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Population genetic data for 20 autosomal STR loci in an Iraqi Arab population: application to the identification of human remains.

Since the 1980s armed conflict and other situations of violence have led to the death of hundreds of thousands of individuals in Iraq. Events range from the Iran Iraq war to more recent atrocities committed during internal unrest, which is still ongoing. Large numbers of individuals are classified as missing and their bodies have never been identified and returned to their families. In an attempt to address this problem the Iraqi Medico Legal Directorate has developed its capacity in forensic anthropology and forensic genetics to recover and identify more individuals. Forensic genetics within the Directorate is split into three sections: Crime Scene, Relationship Testing, and Mass Graves. The Mass Graves Section was formed in 2010 and is tasked with identifying missing individuals. Even after recovery and anthropological assessment the challenges faced are numerous, including DNA recovery from highly degraded remains, some of which are over 35 years post-mortem, to the scale of the numbers of missing persons involved. The Mass Graves Section has adopted the PowerPlex® 21 System (Promega, Madison, USA), which enables 20 STR loci to be amplified in one PCR; DNA can be amplified after extraction or directly from reference samples. In this study the data for an allele reference database for the Iraqi Arab population is presented. In addition some simulations of the theoretical power of the PCR kit for the identification of human remains are presented.

DNA samples were analysed from 1088 healthy, unrelated and consenting adults who were Iraqi nationals and identified themselves as Iraqi Arabs. Samples were collected with informed consent in different parts of Iraq; the majority were from Baghdad (N=293), Babil (N=199), Najaf (N=250), Quadisiya (N=142) and the remaining samples from south of Iraq. Blood samples were collected using finger lancets and stored on FTA® Classic Card (GE Healthcare, Pittsburgh, USA). For each sample a 1.2 mm punch was directly amplified following the manufacturer's recommended conditions. PCR using the PowerPlex 21® System with an Applied Biosystems 9700 thermocycler was carried out using the standard manufacturer's recommended conditions: this co-amplified the amelogenin sex marker and 20 STR Loci (D3S1358, D1S1656, D6S1043, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S39, D19S433, and FGA). Capillary electrophoresis was performed using a 3500 Genetic Analyzer (Applied Biosystems) in accordance with the manufacturer's instructions; ILS-500 (Promega) was used as an internal size standard. Genotyping was performed using GeneMapper ID-X v1.4 software (Applied Biosystems). Alleles were designated according to the published nomenclature; off ladders were called if they fell into a virtual bin as long as the profile as a whole was judged to be of good quality, alleles that were not in a bin or virtual bin were re-run at least once before designation.

Statistical parameters and forensic utility of these markers was evaluated by calculating the allele frequencies, probability of Identity (P_I), major allele frequency (MAF), genotype number, gene diversity, homozygosity, polymorphism information content (PIC), typical paternity index (TPI), power of discrimination (PD) and power of exclusion (PE) using PowerMarker software v. 3.25 [1]. Version 3.5 of Arlequin software [2] was used to perform an exact test to investigate departures from Hardy-Weinberg equilibrium (HWE) and population differentiation; the data generated in this study were compared to 9 population data sets for available loci. The populations assessed were Kurdish [3], Iranian [4], Turkish [5], Syrian [6], Saudi [4], Kuwaiti [7], United Arab Emirates [8], Egyptian [4] and Indian [4].

The observed allele frequencies of 20 STR loci in Arab Iraqis population are summarized in Supplementary Table S1. A total of 286 alleles at these 20 STR loci were found with corresponding allelic frequencies ranging from 0.0005 to 0.5101. The number of alleles varied from 7 (TPOX) to 27 (FGA). Upon analysis of the data Penta E was found to be the most discriminating locus with 0.9113 heterozygosity, PD of 0.9829 and a TPI of 4.3174 – 131 different genotypes were seen at this locus; the least discriminating locus was TPOX with 0.6582 heterozygosity, PD of 0.6946 and a TPI of 1.4353 – 22 genotypes were seen at this locus (Supplementary Table S1). A number of off-ladder variants were identified during the analysis, most of these had been previously reported on STRBase [9], but some uncommon variants were seen in D12S391, with alleles 19.1, 19.2, 19.3 and 20.1 detected in 1, 2, 14 and 10 samples respectively. Other variants not reported in STRBase were D19S433 allele 11.2, and D1S1656, allele 8.

