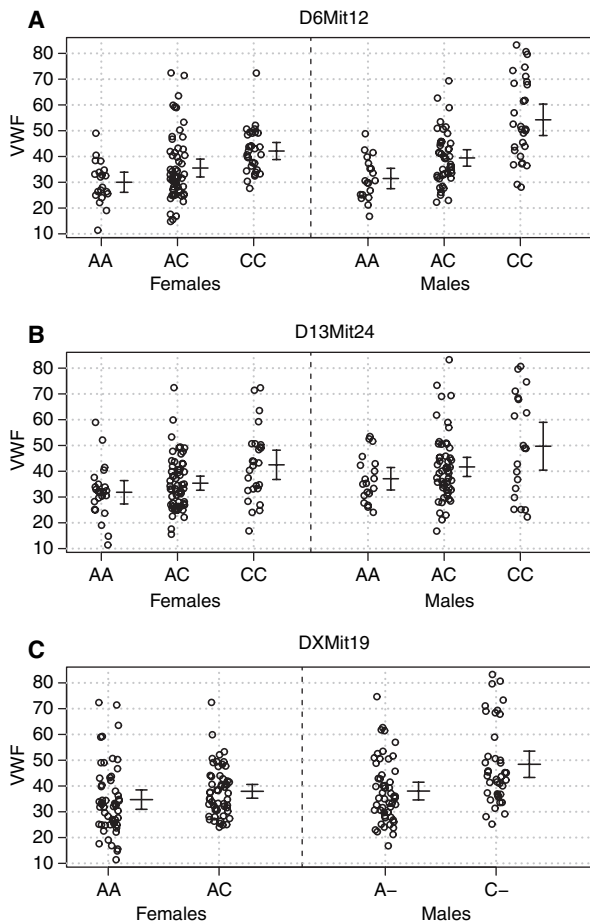


Fig. 3. Genotype and phenotype correlation at three candidate additive von Willebrand factor (VWF) modifier loci. Individual VWF levels and marker genotypes, partitioned by sex, at three candidate murine modifier loci identified via a single quantitative trait locus model. VWF levels are represented as a percentage of CASA/RkJ parental values. Error bars represent 95% confidence intervals.

linkage to *D6Mit12* [24]. These loci were also examined for allele-specific effects on phenotype, as depicted in Fig. 3. Consistent with phenotypes observed in the parental strains, the presence of CASA/RkJ alleles at each candidate locus correlated with an increase in VWF levels. Intermediate phenotypes were noted in heterozygote animals, consistent with additive effects of alleles at each locus.

The molecular mechanism underlying the strong linkage to *D6Mit12* has previously been characterized as a CASA/RkJ-specific coding sequence variant (R2657Q) in the murine *Vwf* gene (*Mvwf2*), which results in higher circulating VWF levels in plasma [24]. The linkage identified on the X chromosome and the potential linkage to chromosome 13 were of particular interest, as they were not detected in our previous analysis of the same sample population and neither has been previously associated with VWF level regulation [24]. The previously identified murine modifier (*Mvwf1*), which is a result of a mutation at the *B4galnt2* locus, is not present in either of the strains studied here [24]. As expected, therefore, no significant evidence for linkage is observed at this locus

(maximum LOD score on murine chromosome 11 is 2.0). Although the ABO blood group is an important VWF modifier in humans, previous studies indicate that the ortholog of the ABO locus is not polymorphic in mice [33,34], which is consistent with the absence of significant linkage at this locus in our study (maximum LOD score on murine chromosome 2 is 1.1).

The very strong effect of the *Vwf* locus led us to repeat the genome scan, including the marker *D6Mit12* as an additive covariate; the intent was to clarify evidence for linkage on chromosomes 13 and X, and to identify additional loci. As shown in Table 1, the inclusion of *D6Mit12* as an additive covariate improved the evidence for chromosome 13, which now achieved significance (*D13Mit196*, LOD = 3.63). However, there was a loss of linkage to the X chromosome, and no other significant linkage was detected (Fig. 2B, Table 1). Together, these data suggest that in an additive model, *Vwf* is the predominant genetic influence on VWF levels between these strains. Chromosome 13 genotype may also influence VWF levels independently, although future validation using a larger sample size is necessary to confirm this effect. Although the evidence for X chromosome linkage in the initial scan appears to result from a chance association in the genotypes between the X chromosome and the *Vwf* locus on chromosome 6, we cannot rule out the possibility that a locus on the X chromosome also contributes to plasma VWF level.

