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SUPPLEMENTAL MATERIAL

Heritability and Preliminary Genome-Wide Linkage Analysis of Arsenic Metabolites in Urine

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Statistical Methods, Variance component models for general pedigrees conducted by SOLAR.

Heritability model

We estimated the heritability of urine arsenic metabolites (%iAs, %MMA and %DMA) by using a general pedigree variance-component method as implemented in the software Sequential Oligogenic Linkage Analysis Routines (SOLAR) (Blangero et al. 2013). SOLAR incorporates the information contained in the participants' pedigrees to obtain maximum likelihood estimates for the proportion of unexplained variance due to additive genetic effects from polygenes and the proportion of variance due to unmeasured environmental covariates, measurement error and non-additive genetic effects. The resultant polygenic model is specified as follows:

$$y_i = \mu + \Sigma^j_1 \beta_j x_{ij} + g_i + e_i \text{ [Equation 1]}$$

where y_i is the observed urine arsenic metabolite (%iAs, %MMA or %DMA) for individual i; μ is the mean when all the covariates in the models are zero; β_j is the vector of regression coefficients; x_{ij} is the value of the covariable j in subject i; and g_i and e_i are the deviations from μ in the individual i which are attributable to additive genetic effects (g_i) and other sources of error (e_i) including unmeasured environmental effects, gene-gene interaction and gene-environment interaction. We assume that g_i and e_i are uncorrelated and normally distributed with mean 0 and variances σ^2_g and $\sigma^2_{e.}$

To enable the analysis of arbitrary pedigree structures, the variance in the model is structured as the following covariance matrix:

$$\Omega = 2\,\Phi\,\sigma^2_g + I\,\sigma^2_e \text{ [Equation 2]}$$

where Φ is the matrix containing the kinship coefficients for all pairs of relatives in the data and *I* is an identity matrix. Subsequently, the expected mean and covariance matrix for each pedigree are defined, and the likelihood of a pedigree is evaluated using the multivariate normal distribution and summing over all pedigrees. The heritability (h^2) is defined as the proportion of unexplained variance in the observed distribution of urine arsenic metabolite that is attributable

to additive genetic effects, or $h^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_e)$. The p-values for h^2 are computed from a likelihood ratio test comparing the likelihood of the model in which the σ^2_g is estimated to a model where σ^2_g is constrained to be 0. These models are more deeply discussed by Hopper JL and Lange K (Hopper and Mathews 1982; Lange and Boehnke 1983).

Quantitative trait locus model

The linkage scan was based on variance component methods as implemented by SOLAR (Almasy and Blangero 1998). The model builds on the model in Equation 1 (see above) adding a term for *k* quantitative trait loci (QTLs), potentially associated to urine arsenic metabolites variability, where q_{ik} is the normally distributed error term due to the k^{th} QTL in individual i. The resulting equations for the observed urine arsenic metabolite values including the QTLs and corresponding variance are specified as follows:

$$y_i = \mu + \Sigma^j_1 \beta_j x_{ij} + \Sigma^k_1 q_{ki} + g_i + e_i \text{ [Equation 3], and}$$

$$\Omega = \Sigma^k_1 \Pi_k \sigma_{qk} + 2 \Phi \sigma^2_g + I \sigma^2_e \text{ [Equation 4],}$$

where Π_k is the identity-by-descent (IBD) matrix whose elements provide the probability of sharing genes identical by descent for a given pair of individuals at a given genetic marker locus potentially linked to a QTL, Φ is the kinship matrix and I is the identity matrix, σ_{qk} refers to genetic variance due to the QTL and σ^2_g refers to the residual additive genetic variance. Almasy L. and Blangero have described the variance component model for QTL linkage analysis in general pedigrees in more detail (Almasy and Blangero 1998; Blangero and Almasy 1997).

References

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Hopper JL, Mathews JD. 1982. Extensions to multivariate normal models for pedigree analysis. Ann Hum Genet 46(Pt 4):373-383.