The exact test for departure from HWE was significant after Bonferroni correction for D21S11 (p -value = 0.0003) and FGA (p -value = 0.0012). Why this is caused is not clear, but it could be due to inbreeding, selection, population substructure, null alleles, or a sampling effect. When comparing different populations there are significant differences: of the neighbouring countries tested the Iranian, Turkish and Syrian populations were similar with between none and two loci significantly different (after Bonferroni correction), whereas the Kuwaiti and Saudi populations were more distant. Notably, the Kurdish population showed significant differences after Bonferroni correction at half the loci tested (Supplementary Table S2), which supports the need for separate population-specific databases within Iraq.

Simulations were carried out in DNA.view [10] to assess the typical likelihood ratios that could be expected when attempting to identify human remains when using different combinations of relatives. A box and whiskers plot is shown in Figure 1, with the distribution of likelihood ratios for 100 simulations using 10 different scenarios. As expected with 20 STR loci the likelihood ratios can

be very high, especially when multiple relatives are available for testing [11] . Median and typical likelihood ratios for different scenarios are presented in Supplementary Table S3. However, care has to be taken when calculating likelihood ratios based on data from the PowerPlex 21® System as four pairs of loci are physically linked: D5S818 and CSF1PO on chromosome 5; D2S1338 and TPOX on chromosome 2; D21S11 and Penta D on chromosome 21; and vWA and D12S391 on chromosome 12. This can lead to co-inheritance in close relatives, which in turn leads to higher likelihood ratios than would be expected with unlinked loci [12] . Another complication that needs to be considered when calculating likelihood ratios, especially in complex identification cases where there are 1000s of missing individuals, is the impact of consanguinity, which is highly relevant in the Iraqi context [13] .

This manuscript of population data follows the journal guidelines [14,15]

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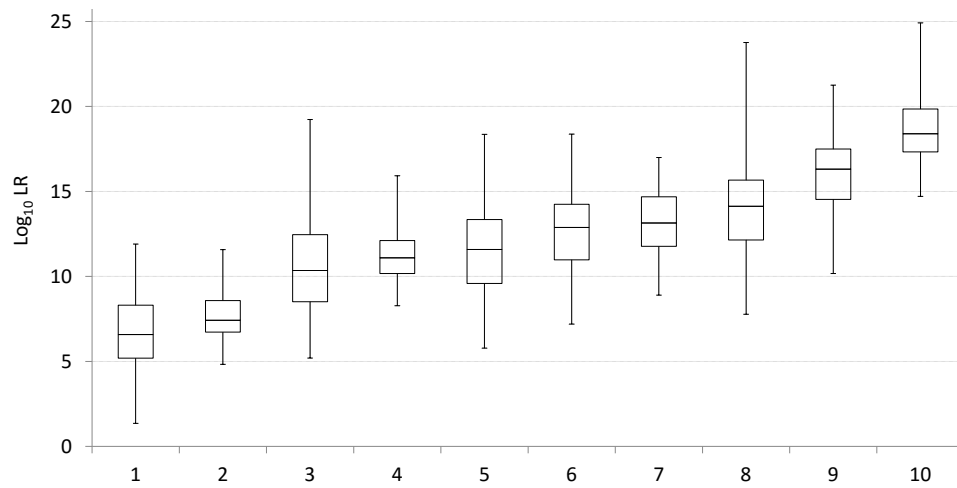


Figure 1 Boxplot comparing the distribution of simulated $\log_{10}$ likelihood ratios for 10 relationship scenarios: 1) one sibling; 2) one parent/child; 3) two siblings; 4) spouse and child; 5) parent and sibling; 6) 2 children; 7) one parent and one child; 8) three siblings; 9) spouse and two children; 10) two parents.

Supplementary Table S1: Allele frequencies and statistical parameters of forensic interest in an Iraqi Arab population for 20 autosomal STR loci amplified using the PowerPlex®21 System