In order to identify additional loci that may interact with *Vwf*, we performed a genome scan with *D6Mit12* included as an interactive covariate; however, no significant interactions between *Vwf* and other loci were detected. To characterize possible interactions between *D6Mit12* and other loci more fully, we partitioned the (A/J × CASA/RkJ) F2 population into three groups according to the genotype of each individual at *D6Mit12* and performed genome scans separately within each group. While no significant linkage was detected within the AA or AC subgroups, two significant linkages were detected in the CC group: a marker on chromosome 13 nearly 40 cM away from the previously identified additive marker *D13Mit196* (*D13Mit248*, LOD = 4.23) and a previously unidentified locus on chromosome 4 (*D4Mit132*, LOD = 4.45; Fig. 4, Table 1). The putative modifier genes underlying these linkage groups were termed *Mvwf3* (chromosome 4) and *Mvwf4* (chromosome 13). These results suggest that loci on chromosomes 13 and 4 may interact with *Vwf* to impact overall VWF levels in mice when the *Vwf* genotype is homozygous for CASA/RkJ alleles (Fig. 5). Mechanistically, these epistatic interactions could impact strain-specific VWF protein production, post-translational modification, intracellular processing, secretion, or clearance from the plasma circulation. We have previously demonstrated that there are not strain-specific transcriptional differences at the *Vwf* allele in (A/J × CASA/RkJ) F1 animals, making an epistatic interaction impacting transcription unlikely [24]. Although we are suggesting an epistatic relationship between *D6Mit12* and these putative loci, we also acknowledge that the power to detect and characterize epistasis in QTL analysis is limited [35]. Further analysis of

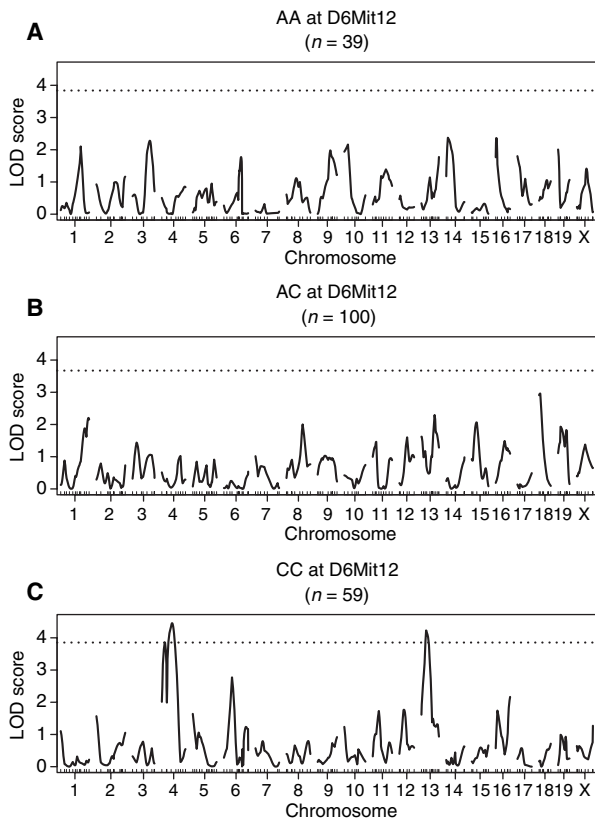


Fig. 4. Logarithm of odds (LOD) scores, partitioned by *D6Mit12* genotype. LOD scores obtained by single quantitative trait locus analyses, performed separately in the three groups defined by the genotype at *D6Mit12*, with sex included as an additive covariate. The dashed horizontal lines indicate the 95% LOD thresholds.

additional F2 offspring will be necessary to confirm the suggested epistasis.

Linkage to chromosomes 4 and 13 was not detected in our previous analysis of the same sample population using a low-density genome scan (45 polymorphic markers) on pooled DNA samples from the phenotypic outliers [24]. Although the use of pooling has been successfully employed as an initial mapping strategy to identify even minor QTL in multiple species [36–39], the power to detect loci that require epistatic interactions for significance is limited. Although the methods employed in our current approach strengthen our power to detect small additive effects and epistatic interactions, failure to detect some of these loci in our original pooling analysis may reflect a combination of insufficient marker density, inconclusive genotyping in some regions of linkage, and/or a masking effect of the *D6Mit12* genotype within the original pools [24]. Even with these two linkage approaches, we have not accounted for the total VWF variance observed between A/J and CASA/RkJ strains. Given the marker density employed, it is unlikely that we have missed any remaining genes of large effect. Further dissection of the genetic variation will require much larger data sets to detect any remaining small-effect modifiers.

