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A Genome Wide Linkage Scan for Cleft Lip and Palate and Dental Anomalies

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Abstract

We revisited 46 families with two or more siblings affected with an orofacial cleft that participated in previous genome wide studies and collected complete dental information. Genotypes from 392 microsatellite markers at 10 cM intervals were reanalyzed. We carried out four sets of genome wide analyses. First, we ran the analysis solely on the cleft status. Second, we assigned to any dental anomaly (tooth agenesis, supernumerary teeth, and microdontia) an affection status, and repeated the analysis. Third, we ran only the 19 families where the proband had a cleft with no dental anomalies. Finally, we ran only the 27 families that had a proband with cleft and additional dental anomalies outside the cleft area. Chromosomes (1, 2, 6, 8, 16, and 19) presented regions with LOD scores >2.0. Chromosome 19 has the most compelling results in our study. The LOD scores increased from 3.11 (in the scan of all 46 families with clefts as the only assigned affection status) to 3.91 when the 19 families whose probands present with no additional dental anomalies were studied, suggesting the interval 19p13.12-19q12 may contain a gene that contributes to clefts but not to dental anomalies. On the other hand, we found a LOD score of 3.00 in the 2q22.3 region when dental anomalies data were added to the analysis to define affection status. Our preliminary results support the hypothesis that some loci may contribute to both clefts and congenital dental anomalies. Also, adding dental anomalies information will provide new opportunities to map susceptibility loci for clefts.

Keywords

cleft lip; cleft palate; tooth agenesis; dental anomalies; linkage

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INTRODUCTION

Isolated or non-syndromic cleft lip and palate (CL/P) is a complex disorder resulting from multiple genetic and environmental factors. CL/P is a common birth defect, and the source of substantial morbidity and mortality worldwide [reviewed by Murray, 2002]. With an average birth prevalence of 1/700 live births; there is remarkable population to population variation [Mossey and Little, 2002]. In general, Asian populations have a higher birth prevalence of orofacial clefts (1/500 births), Caucasians are intermediate (1/1,100), and African populations have the lowest (1/2,500 births). However, the notion that Asians have a higher prevalence of clefts has been challenged based on the evidence that many published prevalence rates included all pregnancies (live and stillbirths) and do not distinguish between syndromic and non-syndromic clefts, or between cleft palate alone and cleft lip with or without cleft palate [Cooper et al., 2006].

An examination of familial recurrence patterns in CL/P indicated that there may be anywhere from 3 to 14 interacting loci involved in clefting [Schliekelman and Slatkin, 2002]. This analysis indicates that very large sample sizes may be necessary to detect the loci involved in CL/P. For a complex genetic disorder such as CL/P, several experimental techniques may be used. These include breakpoint mapping, deletion mapping, direct sequencing of candidate genes/loci, linkage analysis, and linkage disequilibrium analysis [reviewed by Lidral and Murray, 2004]. Genome wide linkage scans of complex traits succeed when heterogeneity is minimized and sample sizes are maximized. The Philippines provides an opportunity to base such a study with the common occurrence of isolated clefting, large average family sizes, and a motivated public health enterprise [Murray et al., 1997]. Our preliminary study indicated three regions that yielded suggestive positive linkage results (LOD scores higher than 1.0) in 220 multiplex extended Filipino kindreds: 2p21, 6q23, and 8p21. The highest signal was in 8p11-23 (single point recessive LOD score =1.2 and multipoint recessive LOD score =2.3) and follow up studies with 271 families showed suggestive linkage disequilibrium results for *FUT10*, *BAG4*, and *FGFR1* [Riley et al., 2007]. Even though this was a large study of 220 families (567 affected and 1,109 unaffected family members genotyped), the results were just modest. We hypothesize that increasing the sophistication of the clinical description would allow reducing misclassification and improving one's ability to see associations that may have been otherwise masked by a larger more heterogeneous classification approach. We proposed to use dental development to subphenotype clefts. Our preliminary analysis suggests dental anomalies are preferentially associated with clefts in some families [Letra et al., 2007]. In order to extend these earlier studies, we proposed to revisit the subset of the initially genotyped families with two or more siblings affected by CL/P and perform a dental examination to broader the phenotypic description of the families.