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Supplemental Material, Table S1. Participant characteristics

Table S1. Characteristics of Strong Heart Study participants with at least one relative within the cohort and STR markers measured (N=487)[a]

	Arizona (N= 178)	Oklahoma (N= 184)	Dakotas (N= 125)	Overall (N= 487)
Age (years)	54.3 (0.5)	55.6 (0.5)	55.2 (0.6)	55.0 (0.3)
Sex (% males)	29.2 (3.4)	39.7 (3.6)	36.0 (4.3)	34.9 (2.2)
Education (%<High School)	62.4 (3.6)	29.9 (3.4)	30.4 (4.1)	41.9 (2.2)
BMI (kg/m^2)	32.7 (0.4)	31.0 (0.4)	30.7 (0.5)	31.6 (0.3)
Smoking status (%)				
Former	33.7 (3.6)	33.7 (3.5)	34.4 (4.3)	33.9 (2.1)
Current	16.9 (2.8)	34.8 (3.5)	40.8 (4.4)	29.8 (2.1)
Current alcohol drinkers (%)				
Former	40.4 (3.7)	41.8 (3.6)	35.2 (4.3)	39.6 (2.2)
Current	41.0 (3.7)	35.3 (3.5)	52.8 (4.5)	41.9 (2.2)
Total arsenic (μg/g)[b]	19.22 (12.70, 26.93)	7.93 (6.14, 11.46)	8.73 (5.62, 15.36)	11.19 (7.03, 18.86)
Arsenobetaine (μg/g) [b]	0.72 (0.47, 1.25)	0.70 (0.47, 1.20)	0.54 (0.37, 1.28)	0.66 (0.45, 1.27)

[a]Percentages (standard errors) for categorical variables or means (standard errors) for continuous variables.

[b]Median (Interquartile range).

Total arsenic was measured directly using inductively coupled plasma mass spectrometry (ICPMS, see methods section).

Supplemental Material, Table S2. Urine arsenic metabolites by participant characteristics

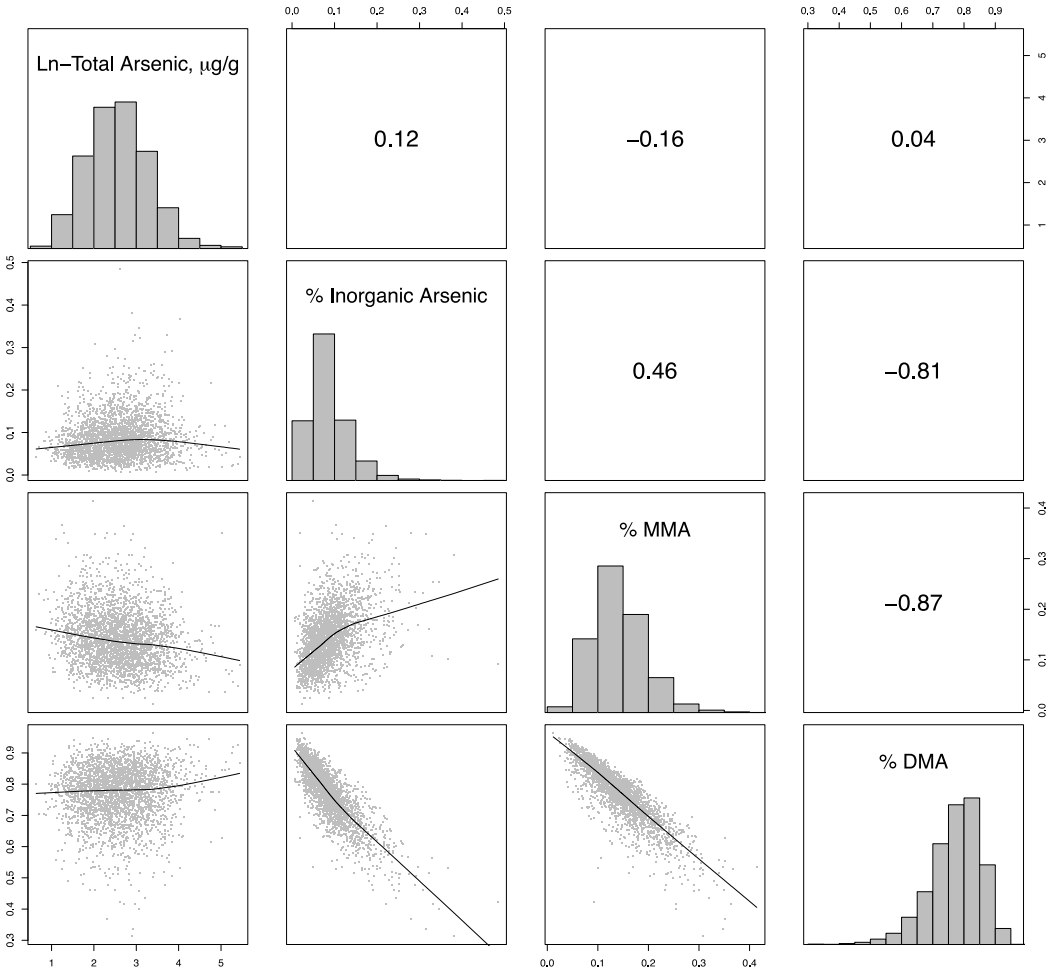
Table S2. Median (interquartile range) of percentage urine arsenic species in the Strong Heart Study participants with at least one relative within the cohort