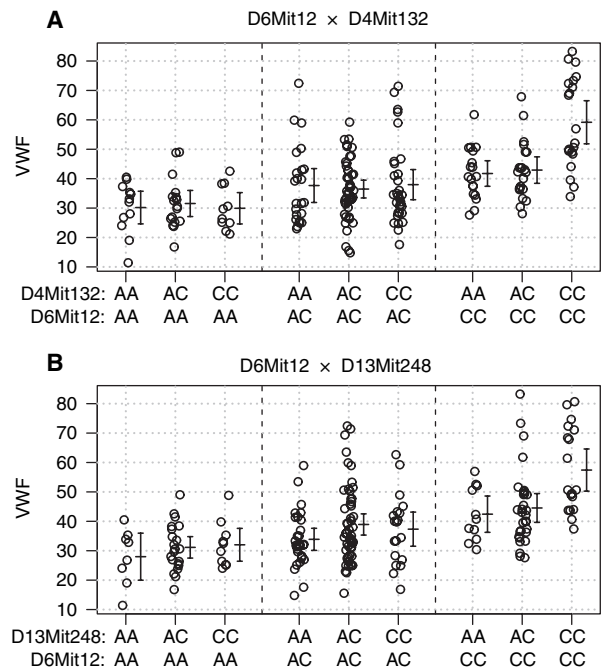


Fig. 5. Phenotype vs. genotype in a two-locus model of epistasis with *D6Mit12*. Individual von Willebrand factor (VWF) levels for individuals partitioned by their two-locus marker genotypes, for markers showing evidence for an epistatic interaction with *D6Mit12*. VWF levels are represented as a percentage of CASA/RkJ parental values. Error bars represent 95% confidence intervals.

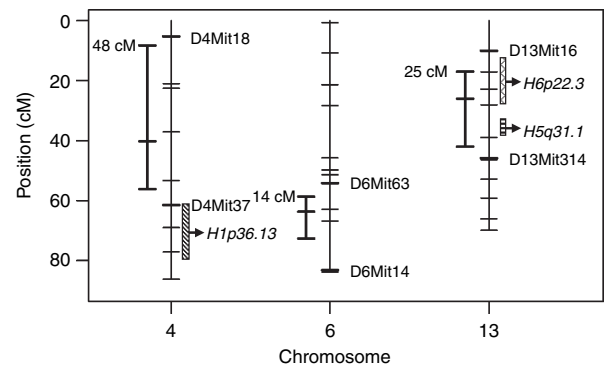


Fig. 6. 1.5-Logarithm of odds (LOD) support intervals for regions of significant linkage and relevant human homology of synteny. The 1.5-LOD support intervals are shown to the left of the chromosomes. Shown to the right (patterned boxes) are relevant regions of human homology of synteny containing quantitative trait loci associated with von Willebrand factor (VWF) level variation in the Genetic Analysis of Idiopathic Thrombophilia (GAIT) study, as reported by Souto *et al.* (*Thromb Haemost* 2003; 89: 468). Additionally, the LOD support interval on murine chromosome 6 shares homology of synteny with the *VWF* locus on human chromosome 12.

The identification of biologically relevant modifier genes in mice can have a significant impact on our understanding of human disease [40,41]. As one example, *Nramp1*, first identified as a murine modifier of tuberculosis susceptibility, has shown direct cross-species relevance for understanding tuberculosis

infection in humans [42,43]. In our investigation of murine VWF level modifiers, we are ultimately interested in the genes which underlie these candidate modifiers, the mechanism by which they may be contributing to VWF level variation in the mouse, and their potential application for understanding of VWF level variation in humans. After correcting for linkage to *ABO*, genome-wide linkage analysis of 21 Spanish families participating in the Genetic Analysis of Idiopathic Thrombophilia (GAIT) project identified significant linkage to five additional candidate VWF modifier loci, located on human chromosomes 1, 2, 5, 6 and 22 [20]. To assess the possible relationship between these human loci and the murine modifiers we have identified, we calculated the 1.5-LOD thresholds for our regions of significant linkage (Fig. 6). Alignment of these intervals with the human database [44] is consistent with a possible homology of synteny between three of the five candidate human VWF modifier loci identified in the GAIT study [20] and the corresponding regions in the mouse genome (Fig. 6). Interestingly, the murine linkage group on chromosome 13 aligns with orthologous linkages on both human chromosomes 5 and 6 (Fig. 6). The *Mvwf4* linkage group could represent two distinct murine VWF modifier loci, perhaps accounting for the distinct additive and epistatic effects we mapped to murine chromosome 13. Taken together, these data suggest that the genetic factors determining VWF levels in the mouse may also be biologically relevant to human VWF level regulation.