SUBJECTS AND METHODS

Dental Assessments

Information on dental anomalies outside the cleft area was collected from the cases and all available relatives. Aside from tooth agenesis, which is the most common congenital anomaly in humans and the one we expected to see the most, other dental anomalies included supernumerary teeth, microdontia, macrodontia, missing cusps, and supernumerary cusps. In many instances, tooth agenesis had to be confirmed by an X-ray exam. We used a portable X-ray (MinXray P200D MarkIII; Toshiba, Tokyo, Japan) to confirm the diagnosis of tooth agenesis. In addition, missing teeth by caries was an important distinction to be made. We conducted careful exams and collected comprehensive caries data (data not shown) to aid in the differential diagnosis.

The University of Iowa IRB and University of Pittsburgh IRB gave approval for the study in conjunction with local approval in the Philippines.

In despite of local political issues, geographic locations, and weather conditions (13 typhoons and severe tropical storms hit the Philippines between May 23rd and December 19th, 2006), we were able to recontact 46 families with two or more affected cleft sibpairs out of 70 families attempted from the subset of 154 families with two or more affected siblings. All 46 families were multiplex (families with additional affected relatives other than the two or more affected siblings). We have collected data on approximately 500 individuals, including 100 unrelated control families that were used to calculate dental anomalies frequency in the general population for our power studies. These control families were used for determining population parameters in the linkage analysis.

Genome Wide Scan Analysis

Genotypes were available for 392 markers from the Marshfield Genetics screening set 8. These genotypes were derived at the Center for Inherited Disease Research (CIDR) [Riley et al., 2007]. We performed a genome wide search for suggestive loci linked to clefts with and without dental anomalies outside the cleft area as an extension of the cleft phenotype. Four sets of genome wide analyses were carried out. First, we ran the analysis solely on the cleft status. Second, we assigned to any dental anomaly (tooth agenesis, supernumerary teeth, and microdontia) an affection status, and repeated the analysis. Third, we ran only the 19 families where the proband had a cleft with no dental anomalies. Finally, we ran only the 27 families that had a proband with cleft and additional dental anomalies outside the cleft area.

Prior to each genome scan; data was assessed with PedCheck [O'Connell and Weeks, 1998] to test for inconsistencies due to non-paternity or other errors. For the parametric linkage analysis, allele frequencies were estimated from unaffected founders in the original group of families [Riley et al., 2007]. The genetic model parameters were taken from segregation analysis results in a sample of the Filipino families (unpublished results). The dominant model was a disease allele frequency of 0.002 with a penetrance of 0.6. The recessive model was a disease frequency of 0.04 with a penetrance of 0.9.

We calculated two-point LOD scores in the extended kindreds employing the LINKAGE program with recent updates to speed calculations (VITESSE and FASTLINK) [Cottingham et al., 1993; Terwilliger and Ott, 1994; O'Connell and Weeks, 1995]. For multipoint LOD and heterogeneity LOD (HLOD) [Smith, 1963] calculations, the descent graph method [Sobel and Lange, 1996; Sobel et al., 1996; Lange, 2002] implemented in computer SIMWALK2 was used. Given the probable complexities in the genetic model for CL/P (e.g., heterogeneity), we also calculated model-free linkage statistics (both two-point and multipoint) using MERLIN [Abecasis et al., 2002].

RESULTS

In the 46 families, there were 628 total individuals. Four hundred eighty-five people are unaffected with cleft (296 have genotyping data). One hundred forty-three people present with the clefts phenotype (140 have genotyping data). Four hundred eighty-five people are cleft unaffected (296 have genotyping data), and of these people 66 individuals have the additional dental affection status (65 have genotyping data). More details are provided in Table I.

Tables II–V describe the most significant linkage results by chromosome in each of the four genome scan analyses (complete results for the four genome wide scans by each STR marker are available as appendices (see the online appendices at http://www.interscience.wiley.com/jpages/1552-4825/suppmat/index.html)). The strongest

linkage signal was found in chromosome 19 for the cleft families whose probands had clefts but no dental anomalies (LOD =3.91). Other regions yielded LOD score results above 2.0: 1q31.3-q32.1, 2q22.3, 6q23.1, 8p23.3-p23.1, and 16q12.1-q22.3.