	N	% Inorganic As	% MMA	% DMA
Overall	487	7.6 (5.4, 10.3)	13.7 (10.6, 16.9)	78.8 (72.1, 83.1)
Age, years				
≤55	271	8.2 (5.7, 10.4)	13.4 (10.5, 17.1)	78.3 (71.9, 82.5)
>55	216	6.9 (4.8, 10.3)	13.9 (10.8, 16.8)	79.6 (72.7, 83.8)
Sex				
Men	170	8.8 (6.2, 12.3)	15.4 (11.7, 18.4)	75.4 (69.7, 81.1)
Women	317	7.1 (4.8, 9.4)	12.8 (10.1, 15.9)	79.9 (74.8, 84.1)
Study region				
Arizona	178	8.7 (5.9, 11.7)	12.7 (10.3, 15.6)	78.9 (72.1, 83.1)
Oklahoma	184	7.0 (4.8, 9.3)	13.3 (10.2, 16.2)	79.2 (73.9, 84.2)
Dakotas	125	7.3 (5.3, 10.3)	15.9 (12.4, 18.5)	77.2 (70.3, 81.1)
Education				
> 12 years	283	7.2 (5.4, 9.8)	13.7 (10.7, 17.1)	78.8 (72.2, 83.3)
≤ 12 years	204	8.3 (5.5, 10.9)	13.6 (10.5, 16.7)	78.6 (72.1, 82.9)
BMI, kg/m^2				
<30	201	8.7 (6.0, 11.8)	15.5 (12.2, 18.4)	76.1 (69.8, 81.1)
≥ 30	286	7.1 (5.1, 9.4)	12.4 (10.0, 15.5)	80.4 (74.9, 84.3)
Smoking				
Never	177	6.8 (5.2, 9.1)	12.2 (9.7, 15.6)	80.9 (75.6, 84.2)
Former	165	7.4 (5.0, 10.1)	13.7 (10.5, 16.8)	78.8 (72.0, 83.7)
Current	145	8.9 (6.1, 12.1)	15.1 (12.4, 18.1)	76.0 (70.4, 80.5)
Alcohol drinking				
Never	90	6.6 (4.9, 8.6)	12.2 (9.4, 15.9)	80.8 (75.8, 85.7)
Former	193	7.9 (5.5, 11.2)	13.9 (11.1, 16.9)	78.4 (71.7, 82.1)
Current	204	8.0 (5.4, 10.5)	14.2 (10.8, 17.3)	78.2 (71.9, 82.7)
Total Arsenic, μg/g				
< 12.9	279	7.3 (5.4, 9.5)	14.6 (11.2, 17.6)	78.4 (71.9, 82.2)
≥ 12.9	208	8.3 (5.4, 11.7)	12.4 (9.9, 15.6)	79.3 (72.4, 83.9)
Arsenobetaine, μg/g				
< 0.7	258	8.1 (5.8, 10.7)	14.4 (11.2, 17.7)	77.4 (71.8, 81.6)
≥ 0.7	229	7.1 (4.7, 10.2)	12.7 (10.0, 16.4)	80.4 (73.7, 84.4)