N=1088	D3S1358	D1S1656	D6S1043	D13S317	Penta_E	D16S539	D18S51	D2S1338	CSF1PO	Penta_D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	D8S1179	D12S391	D19S433	FGA
2.2	-	-	-	-	-	-	-	-	-	0.0147	-	-	-	-	-	-	-	-	-	-
3.2	-	-	-	-	-	-	-	-	-	0.0014	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	0.0519	-	-	-	0.0005	0.0009	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	0.0005	-	-	-	-	0.0023	0.3157	-	-	-	-	0.0037	-	-	-	-
6.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0005	-
7	-	-	0.0009	-	0.0997	-	-	-	0.0009	0.0092	0.1847	-	-	0.0142	-	0.0014	-	-	-	-
8	-	0.0005	-	0.1415	0.0331	0.0418	-	-	0.0069	0.0313	0.1039	-	-	0.1530	0.0110	0.5101	0.0106	-	-	-
8.3	-	-	-	-	-	-	-	-	-	-	0.0018	-	-	-	-	-	-	-	-	-
9	0.0005	0.0005	0.0028	0.0565	0.0138	0.1549	0.0018	-	0.0234	0.1926	0.2514	-	-	0.1218	0.0643	0.1204	0.0092	-	-	-
9.1	-	-	-	-	-	-	-	-	-	-	0.1259	-	-	0.0014	-	-	-	-	-	-
9.3	-	-	-	-	-	0.0005	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9.4	-	-	-	-	-	-	-	-	-	0.0005	-	-	-	-	-	-	-	-	-	-
10	-	0.0014	0.0211	0.0712	0.0712	0.0864	0.0087	-	0.2858	0.1687	0.0156	-	-	0.3088	0.0928	0.0878	0.0735	-	0.0014	-
10.2	-	-	-	-	-	-	0.0014	-	-	-	-	-	-	-	-	-	-	-	-	-
10.3	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0005	-	-	-	-	-	-
11	-	0.0827	0.2895	0.2780	0.1199	0.3396	0.0211	-	0.2941	0.1700	0.0009	-	-	0.2321	0.2886	0.2417	0.0832	-	0.0133	-
11.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0009	-
11.3	-	-	-	-	-	0.0005	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	0.0023	0.1126	0.2670	0.3203	0.1595	0.2385	0.1383	-	0.3323	0.1140	-	0.0005	-	0.1484	0.3483	0.0349	0.1131	-	0.1034	-
12.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0032	-
12.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0009	-	-	-	-	-
13	0.0028	0.0956	0.0731	0.0947	0.1103	0.1135	0.1733	0.0005	0.0441	0.1641	-	0.0069	-	0.0179	0.1811	-	0.2592	0.0005	0.2183	-
13.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0248	-
13.4	-	-	-	-	-	-	-	-	-	0.0005	-	-	-	-	-	-	-	-	-	-
14	0.0515	0.1085	0.0469	0.0363	0.0496	0.0234	0.1631	-	0.0115	0.0694	-	0.0648	-	0.0018	0.0124	-	0.2100	0.0018	0.2762	-
14.1	-	-	-	-	-	-	-	-	-	0.0083	-	-	-	-	-	-	-	-	-	-
14.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0473	-
14.3	-	0.0005	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	0.2495	0.1792	0.0064	0.0014	0.0662	0.0009	0.1259	0.0032	0.0005	0.0414	-	0.1131	-	-	0.0005	-	0.1742	0.0175	0.1121	-

[illegible]

24	-	-	-	-	-	-	0.0005	0.0767	-	-	-	-	-	-	-	-	-	0.0418	-	0.1618
24.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0023
24.3	-	-	-	-	-	-	-	-	-	-	-	-	0.0005	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	0.0565	-	-	-	-	0.0005	-	-	-	-	0.0234	-	0.1071
25.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0005
26	-	-	-	-	-	-	-	0.0110	-	-	-	-	0.0009	-	-	-	-	0.0041	-	0.0395
26.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0005
26.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0005
27	-	-	-	-	-	-	-	0.0018	-	-	-	-	0.0147	-	-	-	-	-	-	0.0069
28	-	-	-	-	-	-	-	0.0009	-	-	-	-	0.1484	-	-	-	-	-	-	0.0018
28.1	-	-	-	-	-	-	-	-	-	-	-	-	0.0005	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-	-	-	0.2390	-	-	-	-	-	-	0.0028
29.1	-	-	-	-	-	-	-	-	-	-	-	-	0.0046	-	-	-	-	-	-	-
29.2	-	-	-	-	-	-	-	-	-	-	-	-	0.0018	-	-	-	-	-	-	-
29.3	-	-	-	-	-	-	-	-	-	-	-	-	0.0014	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	0.2252	-	-	-	-	-	-	0.0005
30.2	-	-	-	-	-	-	-	-	-	-	-	-	0.0253	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-	-	-	-	-	-	0.0427	-	-	-	-	-	-	-
31.2	-	-	-	-	-	-	-	-	-	-	-	-	0.1034	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-	0.0060	-	-	-	-	-	-	-
32.2	-	-	-	-	-	-	-	-	-	-	-	-	0.1319	-	-	-	-	-	-	-
33.1	-	-	-	-	-	-	-	-	-	-	-	-	0.0014	-	-	-	-	-	-	-
33.2	-	-	-	-	-	-	-	-	-	-	-	-	0.0460	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-	-	-	-	-	-	0.0005	-	-	-	-	-	-	-
34.2	-	-	-	-	-	-	-	-	-	-	-	-	0.0028	-	-	-	-	-	-	-
35	-	-	-	-	-	-	-	-	-	-	-	-	0.0009	-	-	-	-	-	-	-
35.2	-	-	-	-	-	-	-	-	-	-	-	-	0.0014	-	-	-	-	-	-	-
36	-	-	-	-	-	-	-	-	-	-	-	-	0.0005	-	-	-	-	-	-	-