DISCUSSION

There are substantial data supporting a role for particular genes affecting tooth, lip, and palate development based on the occurrence of anomalies in all these single gene disorders (*FGFR1* and Kallmann syndrome [Dodé et al., 2003]; *IRF6* and Van der Woude syndrome [Kondo et al., 2002]; *MSX1* and cleft lip and palate/oligodontia syndrome [van den Boogard et al., 2000]; *p63* and ectrodactyly-ectodermal dysplasia clefting syndrome [Celli et al., 1999]; and, *PVRL1* and cleft lip and palate-ectodermal dysplasia syndrome [Suzuki et al., 2000]). Expression analysis of these tissues during development also supports an overlapping role [Vieira et al., 2007b]. Our study provides further evidence that common genetic factors may contribute to both CL/P and dental anomalies.

There are limitations in our study. Although the Filipino families included in our study tend to have large sibships, it was not always possible to examine all potential subjects in all families. A number of reasons account for that, such as having a job in another city and not being available at the time of data collection, or have chosen to not participate in the study. Another limitation is that this family dataset is probably not representative of the Filipino population. Although it is possible that this group of families may be representative of the Cebu province or even the Central Visayas region, the lack of official population-based records of birth defects in the Philippines prevents us to make any further assumptions regarding the Filipino population as a whole.

Chromosome 19 has the most compelling results in our study. The LOD scores increased from 3.11 (in the scan of all 46 families with clefts as the only assigned affection status) to 3.91 when the 19 families whose probands present with no additional dental anomalies were studied, suggesting the interval 19p13.12-19q12 may contain a gene that contributes to clefts but not to dental anomalies. We have previously studied the 19q13 region and have found a strong association with a marker in *PVR* in two independent cleft populations (South America, P =0.0007; and Iowa, P =0.0009) [Warrington et al., 2006]. Previous publications have also suggested the 19q13 region and the *BCL3* gene [Stein et al., 1995; Amos et al., 1996; Maestri et al., 1997; Wyszynski et al., 1997; Martinelli et al., 1998; Yoshiura et al., 1998; Carreño et al., 2002; Gaspar et al., 2002; Marazita et al., 2002a, b; Blanco et al., 2004; Morkûnienė et al., 2007] although a few studies did not replicate the association between the 19q13 region and clefts [Wong et al., 2000; Beaty et al., 2001; Fujita et al., 2004; Pezzetti et al., 2007]. Our results further support a gene contributing to clefts resides in this region, which appears to be upstream from 19q13 based on our preliminary linkage results.

We found a LOD score of 3.00 in the 2q22.3 region when dental anomalies data were added to the analysis to define affection status. Previous work suggested that the 2q32-35 region could have a role in clefts, based on the meta-analysis approach (P =0.0004) [Marazita et al., 2004]. There are no obvious candidate genes for clefts lying in the 2q22.3 region. Our results suggest that a gene in the region may contribute to clefts with an associated dental abnormality.

Another four loci demonstrated suggestive linkage results (LOD scores 2.00 or above). On chromosome 1q31.3-1q32.1, a LOD score of 2.17 was found in the scan of clefts and a LOD score of 2.00 when the information of dental anomalies was added in the analysis. This region is relatively close to *IRF6* (1q32.2). Our previous work found a strong association between *IRF6* and clefts [Zucchero et al., 2004; Vieira et al., 2007a], and isolated tooth agenesis [Vieira et al., 2007b]. However, we were not able to identify the functional genetic variant yet. The

association with *IRF6* was seen in markers in the gene and 100 kb downstream the gene (towards the centromere) [Zucchero et al., 2004]. In our results, another linkage signal could be seen for the clefts data at D1S3462 (LOD score 2.85). This marker is upstream of *IRF6*, at 1q42.2. These data add more complexity to this region, and suggests that either the functional variant could be anywhere between D1S1660 and D1S3462, or more than one functional variant exists in the region. More recently, a large Danish family segregating cleft lip and palate was linked to a 6.5 Mb interval at 1q32.1-q32.3, which is inside the interval described above [Jakobson et al., 2007] (Fig. 1).

Another suggestive linkage signal was seen at 6q23.1 (LOD score 2.22) in the clefts data. This peak (at 129 cM) is near to the 123 cM peak reported by Marazita et al. [2004] (LOD score 3.55). These results suggest that a gene in 6q contributes to clefts but not to associated dental anomalies.

Chromosome 8p23.3–p23.1 also has positive signals in the data (2 cM, LOD score =2.41; 7 cM, LOD score =2.15, and 22 cM, LOD score =2.47). These signals are near to the 48 cM signal we previously reported [Riley et al., 2007] with a LOD score of 2.38 in 220 Filipino families. Fine mapping work also presented in Riley et al. [2007] showed both linkage and association positive results for markers in *FGFR1* and *BAG4*, while either linkage or association showed positive results for markers in *SLC18A1*, *EPHX2*, and *FZD3*. However, under a more strict criteria correcting for multiple testing, none of these results would be statistically significant. These results, in combination with our more recent linkage data suggest we could focus on a more upstream region of chromosome 8, where our peak results are more significant.