For BMI we selected 30 kg/m^2, a cut-off commonly used to classify individuals as obese and non-obese. For education we selected 12 years of education, as at least 12 years of education is consistent with the completion of high school. Total arsenic was measured directly using inductively coupled plasma mass spectrometry (ICPMS, see methods section).

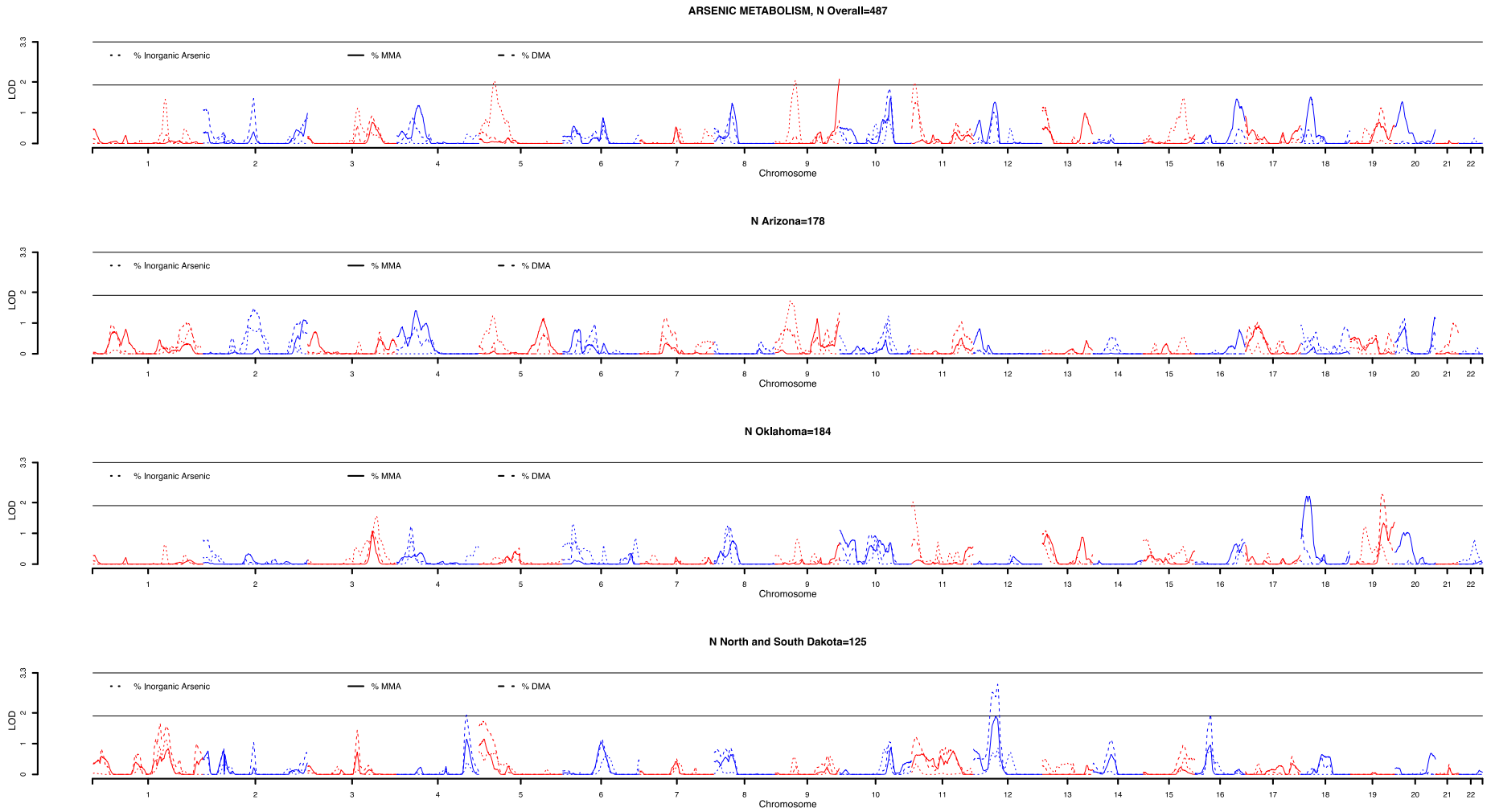
Supplemental Material, Figure S1. Correlation matrix

