Linkage Disequilibrium Analysis of D12S391 and vWA

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European Standard Set of Forensic Loci

• Prior to April 2009, European Standard Set consisted of seven STR loci
  – TH01, vWA, FGA, D8S1179, D18S51, D21S11, and D3S1358

• In November 2009, European Union adopted five additional STRs
  – D12S391, D1S1656, D2S441, D10S1248, and D22S1045

• These loci are included in the next generation of multiplex PCR kits
  – PowerPlex® ESX and ESI Systems (Promega)
  – AmpF/STR® NGM™ (Applied Biosystems)
  – Investigator ESSplex and ESSplex SE Kits (Qiagen)
Chromosomal Positions for the European Standard Set and Other Common STR Markers Used

European Standard Set + D16S539, D2S1338, D19S433, SE33

6.3 Mb apart
Genetic Markers for Forensic Use

• Ensure full recombination and independent inheritance

• Markers on the same chromosome should be at least 50 Mb apart (ideal for forensic use)

• CODIS loci CSF1PO and D5S818 (26 Mb apart) are considered statistically independent
  – No deviation from Hardy-Weinberg equilibrium, no linkage disequilibrium in population samples
    J.W. Bacher et al., Chromosome localization of CODIS loci and new pentanucleotide repeat loci, Progress in Forensic Genetics 8 (2000) 33–36
  – Small increased effect of linkage on match probabilities noted in full and half sibling pairs

• Are vWA and D12S391 (6.3 Mb apart) independent?

• Should vWA and D12S391 be used with the product rule for match probability calculations?
Research Design

• NIST U.S. population samples
  – 254 African American, 261 Caucasian, 139 Hispanic

• U.S. father/son samples
  – 178 African American, 198 Caucasian, 190 Hispanic, 198 Asian

• Previously genotyped with PowerPlex® ESI/ESX 17

• Father/son genotypes phased to identify paternally transmitted alleles

Statistical Tests using Arlequin v. 3.5

• Hardy-Weinberg Equilibrium (population samples)
  – Exact test = test the hypothesis that the observed genotypes are the product of a random union of gametes

• Linkage Disequilibrium (population samples, phase unknown)
  – Likelihood ratio test = likelihood of sample when hypothesis of no association between loci vs. likelihood of sample when association is allowed

• Linkage Disequilibrium (paternity samples, phase known)
  – Exact test = test for the presence of significant association between pairs of loci

L. Excoffier, G. Laval, S. Schneider, Arlequin ver. 3.0: An integrated software package for population genetics data analysis, Evolutionary Bioinformatics Online 1 (2005) 47–50.
\(P\)-values from analyses of Hardy-Weinberg equilibrium and linkage disequilibrium of the D12S391 and vWA loci using the unrelated NIST U.S. population samples

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>Hardy-Weinberg Equilibrium</th>
<th>Linkage Disequilibrium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D12S391</td>
<td>vWA</td>
</tr>
<tr>
<td>African American</td>
<td>254</td>
<td>0.0982</td>
<td>0.9853</td>
</tr>
<tr>
<td>Caucasian</td>
<td>261</td>
<td>0.7814</td>
<td>0.1381</td>
</tr>
<tr>
<td>Hispanic</td>
<td>139</td>
<td>0.6434</td>
<td>0.9329</td>
</tr>
</tbody>
</table>

Significance level, \(p < 0.05\).

No significant departure from HWE for D12S391 or vWA (\(p > 0.05\))

No significant linkage disequilibrium between the loci (\(p > 0.05\))

Consistent with results from seven worldwide populations

$P$-value results from analysis of linkage disequilibrium of the D12S391 and vWA loci using **U.S. father/son paternity samples**

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>Linkage Disequilibrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>African American</td>
<td>178</td>
<td>0.0275</td>
</tr>
<tr>
<td>Caucasian</td>
<td>198</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hispanic</td>
<td>190</td>
<td>0.0915</td>
</tr>
<tr>
<td>Asian</td>
<td>198</td>
<td>0.0031</td>
</tr>
</tbody>
</table>

$N =$ number of father/son samples. Significance level, $p < 0.05$.

Evidence of LD in African American, Caucasian, and Asian paternity samples

No significant LD detected in Hispanic paternity samples
  • Population effect is possible
Linkage Disequilibrium between D12S391 and vWA

• Use of father/son pairs allowed for allelic phase to be determined
  – Significant LD was detected
  – Non-random association of alleles at D12S391 and vWA

• LD is more difficult to detect in unrelated population samples due to less power
  – Unknown allelic phase
  – Large number of possible haplotypes
Profile Probability Calculations

For casework analysis that involves unrelated or related individuals:

• Single-locus genotype probabilities of D12S391 and vWA should not be multiplied to determine the STR profile probability

• Possible solutions:
  1. Choose one locus for profile probability calculations
  2. Use haplotype frequencies of D12S391/vWA diplotype

A diplotype consists of two haplotypes, which are phased multilocus genotypes
Single Locus vs. Haplotype Approach

• African American allele frequencies
  – Most common allele for D12S391 = 0.267
  – Most common allele for vWA = 0.254

• African American haplotype frequencies in phased paternity samples*
  – Most common haplotype for D12S391/vWA = 0.087

Haplotype frequencies are generally rarer than the allele frequencies of a single locus

Haplotype Approach with Unphased Alleles

Family reference data may not be available to infer the gametic phase of alleles at D12S391 and vWA

<table>
<thead>
<tr>
<th>Unphased genotype: A,B at D12S391</th>
<th>Two possible diplotypes from four haplotype combinations</th>
<th>Probability of observing either one of the possible diplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>vWA D12S391</td>
<td>C,D at vWA</td>
<td>If a diplotype is comprised of two different haplotypes (i.e., AC ≠ BD and AD ≠ BC), then</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[ \text{Pr}(AC/BD \text{ or } AD/BC) = 2(p_{AC}p_{BD} + p_{AD}p_{BC}) ]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>if a diplotype is comprised of the same haplotypes (i.e., AC = BD = AD = BC), then</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[ \text{Pr}(AC/BD \text{ or } AD/BC) = 2p_{AC}^2 ]</td>
</tr>
</tbody>
</table>
Summary

• U.S. is looking to expand the core loci to provide more international overlap (18-20 loci total)

• Single-locus genotype probabilities **should not** be multiplied for match probability calculations

• Recommend using haplotype frequencies of a D12S391/vWA diplotype for profile probability calculations
  – Allows for consideration of genotype data from both loci without statistical bias

• Further work with multi-generation families is needed to determine the actual recombination fraction between the linked D12S391 and vWA loci
  – Assess the impact of the linkage effect on match probability calculations

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http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
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