Finally, we had suggestive linkage signals in chromosome 16q12.2 (81 cM, LOD score =2.55), 16q21 (83 cM, LOD score =2.62), and 16q22.3 (88 cM, LOD score =2.35) in the clefts data and 16q12.1 in the families with probands with dental anomalies (58 cM, LOD score =2.04). Previous work has also found association with a marker at 51 cM (D16S769, P =0.009) in Chinese families [Marazita et al., 2002b]. These peaks are not far from the 16q24.1 association signal for D16S3037 (P =0.000063) [Chiquet et al., 2007]. The proximity of *CRISPLD2* to D16S3037 made it a prime candidate gene for further studies. The authors found association also with a marker in the exon 2 of *CRISPLD2* (rs1546124, P = 0.00167), which is 51 base pairs prior the initiation codon of the gene. These findings in combination with our data suggest that chromosome 16 has a gene that contributes to clefts. Also, our data suggest the genetic factor in chromosome 16 may be involved not only with clefts, but also with clefts associated with dental anomalies.

In summary, our results support the hypothesis that increasing the complexity of the clinical description by adding dental anomalies information will provide new opportunities to map susceptibility loci for clefts. Here we report, for the first time, a series of genome wide searches for cleft susceptibility loci using dental anomalies to subphenotype clefts. This approach appears to be a promising one and may help in the identification of genetic variants that increase cleft susceptibility, which would be a crucial step that may allow better estimates of recurrence risks for individual families.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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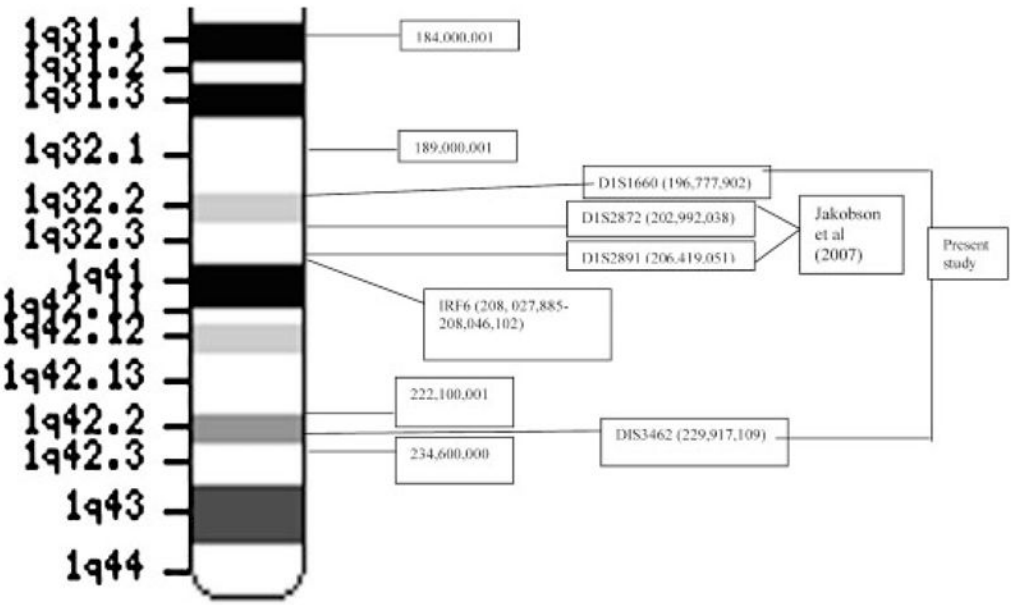

Fig. 1.

Diagram of the 1q31-1q44 segment. Locations are described in base pairs, based on the UCSC Genome Browser on Human Ma. 2006 Assembly (http://genome.ucsc.edu). Our linkage signals are located between the markers D1S1660 and D1S3462. Jakobson et al. [2007] linkage region is located between D1S2872 and D1S2891.