In summary, we have used high-density genotyping and QTL analysis both to confirm the influence of *Vwf* (*Mvwf2*) on murine VWF levels and to identify two new regions of the mouse genome which co-segregate with VWF level variation in the A/J and CASA/RkJ strains. The influence of candidate loci on chromosomes 4 and 13 is most likely through epistatic interactions with the *Vwf* CASA/RkJ allele, although chromosome 13 may also exert a small independent, additive effect. We have designated the underlying modifier loci as *Mvwf3* and *Mvwf4*, respectively. Initial linkage to the X chromosome was not confirmed after the inclusion of *D6Mit12* as a covariate, making it difficult to discern how much effect, if any, is attributable to an X chromosome locus. Collectively, these putative modifier loci and *Mvwf2* (*Vwf*) account for approximately 29% of the total VWF level variation (or ~45% of the genetic variation) observed between these populations. Identification of the specific genes underlying *Mvwf3* and *Mvwf4* may provide novel insight into the mechanisms by which VWF levels are regulated in the mouse and may also have direct relevance as modifiers of VWF plasma level and VWD penetrance and expressivity in humans.

Acknowledgements

Support from the National Institute of Health to D. Ginsburg (R37-HL39693) and K. W. Broman (GM074244), the NHLBI Mammalian Genotyping Service (Contract Number HV48141), and the University of Michigan Geno-

typing Core (with support from the University of Michigan Endowment for the Basic Sciences) is gratefully acknowledged. H. L. Lemmerhirt was also supported by a National Science Foundation (NSF) Graduate Fellowship Award. D. Ginsburg is an investigator of the Howard Hughes Medical Institute.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

Supplementary material

The following supplementary material is available for this article:

Table S1. High-density microsatellite panel for (A/J × CASA/RkJ) F2 genotyping.

Fig. S1. High-density microsatellite panel for F2 genotyping. A total of 175 polymorphic markers (indicated by the hash marks) were used in a secondary screen for linkage in the F2 population. The average distance between markers was 8.7 cM.

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1538-7836.2006.02325.x>

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

- Nichols WC, Ginsburg D. Willebrand disease. *Medicine* 1997; **76**: 1–20.
- Koster T, Blann AD, Briët E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. *Lancet* 1995; **345**: 152–5.
- Kraaijenhagen RA, in't Anker PS, Koopman MM, Reitsma PH, Prins MH, van den Ende A, Büller HR. High plasma concentration of factor VIIIc is a major risk factor for venous thromboembolism. *Thromb Haemost* 2000; **83**: 5–9.
- Rosendaal FR. High levels of factor VIII and venous thrombosis. *Thromb Haemost* 2000; **83**: 1–2.
- Vossen CY, Hasstedt SJ, Rosendaal FR, Callas PW, Bauer KA, Broze GJ, Hoogendoorn H, Long GL, Scott BT, Bovill EG. Heritability of plasma concentrations of clotting factors and measures of a pre-thrombotic state in a protein C-deficient family. *J Thromb Haemost* 2004; **2**: 242–7.
- Souto JC, Almasy L, Borrell M, Gari M, Martinez E, Mateo J, Stone WH, Blangero J, Fontcuberta J. Genetic determinants of hemostasis phenotypes in Spanish families. *Circulation* 2000; **101**: 1546–51.
- Orstavik KH, Magnus P, Reisner H, Berg K, Graham JB, Nance W. Factor VIII and factor IX in a twin population. Evidence for a major effect of ABO locus on factor VIII level. *Am J Hum Genet* 1985; **37**: 89–101.
- de Lange M, Snieder H, Ariens RA, Spector TD, Grant PJ. The genetics of haemostasis: a twin study. *Lancet* 2001; **357**: 101–5.
- Levy GG, Ginsburg D. Getting at the variable expressivity of von Willebrand disease. *Thromb Haemost* 2001; **86**: 144–8.