Marker	D3S1358	D1S1656	D6S1043	D13S317	Penta_E	D16S539	D18S51	D2S1338	CSF1PO	Penta_D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	D8S1179	D12S391	D19S433	FGA
Ho	0.7417	0.8603	0.7923	0.7638	0.8842	0.7583	0.8548	0.8447	0.6912	0.8143	0.7399	0.7574	0.8235	0.7702	0.7408	0.6517	0.8097	0.8603	0.8061	0.8474
He	0.7667	0.8792	0.8115	0.7819	0.9113	0.7815	0.8751	0.8786	0.719	0.8581	0.7765	0.799	0.8376	0.7903	0.7499	0.6582	0.8298	0.8858	0.8372	0.8661
Homozygosity	0.2583	0.1397	0.2077	0.2362	0.1158	0.2417	0.1452	0.1553	0.3088	0.1857	0.2601	0.2427	0.1765	0.2298	0.2592	0.3484	0.1903	0.1397	0.1939	0.1526
TPI	1.936	3.5788	2.4071	2.1168	4.3174	2.0684	3.4431	3.219	1.6191	2.6931	1.9223	2.0606	2.8333	2.176	1.9291	1.4353	2.628	3.5788	2.5782	3.2772
PD	0.875	0.9677	0.9163	0.8875	0.9829	0.8869	0.966	0.9675	0.8154	0.9556	0.8843	0.9071	0.9402	0.8985	0.8517	0.6946	0.9342	0.9716	0.9383	0.9607
PE	0.472	0.6636	0.5459	0.503	0.711	0.4951	0.6531	0.6344	0.4072	0.5815	0.4695	0.4937	0.597	0.5124	0.4707	0.3618	0.5739	0.6636	0.5679	0.6395
PIC	0.7275	0.8671	0.7879	0.7508	0.9042	0.7506	0.8616	0.8661	0.6659	0.8414	0.7414	0.7698	0.8174	0.7591	0.7096	0.6123	0.8079	0.8744	0.8186	0.8509
Pr	0.0914	0.0269	0.0588	0.0801	0.0151	0.0795	0.0287	0.0274	0.1305	0.0357	0.0848	0.0682	0.0481	0.0755	0.1041	0.1629	0.0509	0.0244	0.0444	0.0334
MAF	0.2923	0.1958	0.2895	0.3203	0.1595	0.3396	0.1733	0.1999	0.3323	0.1926	0.3157	0.2813	0.239	0.3088	0.3483	0.5101	0.2592	0.1751	0.2762	0.1705
GenotypeNo	27	87	68	31	131	31	84	74	28	78	25	37	70	34	26	22	50	105	79	91
GeneDiversity	0.7663	0.8788	0.8112	0.7815	0.9109	0.7811	0.8747	0.8782	0.7187	0.8577	0.7761	0.7986	0.8372	0.79	0.7495	0.6579	0.8294	0.8854	0.8368	0.8657
P-value	0.0057	0.1567	0.0691	0.647	0.4513	0.0034	0.4224	0.054	0.0128	0.023	0.0386	0.0341	0.0003	0.0101	0.1912	0.2121	0.1507	0.1038	0.0845	0.0012

Ho: observed heterozygosity, He: expected heterozygosity, TPI: typical paternity index, PD: power of discrimination, PE: power of exclusion, PIC: polymorphic information content, Pr: probability of Identity, MAF: major allele frequency

Supplementary Table S2 Population differentiation between Iraqi Arabs and nine regional populations