TABLE I

Details on the Number of Study Participants and Their Status

Families	Cleft Status	Dental anomalies status	Individuals with no genotypes available	Individuals with genotypes available	Total
All 46 families	Unaffected	Unaffected	188	231	419
	Unaffected	Affected	1	65	66
	Affected	Unaffected	3	106	109
	Affected	Affected	0	34	34
	Total		192	436	628
27 families whose cleft probands have dental anomalies outside the cleft area	Unaffected	Unaffected	52	90	142
	Unaffected	Affected	0	25	25
	Affected	Unaffected	0	55	55
	Affected	Affected	0	0	0
	Subtotal		52	170	222
19 families whose cleft probands do not have dental anomalies outside the cleft area	Unaffected	Unaffected	136	141	277
	Unaffected	Affected	1	40	41
	Affected	Unaffected	3	51	54
	Affected	Affected	0	34	34
	Subtotal		140	266	406

TABLE II

Genome Wide Linkage Summary of the Most Significant Results by Chromosome for Clefts as Positive Affection Status (Complete Results, Including STR Information Is Available Online in an Appendix)

Chromosome	Single point analysis			Multipoint analysis		
	Maximum LOD score, dominant model	Maximum LOD score, recessive model	Non-parametric, linkage *P*-value	Maximum HLOD, dominant model	Maximum HLOD, recessive model	Non-parametric, linkage *P*-value
1	0.94	2.85	0.008	0.91	2.17	0.016
2	0.46	1.51	0.04	0.72	1.03	0.009
3	0.56	0.72	0.02	0.42	0.57	0.110
4	0.45	0.38	0.08	0.20	0.98	0.191
5	1.73	0.85	0.008	1.05	1.58	0.005
6	1.40	2.22	0.03	0.87	1.49	0.030
7	0.10	0.18	0.05	0.37	0.46	0.020
8	2.47	1.83	0.005	2.41	1.30	0.010
9	1.96	1.28	0.08	0.83	1.41	0.023
10	0.99	0.89	0.11	0.20	0.65	0.123
11	0.67	0.83	0.12	0.68	0.52	0.066
12	0.57	0.71	0.05	0.68	0.54	0.041
13	0.44	0.84	0.07	0.73	1.51	0.125
14	0.44	1.22	0.01	0.34	0.42	0.020
15	0.14	0.10	0.11	0.00	0.44	0.192
16	0.73	1.78	0.03	1.42	2.62	0.063
17	0.56	0.57	0.08	0.59	1.06	0.064
18	0.79	1.14	0.09	0.40	0.96	0.076
19	1.05	1.25	0.06	0.97	3.11	0.042
20	0.04	0.50	0.20	0.08	0.21	0.246
21	0.56	0.09	0.30	0.15	0.00	0.279
22	0.55	0.37	0.20	0.20	0.54	0.155
X	0.66	0.24	0.07	—	—	—

TABLE III

Genome Wide Linkage Summary of the Most Significant Results by Chromosome for Clefts and/or Dental Anomalies as Positive Affection Status (Complete Results, Including STR Information Is Available Online in an Appendix)

Chromosome	Single point analysis			Multipoint analysis		
	Maximum LOD score, dominant model	Maximum LOD score, recessive model	Non-parametric, linkage *P*-value	Maximum HLOD, dominant model	Maximum HLOD, recessive model	Non-parametric, linkage *P*-value
1	0.90	2.00	0.006	1.10	1.34	0.007
2	1.51	2.42	0.0008	1.30	3.00	0.004
3	0.89	0.38	0.01	1.14	0.54	0.077
4	0.36	0.34	0.11	0.25	0.51	0.147
5	0.84	0.82	0.02	1.16	0.78	0.009
6	0.47	0.83	0.13	0.18	0.24	0.135
7	0.65	0.22	0.04	0.78	0.50	0.037
8	1.15	1.11	0.11	0.58	0.28	0.095
9	1.75	1.78	0.04	1.53	1.25	0.012
10	0.85	0.25	0.03	0.47	0.08	0.139
11	1.26	0.35	0.11	0.78	0.07	0.020
12	1.32	0.32	0.009	0.32	0.23	0.057
13	0.63	1.63	0.04	0.62	1.05	0.049
14	0.70	1.18	0.06	0.22	0.28	0.019
15	0.45	0.01	0.20	0.20	0.42	0.377
16	2.33	0.21	0.04	1.03	1.00	0.090
17	0.54	0.03	0.04	0.50	0.21	0.038
18	0.48	0.20	0.07	0.24	0.06	0.269
19	0.35	0.56	0.02	0.24	0.76	0.058
20	0.15	0.70	0.30	0.18	1.02	0.325
21	0.16	0.50	0.20	0.20	0.21	0.314
22	0.45	0.32	0.30	0.01	0.04	0.280
X	0.57	0.30	0.20	—	—	—