- 10 International Society on Thrombosis and Haemostasis Scientific and Standardization Committee. VWF Information Homepage. <http://www.vwf.group.shef.ac.uk/> (Accessed 5 January 2006).
- 11 Bodo I, Katsumi A, Tuley EA, Eikenboom JC, Dong Z, Sadler JE. Type 1 von Willebrand disease mutation Cys1149Arg causes intracellular retention and degradation of heterodimers: a possible general mechanism for dominant mutations of oligomeric proteins. *Blood* 2001; **98**: 2973–9.
- 12 O'Brien LA, James PD, Othman M, Berber E, Cameron C, Notley CR, Hegadorn CA, Sutherland JJ, Hough C, Rivard GE, O'Shaunessey D, Lillicrap D. Founder von Willebrand factor haplotype associated with type 1 von Willebrand disease. *Blood* 2003; **102**: 549–57.
- 13 Kunicki TJ, Federici AB, Salomon DR, Koziol JA, Head SR, Mondala TS, Chismar JD, Baronciani L, Canciani MT, Peake IR. An association of candidate gene haplotypes and bleeding severity in von Willebrand disease (VWD) type 1 pedigrees. *Blood* 2004; **104**: 2359–67.
- 14 Casana P, Martinez F, Haya S, Espinos C, Aznar JA. Significant linkage and non-linkage of type 1 von Willebrand disease to the von Willebrand factor gene. *Br J Haematol* 2001; **115**: 692–700.
- 15 Castaman G, Eikenboom JC, Bertina RM, Rodeghiero F. Inconsistency of association between type 1 von Willebrand disease phenotype and genotype in families identified in an epidemiological investigation. *Thromb Haemost* 1999; **82**: 1065–70.
- 16 Lanke E, Johansson A, Hallden C, Lethagen S. Genetic analysis of 31 Swedish type 1 von Willebrand disease families reveals incomplete linkage to the von Willebrand factor gene and a high frequency of a certain disease haplotype. *J Thromb Haemost* 2005; **3**: 2656–63.
- 17 De Visser MC, Sandkuijl LA, Lensen RP, Vos HL, Rosendaal FR, Bertina RM. Linkage analysis of factor VIII and von Willebrand factor loci as quantitative trait loci. *J Thromb Haemost* 2003; **1**: 1771–6.
- 18 Keightley AM, Lam YM, Brady JN, Cameron CL, Lillicrap D. Variation at the von Willebrand Factor (vWF) gene locus is associated with plasma vWF:Ag levels: identification of three novel single nucleotide polymorphisms in the vWF gene promoter. *Blood* 1999; **93**: 4277–83.
- 19 Harvey PJ, Keightley AM, Lam YM, Cameron C, Lillicrap D. A single nucleotide polymorphism at nucleotide-1793 in the von Willebrand factor (VWF) regulatory region is associated with plasma VWF:Ag levels. *Br J Haematol* 2000; **109**: 349–53.
- 20 Souto JC, Almasy L, Soria JM, Buil A, Stone W, Lathrop M, Blangero J, Fontcuberta J. Genome-wide linkage analysis of von Willebrand factor plasma levels: results from the GAIT project. *Thromb Haemost* 2003; **89**: 468–74.
- 21 Eikenboom J, van Marion V, Putter H, Goodeve A, Rodeghiero F, Castaman G, Federici AB, Battle J, Meyer D, Mazurier C, Goude-mand J, Schneppenheim R, Budde U, Ingerslev J, Vorlova Z, Habart D, Holmberg L, Lethagen S, Pasi J, Hill F, Peake I. Linkage analysis in families diagnosed with type 1 von Willebrand disease in the European study, molecular and clinical markers for the diagnosis and management of type 1 VWD. *J Thromb Haemost* 2006; **4**: 774–82.
- 22 James PD, Paterson AD, Notley C, Cameron C, Hegadorn C, Tinlin S, Brown C, O'Brien L, Leggo J, Lillicrap D. Genetic linkage and association analysis in type 1 von Willebrand disease: results from the Canadian Type 1 VWD Study. *J Thromb Haemost* 2006; **4**: 783–92.
- 23 Mohlke KL, Nichols WC, Westrick RJ, Novak EK, Cooney KA, Swank RT, Ginsburg D. A novel modifier gene for plasma von Willebrand factor level maps to distal mouse chromosome 11. *Proc Natl Acad Sci USA* 1996; **93**: 15352–7.