	Kurdish [3]	Iranian [4]	Turkish [5]	Syrian [6]	Saudi [4]	Kuwaiti [7]	UAE [8]	Egyptian [4]	Indian [4]
CSF1PO	0.00008+- 0.0001	0.23243+- 0.0282	0.61066+- 0.0226	0.45671+- 0.0230	0.00005+- 0.0000	0.24985+- 0.0400	0.13280+- 0.0127	0.05004+- 0.0064	0.00000+- 0.0000
D3S1358	0.48105+- 0.0461	0.04174+- 0.0093	0.99650+- 0.0009	0.30600+- 0.0193	0.15830+- 0.0119	0.02870+- 0.0080	0.32249+- 0.0206	0.05216+- 0.0101	0.00050+- 0.0004
THO1	0.00160+- 0.0014	0.12769+- 0.0142	0.01969+- 0.0017	0.03662+- 0.0081	0.56567+- 0.0138	0.40795+- 0.0288	0.08354+- 0.0165	0.00000+- 0.0000	0.00138+- 0.0006
FGA	0.02809+- 0.0083	0.65757+- 0.0261	0.91031+- 0.0143	0.02160+- 0.0061	0.02589+- 0.0060	0.00177+- 0.0007	0.01492+- 0.0052	0.00000+- 0.0000	0.41481+- 0.0443
TPOX	0.02258+- 0.0032	0.46755+- 0.0222	0.89500+- 0.0104	0.00460+- 0.0015	0.01682+- 0.0034	0.00393+- 0.0022	0.00078+- 0.0003	0.00171+- 0.0005	0.00000+- 0.0000
vWA	0.00194+- 0.0012	0.46496+- 0.0271	0.01336+- 0.0035	0.13746+- 0.0133	0.40483+- 0.0391	0.00000+- 0.0000	0.05558+- 0.0137	0.23278+- 0.0238	0.00000+- 0.0000
D5S818	0.03116+- 0.0111	0.13438+- 0.0275	0.01015+- 0.0033	0.23733+- 0.0310	0.45080+- 0.0232	0.22637+- 0.0412	0.02316+- 0.0063	0.00976+- 0.0047	0.00000+- 0.0000
D7S820	0.00000+- 0.0000	0.00841+- 0.0024	0.71170+- 0.0177	0.63007+- 0.0274	0.00290+- 0.0010	0.00057+- 0.0004	0.02870+- 0.0125	0.02013+- 0.0088	0.00000+- 0.0000
D13S317	0.20158+- 0.0272	0.90518+- 0.0099	0.08274+- 0.0113	0.09757+- 0.0098	0.00000+- 0.0000	0.01098+- 0.0080	0.19327+- 0.0178	0.00002+- 0.0000	0.00000+- 0.0000
D21S11	0.00000+- 0.0000	0.07181+- 0.0120	0.22707+- 0.0308	0.00005+- 0.0001	0.00002+- 0.0000	0.02689+- 0.0057	0.02433+- 0.0055	0.00009+- 0.0001	0.00009+- 0.0001
D2S1338	-	0.00187+- 0.0008	0.87578+- 0.0091	-	0.00000+- 0.0000	0.00000+- 0.0000	0.29853+- 0.0505	0.20480+- 0.0433	0.00000+- 0.0000
D8S1179	-	0.00087+- 0.0004	0.03021+- 0.0098	0.38208+- 0.0154	0.00000+- 0.0000	0.00000+- 0.0000	0.00241+- 0.0022	0.01753+- 0.0069	0.00000+- 0.0000
D16S539	-	0.83852+- 0.0200	0.28813+- 0.0188	0.01149+- 0.0028	0.50848+- 0.0305	0.01356+- 0.0054	0.02848+- 0.0088	0.09778+- 0.0263	0.00000+- 0.0000
D18S51	-	0.32138+- 0.0251	0.20565+- 0.0250	0.54512+- 0.0213	0.00733+- 0.0033	0.00158+- 0.0009	0.26501+- 0.0415	0.00000+- 0.0000	0.00000+- 0.0000
D19S433	-	0.57518+- 0.0360	0.28224+- 0.0275	-	0.00000+- 0.0000	0.00117+- 0.0006	0.00000+- 0.0000	0.10255+- 0.0274	0.00000+- 0.0000

Figures in bold indicate a p -value less than 0.05, figures in bold and italic indicate a significant p -value after Bonferroni correction for 10 populations (i.e. 0.005). The exact test was carried out with 100,000 Markov steps.

Supplementary Table S3 Median and typical likelihood for 10 scenarios using different relatives for the identification of human remains. Typical likelihood ratios were calculated for each scenario by taking the mean of the log values of individual LRs. The antilog of the mean was the typical LR.

	One sibling	One parent or child	Two siblings	Spouse + child	Parent + sibling	2 Children	1 Parent+1 child	Three sibling	Spouse + 2 children	2 Parents
Median	3834058	26495282	22494443758	1.249E+11	3.85E+11	7.64E+12	1.39642E+13	1.34164E+14	2.0445E+16	2.498E+18
Typical LR	4408092	38373812	37879817004	1.66293E+11	5.66E+11	4.19E+12	1.86114E+13	1.13846E+14	1.44182E+16	5.698E+18