Figure S1. Distribution and relationship of total arsenic concentrations (μg/g creatinine) and arsenic metabolites (%iAs, %MMA and %DMA) in urine (n=2,907).



The diagonal shows the distribution of the variables in the correlation matrix. The upper diagonal panel shows the Spearman correlation coefficients for the corresponding variables in the correlation matrix. The lower diagonal panel shows the scatterplots and the smoothed relationship for the corresponding variables in the correlation matrix using the lowess command in R software (R-Development Core Team 2012, available at: http://cran.r-project.org/).

Supplemental Material, Figure S2. Linkage scan of genetic loci associated with urine arsenic metabolites in Strong Heart Study participants with short tandem repeat markers genotyped, stratified by study region



Models adjusted for age, age^2, sex, age*sex, age2*sex, smoking status (never, former, current), education (> 12 years, ≤ 12 years), body mass index (<30 and ≥30 kg/m^2), alcohol status (never, former, current), region (Dakotas, Oklahoma and Arizona) and total arsenic (log μg/g). Residual kurtosis was -0.05 for % inorganic arsenic, -0.05 for %MMA and -0.04 for %DMA. The horizontal lines represent LOD scores of 1.9 and 3.3, which are considered as suggestive and confirmed evidence in favor of linkage with a causal locus (Lander E and Kruglyak L. Nat Genet 1995;11:241-247).

Supplemental Material, Table S3. List of short tandem repeat (STR) markers used in the Strong Heart Family Study. The chromosomal location is based on Haldane centimorgans.

Chromosome	Marker	Location
1	D1S468	4.16
	D1S214	12.35
	D1S450	16.95
	D1S2667	20.32
	D1S2697	29.88
	D1S199	38.34
	D1S234	45.44
	D1S255	59.79
	D1S2797	70.2
	D1S2890	82.35
	D1S230	89.57
	D1S2841	105.49
	D1S207	108.87
	D1S2868	118.5
	D1S206	124.55
	D1S2726	134.14
	D1S252	141.25
	D1S498	147.13
	D1S484	159.84
	D1S2878	168.29
	D1S196	171.94
	D1S218	178.92
	D1S238	191.32
	D1S413	197.84
	D1S249	210.08
	D1S425	218.18
	D1S213	228.76
	D1S2800	244.37
	D1S2785	261.73
	D1S2842	266.17
	D1S2836	276.53
2	D2S319	12.31
	D2S2211	18.3
	D2S162	23.43
	D2S168	29.74
	D2S305	42.37
	D2S165	52.05
	D2S367	59.1

	D2S2259	68.38
	D2S391	73.72
	D2S337	85.62
	D2S2368	90.93
	D2S286	101.9
	D2S2333	110.84
	D2S2216	113.54
	D2S160	127.14
	D2S347	137.61
	D2S112	148.29
	D2S151	160.24
	D2S142	168.06
	D2S2330	174.06
	D2S335	181.11
	D2S364	191.09
	D2S117	198.11
	D2S325	208.47
	D2S2382	217.52
	D2S126	227.19
	D2S396	239.32
	D2S206	244.07
	D2S338	252.65
	D2S125	262.74
3	D3S1297	4.95
	D3S1304	20.31
	D3S1263	30.53
	D3S3659	45.15
	D3S1266	51.92
	D3S1277	61.67
	D3S1289	75.46
	D3S1300	84.03
	D3S1285	91.18
	D3S1566	96.72
	D3S3681	109.69
	D3S1271	114.57
	D3S1278	125.11
	D3S1267	132.82
	D3S1292	141.43
	D3S1569	153.31
	D3S1279	163.02
	D3S1614	172.88
	D3S1565	181