- 24 Lemmerhirt HL, Shavit JA, Levy GG, Cole SM, Long JC, Ginsburg D. Enhanced VWF biosynthesis and elevated plasma VWF due to a natural variant in the murine Vwf gene. *Blood* 2006; **108**: 3061–7.
- 25 Mohlke KL, Purkayastha AA, Westrick RJ, Smith PL, Petryniak B, Lowe JB, Ginsburg D. MvWF, a dominant modifier of murine von Willebrand factor, results from altered lineage-specific expression of a glycosyltransferase. *Cell* 1999; **96**: 111–20.
- 26 Hsu TM, Chen X, Duan S, Miller RD, Kwok PY. Universal SNP genotyping assay with fluorescence polarization detection. *BioTechniques* 2001; **31**: 560, 562, 564–8, passim.
- 27 Broman KW, Wu H, Sen S, Churchill GA. R/qtl: QTL mapping in experimental crosses. *Bioinformatics* 2003; **19**: 889–90.
- 28 Ihaka R, Gentleman R. R: a language for data analysis and graphics. *J Comput Graphical Stat* 1996; **5**: 299–314.
- 29 Lander ES, Botstein D. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 1989; **121**: 185–99.
- 30 Churchill GA, Doerge RW. Empirical threshold values for quantitative trait mapping. *Genetics* 1994; **138**: 963–71.
- 31 Conlan MG, Folsom AR, Finch A, Davis CE, Sorlie P, Marcucci G, Wu KK. Associations of factor VIII and von Willebrand factor with age, race, sex, and risk factors for atherosclerosis. The Atherosclerosis Risk in Communities (ARIC) study. *Thromb Haemost* 1993; **70**: 380–5.
- 32 The Jackson Laboratory. *Mouse Phenome Database*. <http://www.jax.org/phenome/>. Accessed 24 January 2006.
- 33 Yamamoto M, Lin XH, Kominato Y, Hata Y, Noda R, Saitou N, Yamamoto F. Murine equivalent of the human histo-blood group ABO gene is a cis-AB gene and encodes a glycosyltransferase with both A and B transferase activity. *J Biol Chem* 2001; **276**: 13701–8.
- 34 Larkin JM, Porter CD. Mice are unsuitable for modelling ABO discordance despite strain-specific A cross-reactive natural IgM. *Br J Haematol* 2005; **130**: 310–7.
- 35 Purcell S, Sham PC. Epistasis in quantitative trait locus linkage analysis: interaction or main effect? *Behav Genet* 2004; **34**: 143–52.
- 36 Taylor BA, Phillips SJ. Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling. *Genomics* 1996; **34**: 389–98.
- 37 Taylor BA, Phillips SJ. Obesity QTLs on mouse chromosomes 2 and 17. *Genomics* 1997; **43**: 249–57.
- 38 Butcher LM, Meaburn E, Knight J, Sham PC, Schalkwyk LC, Craig IW, Plomin R. SNPs, microarrays and pooled DNA: identification of four loci associated with mild mental impairment in a sample of 6000 children. *Hum Mol Genet* 2005; **14**: 1315–25.
- 39 Fisher PJ, Turic D, Williams NM, McGuffin P, Asherson P, Ball D, Craig I, Eley T, Hill L, Chorney K, Chorney MJ, Benbow CP, Lubinski D, Plomin R, Owen MJ. DNA pooling identifies QTLs on chromosome 4 for general cognitive ability in children. *Hum Mol Genet* 1999; **8**: 915–22.
- 40 Nadeau JH. Modifier genes and protective alleles in humans and mice. *Curr Opin Genet Dev* 2003; **13**: 290–5.
- 41 Nadeau JH. Modifier genes in mice and humans. *Nat Rev Genet* 2001; **2**: 165–74.
- 42 Vidal SM, Malo D, Vogan K, Skamene E, Gros P. Natural resistance to infection with intracellular parasites: isolation of a candidate for Bcg. *Cell* 1993; **73**: 469–85.
- 43 Bellamy R, Ruwende C, Corrah T, McAdam KPWJ, Whittle HC, Hill AVS. Variations in the *NRAMP1* gene and susceptibility to tuberculosis in West Africans. *N Engl J Med* 1998; **338**: 640–4.
- 44 The Jackson Laboratory. *Mammalian Orthology and Comparative Maps*. http://www.informatics.jax.org/menus/homology_menu.shtml. Accessed 24 January 2006.