TABLE IV

Genome Wide Linkage Summary of the Most Significant Results by Chromosome for 27 Families Whose Cleft Probands Have Dental Anomalies Outside the Cleft Area (Clefts and/or Dental Anomalies Assigned as Positive Affection Status)

Chromosome	Single point analysis			Multipoint analysis		
	Maximum LOD score, dominant model	Maximum LOD score, recessive model	Non-parametric, linkage *P*-value	Maximum HLOD, dominant model	Maximum HLOD, recessive model	Non-parametric, linkage *P*-value
1	0.49	0.85	0.004	0.59	0.78	0.009
2	1.08	1.75	0.006	1.34	1.02	0.011
3	0.51	0.64	0.05	0.28	1.01	0.099
4	0.67	0.81	0.02	1.09	0.47	0.037
5	1.09	0.96	0.01	1.73	0.55	0.010
6	0.68	0.39	0.12	0.10	0.11	0.084
7	0.54	0.12	0.04	0.56	0.35	0.050
8	1.51	0.85	0.08	0.57	0.00	0.091
9	1.09	1.51	0.01	1.32	1.29	0.009
10	0.61	0.20	0.06	0.28	0.00	0.255
11	0.97	0.33	0.15	0.31	0.05	0.044
12	1.71	1.12	0.008	0.13	0.16	0.076
13	0.24	0.47	0.20	0.32	1.03	0.270
14	1.35	0.67	0.06	0.00	0.06	0.119
15	0.12	0.02	0.30	0.14	0.02	0.329
16	2.04	0.41	0.11	1.28	0.65	0.067
17	0.40	0.07	0.11	0.75	0.23	0.069
18	0.27	0.11	0.12	0.12	0.00	0.279
19	0.18	0.59	0.09	0.13	0.28	0.174
20	0.05	0.49	0.30	0.02	0.04	0.338
21	0.05	0.03	0.30	0.00	0.25	0.405
22	0.26	0.19	0.20	0.13	0.07	0.266
X	1.09	0.34	0.14	—	—	—

Complete results, including STR information is available online in an appendix.

TABLE V

Genome Wide Linkage Summary of the Most Significant Results by Chromosome for 19 Families Whose Cleft Probands Do Not Have Dental Anomalies Outside the Cleft Area (Clefts Assigned as Positive Affection Status)

Chromosome	Single point analysis			Multipoint analysis		
	Maximum LOD score, dominant model	Maximum LOD score, recessive model	Non-parametric, linkage *P*-value	Maximum HLOD, dominant model	Maximum HLOD, recessive model	Non-parametric, linkage *P*-value
1	0.54	1.51	0.03	0.56	1.27	0.012
2	0.62	1.00	0.13	0.54	1.03	0.160
3	0.80	1.72	0.04	0.45	1.59	0.033
4	0.80	0.96	0.03	0.46	0.68	0.089
5	1.39	0.39	0.008	1.43	0.79	0.029
6	0.94	1.29	0.09	0.62	1.08	0.050
7	0.56	0.41	0.05	1.34	0.62	0.008
8	0.86	1.31	0.06	1.24	0.36	0.082
9	0.71	0.58	0.30	1.00	0.82	0.077
10	1.75	1.12	0.06	0.52	0.48	0.052
11	0.47	0.58	0.10	0.66	0.51	0.114
12	0.98	1.11	0.13	0.19	0.85	0.156
13	0.11	1.31	0.03	0.21	1.92	0.096
14	0.75	0.80	0.03	0.95	1.01	0.005
15	0.50	0.81	0.06	0.05	0.24	0.137
16	0.77	1.11	0.11	0.61	1.59	0.111
17	1.02	1.04	0.03	0.59	1.56	0.133
18	1.19	1.71	0.04	0.64	1.04	0.018
19	0.66	1.82	0.06	1.67	3.91	0.005
20	0.24	0.66	0.14	0.00	0.40	0.201
21	1.59	0.43	0.12	1.21	0.20	0.209
22	0.47	1.22	0.08	0.66	0.82	0.046
X	0.93	0.70	0.08	—	—	—

Complete results, including STR information is available online in an appendix.