	D3S1262	197.62
	D3S1580	205.46
	D3S1601	211.65
	D3S1311	223.98
4	D4S412	4.52
	D4S2935	13.24
	D4S403	27.62
	D4S419	36.07
	D4S391	48.62
	D4S405	61.72
	D4S1592	74.36
	D4S392	82.1
	D4S2964	90.26
	D4S1534	95.04
	D4S1572	109.5
	D4S406	116.82
	D4S402	124.26
	D4S1575	133.91
	D4S424	141.27
	D4S413	154.83
	D4S1597	166.41
	D4S1539	172.85
	D4S415	176.12
	D4S1535	192.54
	D4S426	206.46
5	D5S1981	1.2
	D5S406	12.98
	D5S630	24.73
	D5S416	37.82
	D5S419	47.48
	D5S426	58.25
	D5S418	66.17
	D5S407	73.57
	D5S647	80.58
	D5S424	92.74
	D5S641	101.1
	D5S428	103.9
	D5S644	110.69
	D5S433	114.77
	D5S2027	120.17
	D5S471	127.38

	D5S2115	139.28
	D5S436	150
	D5S410	161.55
	D5S422	169.32
	D5S400	180.4
	D5S408	209.3
6	D6S1574	15.95
	D6S309	21.34
	D6S470	24.65
	D6S289	35.97
	D6S422	44.27
	D6S276	49.4
	D6S1610	60.02
	D6S257	81.56
	D6S460	92.65
	D6S462	100.04
	D6S300	103.02
	D6S287	122.15
	D6S262	133.64
	D6S292	139.36
	D6S308	147.97
	D6S441	163.69
	D6S1581	173.43
	D6S264	184.73
	D6S281	192.35
	D6S446	193.14
7	D7S531	7.67
	D7S517	8.86
	D7S513	23.1
	D7S507	32.2
	D7S493	37.65
	D7S516	45.07
	D7S484	55.58
	D7S510	61.59
	D7S519	69.87
	D7S502	80.58
	D7S669	90.83
	D7S630	101.52
	D7S657	105.09
	D7S515	113.01
	D7S486	124.27

	D7S530	132.61
	D7S640	140.97
	D7S684	149.07
	D7S661	154.31
	D7S636	165.35
	D7S798	172.99
	D7S2423	186.83
8	D8S264	3.47
	D8S277	15.76
	D8S550	21.88
	D8S549	26.98
	D8S258	35.88
	D8S1771	44.92
	D8S505	55.63
	D8S285	69.83
	D8S260	75.18
	D8S270	99.18
	D8S1784	114.59
	D8S514	126.9
	D8S284	142.34
	D8S272	152.77
9	D9S288	8.53
	D9S286	18.34
	D9S285	34.59
	D9S157	37.33
	D9S171	46.84
	D9S161	52.43
	D9S1817	57.85
	D9S273	68.12
	D9S175	72.25
	D9S167	82.73
	D9S283	95
	D9S287	100.82
	D9S1690	106.26
	D9S1677	115.15
	D9S1776	124
	D9S1682	131.25
	D9S290	138.99
	D9S164	149.72
	D9S1826	161.18
	D9S158	161.76

10	D10S249	1.19
	D10S591	14.76
	D10S189	20.36
	D10S547	28.5
	D10S1653	38.66
	D10S548	44.41
	D10S197	51.11
	D10S208	61.08
	D10S196	71.44
	D10S1652	82.09
	D10S537	90.78
	D10S1686	106.69
	D10S185	115.25
	D10S192	122.74
	D10S597	128.73
	D10S1693	140.61
	D10S587	151.27
	D10S217	162.82
	D10S1651	175.11
	D10S212	180.5
11	D11S4046	0
	D11S1338	9.97
	D11S902	26.11
	D11S904	44.32
	D11S935	53.7
	D11S905	58.2
	D11S4191	65.85
	D11S987	73.34
	D11S1314	79.88
	D11S937	84.91
	D11S901	89.71
	D11S4175	94.3
	D11S898	105
	D11S908	117.36
	D11S925	125.75
	D11S4151	134.91
	D11S1320	149.32
	D11S968	154.92
12	D12S352	0
	D12S99	15.49