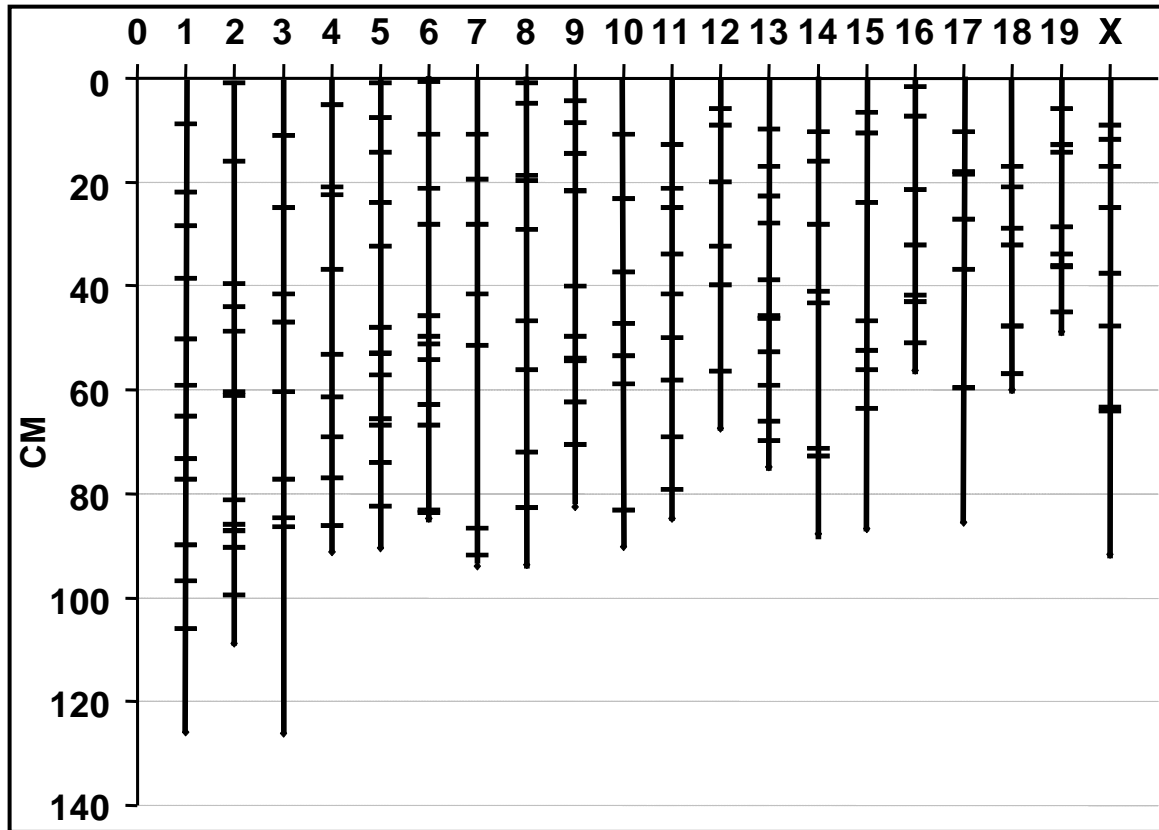


Figure S1: High-density microsatellite panel for F2 genotyping.

A total of 175 polymorphic markers (indicated by the hash marks) were used in a secondary screen for linkage in the F2 population. The average distance between markers was 8.7 cM.