	D12S336	24.55
	D12S364	31.91
	D12S310	36.86
	D12S1617	46.67
	D12S345	56.05
	D12S85	61.41
	D12S368	67.52
	D12S83	75.06
	D12S326	92.48
	D12S351	102.6
	D12S346	112.78
	D12S78	117.92
	D12S79	133.95
	D12S86	140.28
	D12S324	151.13
	D12S1659	162.27
	D12S1723	172.4
13	D13S175	3.47
	D13S217	22.94
	D13S171	31.89
	D13S218	40.33
	D13S263	44.28
	D13S153	52.87
	D13S156	71.16
	D13S170	77.67
	D13S265	82.35
	D13S159	93.61
	D13S158	100.73
	D13S173	111.41
	D13S1265	116.67
	D13S285	126.4
14	D14S261	4.52
	D14S283	15.3
	D14S275	22.95
	D14S70	37.43
	D14S288	45.16
	D14S276	55.74
	D14S63	64.95
	D14S258	70.03
	D14S74	79.76
	D14S280	96.33

	D14S65	108.62
	D14S985	118.3
	D14S292	125.64
15	D15S128	6.06
	D15S1002	15.51
	D15S165	23.42
	D15S1007	29.75
	D15S1012	39.25
	D15S994	43.89
	D15S978	49.51
	D15S117	57.9
	D15S153	69.69
	D15S131	78.27
	D15S205	91.16
	D15S127	99.44
	D15S130	111.11
	D15S120	129.9
16	D16S423	14.64
	D16S404	25.69
	D16S3075	30.38
	D16S3103	39.13
	D16S3046	44.2
	D16S3068	50.81
	D16S3136	61.8
	D16S415	68.52
	D16S503	83.55
	D16S515	93.91
	D16S516	100.45
	D16S3091	111.96
	D16S520	125.99
17	D17S849	0.63
	D17S831	7.35
	D17S938	17.45
	D17S1852	31.26
	D17S799	37.74
	D17S921	42.34
	D17S1857	47.4
	D17S798	56.73
	D17S1868	75.89
	D17S787	82.77

	D17S944	94.6
	D17S949	104.95
	D17S785	117.48
	D17S784	132.09
	D17S928	138.44
18	D18S59	1.4
	D18S63	9.74
	D18S452	18.14
	D18S464	33.23
	D18S53	38.66
	D18S478	52.14
	D18S1102	59.5
	D18S474	74.33
	D18S64	83.8
	D18S68	90.54
	D18S61	99.56
	D18S1161	110.48
	D18S462	118.57
	D18S70	123.94
19	D19S209	11.89
	D19S216	17.38
	D19S884	26.62
	D19S221	33.51
	D19S226	37.53
	D19S414	55.22
	D19S220	65.1
	D19S420	70.51
	D19S902	77.81
	D19S888	96.51
	D19S418	106.92
	D19S210	111.68
20	D20S117	2.94
	D20S889	12.15
	D20S115	25.13
	D20S186	35.24
	D20S112	45.1
	D20S195	57.3
	D20S107	62.77
	D20S119	70.48
	D20S178	74.95

	D20S196	79.69
	D20S100	90.29
	D20S171	100.25
	D20S173	101.32
21	D21S1256	13.2
	D21S1899	16.08
	D21S1914	24.9
	D21S263	33.15
	D21S1252	43.79
	D21S266	57.06
22	D22S420	3.05
	D22S539	15.63
	D22S315	23.71
	D22S280	38.53
	D22S283	43.61
	D22S423	50.75
	D22S274	58.24