Chr.	Marker	cM	Chr.	Marker	cM	Chr.	Marker	cM	Chr.	Marker	cM
1	D1MIT66	9.0	5	D5MIT345	1.0	9	HR192-194*	4.4	14	D14MIT174	10.5
	D1MIT122	22.1		D5MIT251	7.6		D9MIT64	8.8		D14MIT60	16.1
	D1MIT236	28.6		D5MIT352	14.4		HR132-133†	14.5		D14MIT64	28.2
	D1MIT303	38.7		D5MIT11	24.0		D9MIT285	21.9		D14MIT39	41.1
	D1MIT134	50.3		D5MIT301	32.4		D9MIT302	40.2		D14MIT68	43.5
	D1MIT185	59.2		D5MIT309	48.0		D9MIT198	49.8		D14MIT170	71.3
	D1MIT495	65.1		D5MIT155	52.9		D9MIT355	54.1		D14MIT75	72.8
	D1MIT102	73.4		D5MIT158	57.2		D9MIT183	54.6		15	D15MIT13
	D1MIT425	77.4		D5MIT95	65.7		D9MIT350	62.5	D15MIT252		10.7
	D1MIT270	90.0		D5MIT161	66.8		D9MIT279	70.6	D15MIT143		24.1
	D1MIT406	96.8		D5MIT167	74.1	10	D10MIT213	11.0	D15MIT68		46.8
	D1MIT292	106.2		D5MIT409	82.5		D10MIT184	23.4	D15MIT159		52.6
	2	D2MIT1		1.0	6	D6MIT138	0.7	D10MIT115	37.5	D15MIT242	56.3
D2MIT295		16.1	D6MIT116	10.8		D10MIT95	47.3	D15MIT193	63.7		
D2MIT61		39.5	D6MIT272	21.3		D10MIT96	53.4	16	D16MIT32	1.7	
D2MIT349		44.0	D6MIT123	28.3		D10MIT233	59.0		D16MIT81	7.5	
D2MIT126		48.8	D6MIT67	45.8		D10MIT103	83.2		D16MIT58	21.5	
D2MIT62		60.3	D6Mit106	49.8		11	D11MIT152		13.0	D16MIT157	32.2
D2MIT395		61.1	D6MIT328	51.4			D11MIT51		21.4	D16MIT217	41.9
D2MIT285		81.3	D6MIT63	54.1		D11MIT189	25.0	D16MIT189	43.2		
D2MIT411		85.9	D6MIT12	62.9		D11MIT86	34.0	D16MIT52	51.0		
D2MIT49		87.2	D6MIT194	66.9		D11MIT4	41.7	17	D17MIT133	10.4	
D2MIT145		90.4	D6MIT14	83.2	D11MIT36	50.1	D17MIT176		18.1		
D2MIT148		99.7	D6MIT15	83.8	D11MIT289	58.1	D17MIT51		18.6		
3		D3MIT203	11.2	7	D7MIT267	11.0	D11MIT180		69.2	D17MIT180	27.3
	D3MIT6	25.0	D7MIT228		19.5	D11MIT203	79.2		D17MIT53	37.0	
	D3MIT22	41.6	D7MIT198		28.2	12	D12MIT58	6.0	D17MIT155	59.6	
	D3MIT49	47.2	D7MIT350		41.5		D12MIT136	9.2	18	D18MIT12	17.0
	D3MIT315	60.5	D7MIT98		51.5	D12MIT285	20.1	D18MIT202		21.1	
	D3MIT194	77.2	D7MIT109		86.8	D12MIT143	32.5	D18MIT123		29.0	
	D3MIT200	84.8	D7MIT259		92.0	D12MIT194	39.9	D18MIT152		32.3	
D3MIT147	86.4	D8MIT155	1.0	D12MIT141	56.5	D18MIT186	47.7				
4	D4MIT18	5.2	8	D8MIT124	5.0	13	D13MIT16	10.0	D18MIT188	47.7	
	D4MIT196	21.0		D8MIT24	18.8		D13MIT275	17.0	D18MIT4	57.1	
	D4MIT238	22.5		D8MIT292	19.8		D13MIT138	22.8	19	D19MIT68	6.0
	D4MIT132	37.0		D8MIT205	29.2		D13MIT248	28.1		D19MIT28	12.8
	D4MIT255	53.3		D8MIT50	46.8		D13MIT24	38.9		D19MIT16	14.4
	D4MIT37	61.5		D8MIT211	56.3		D13MIT314	45.8		D19MIT82	28.8
	D4MIT203	69.0		D8MIT215	72.0		D13MIT202	46.4		D19MIT90	33.9
	D4MIT251	77.1		D8MIT42	82.8		D13MIT106	46.4	D19MIT53	36.2	
	D4MIT190	86.2					D18MIT223	52.8	D19MIT17	36.5	
							D13MIT287	59.1	D19MIT103	45.1	
				D13MIT196	66.1	X	DXMIT81	9.3			
				D13MIT78	69.9		DXMIT50	11.8			
							DXMIT68	17.2			
							DXMIT119	25.0			
							DXMIT19	37.8			

*	(F): GGAAGGTGGACTGTGGACAT
	(R): TGCCACAATCCTTGTTTCAGA
	(F _{snp}): GCATTACATCTGTTTCTAGCGCCATGTG
†	(F): GACCTTGCTGAAAAAGTGC
	(R): GGTTCATTTCGAATCCATCA

Table S1: High-density microsatellite panel for (A/J×CASA/RkJ) F2 genotyping.