

Parentage testing anomalies in Hong Kong SAR of China

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Objective To determine if there are any differences in the number of exclusions from paternity of men using an anonymous parentage testing service compared with that of men using an irperson parentage testing service provided by the same company in Hong Kong SAR of China.

Methods Comparable numbers of consecutive anonymous and irperson parentage tests conducted by the same company were analyzed.

Results Men using an anonymous parentage testing service were excluded from paternity at a significantly greater rate ($P < 0.001$), compared with men using an irperson parentage test service.

Conclusions The results obtained from anonymous parentage testing indicate that the number of families containing children of doubtful parentage is much greater than expected previously. As illegitimate children are known to suffer greater degrees of abuse and neglect, this finding poses serious social questions regarding the welfare of families, which the relevant authorities should acknowledge and address.

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The forensic use of DNA technology began in the United Kingdom in 1987, with the introduction of DNA fingerprinting based on restriction fragment length polymorphisms.¹ Subsequent developments included the use of variable number tandem repeat and short tandem repeat (STR) loci located in the non-transcribed regions of the genome.² The latter are particularly amenable to analysis by the polymerase chain reaction (PCR). This has led to the rapid introduction of DNA fingerprinting both as a forensic tool and as a commercial activity with a particular association to parentage testing.

In many DNA-based parentage test services, a panel of 9 - 13 STR loci are analysed for each sample donor. Reagents for STR PCR testing are available from several commercial sources. A typical parentage examination case includes an alleged father, and a biological mother and child. Under standard test conditions, if all STR loci from the child match those observed in the alleged father a combined probability index (CPI) is calculated based on the frequency of occurrence of the STR alleles in the alleged father's ethnic group. Using the CPI and a prior probability of 0.5, a probability of paternity can be calculated as follows:

$$\text{probability of paternity} = \text{CPI} / (\text{CPI} + 1)$$

If two or more loci from the child do not match those observed in the alleged father, then the tested man is excluded as the biological parent. Occasionally, a mutation occurs in one or more of the child's STR loci resulting in an apparent mismatch between child and alleged parent. This inconclusive test reduces the CPI below the threshold needed to affirm a probability of paternity greater than 99.9%. An inconclusive result must be resolved by analysing further STR loci.

As a company providing both irperson testing (where all tested persons visit our offices for tissue sampling) and anonymous parentage testing services (where applicants post samples for testing to our laboratory) based on STR PCR analysis, we compared the results obtained from each to see if there were any significant differences in the frequency of excluded, non-excluded and inconclusive results.

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METHODS

Sample collection

In-person test

Test subjects provided cheek cells by performing a buccal scrape using a sterile nylon brush under the direction of a trained laboratory assistant. Samples were sealed in sterile containers and transferred to the laboratory for DNA extraction and STR PCR. The movements of the samples were continuously documented throughout the laboratory analysis.

Anonymous test

Applicants collected buccal scrapes by themselves using sterile nylon brushes provided in the GeneTek DNA Parentage Testing Sample Collection Kit (DNA-TECH Ltd., Hong Kong, China) obtained from local pharmacies in Hong Kong. Applicants returned the samples to DNA-TECH Ltd. for testing. Samples were transferred to the laboratory for DNA extraction and STR PCR.

DNA extraction

The used buccal swabs were removed from the transport container, placed in a 1.5-ml screw-cap microcentrifuge tube containing DNA extraction solution (MasterAmp Buccal Swab DNA Extraction Kit, Epicentre Technologies, USA) and rotated at least 10 times to remove adhered cells. The tube was sealed, vortex mixed for 10 seconds and incubated in a 60 °C water bath for 30 minutes. After incubation, the tube was vortex mixed for 15 seconds and incubated in a 98 °C water bath for 8 minutes, followed by a 15 seconds vortex mix and further incubation at 98 °C for 8 minutes. After incubation, the tube was vortex mixed for 15 seconds and chilled on ice. Cellular debris was pelleted in a microcentrifuge (3000 r/min, 5 minutes, 4 °C). The supernatant (containing DNA) was transferred to a clean 1.5 ml microcentrifuge tube and 3 volumes of absolute ethanol were added. The tube was stored at -80 °C for 1 hour. After incubation, the tube was centrifuged (13 800 r/min, 15 minutes). The supernatant was discarded and the DNA pellet carefully washed with 1 ml 70 % ethanol. The pellet was resuspended in 20 µl DEPC-treated water. The pellet was stored at -70 °C prior to use.

STR polymerase chain reaction

For each sample, the following STR loci were analysed: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820. If necessary, following alleles were analysed including: D16S539, TH01, TPOX, CSF1PO using the AmpFISTR COfiler and AmpFISTR Profiler Plus amplification kits (Applied Biosystems, Inc.,

Foster City, CA, USA). Amplified products were analysed on an ABI Prism PE310 Genetic Analyzer (Applied Biosystems, Inc., USA) according to the protocol included in the kit. The PCR mix contained AmpFISTR PCR Reaction Mix, AmpFISTR Primer Set and AmpliTaq Gold DNA polymerase (Roche Molecular Systems, Inc., Pleasanton, CA, USA), 1.25 ng sample DNA, and DEPC-treated water to 25 µl. The reaction tubes were placed in a Robocycler Gradient 96 (Stratagene, La Jolla, CA, USA), and the following amplification programme was applied: denaturation (95 °C, 11 minutes), followed by 28 cycles of denaturation (94 °C, 1 minute), annealing (59 °C, 1 minute), extension (72 °C, 1 minute) followed by a final extension at 60 °C for 45 minutes and ending at 25 °C.

Exclusion from paternity

Alleged fathers were excluded from biological paternity if two or more amplified STR loci observed in the child failed to correspond with those observed in the alleged father.

Calculating CPI

In cases of non-exclusion, the CPI was calculated using a prior probability of 0.5. The frequency of occurrence of STR alleles in the alleged father's ethnic group was obtained from published data.³⁻⁵

Statistical analysis

All data were compared using binomial distribution theorem for unpaired groups.

RESULTS

Exclusion from paternity by test service

Between late 2000 and early 2002, 76 consecutive in-person parentage test cases and 75 consecutive anonymous parentage test cases submitted for study were analysed. The results are presented in Table 1.

Table 1. Comparison of in-person and anonymous parentage test results

Possible parentage testing outcome	In-person testing n (%)	Anonymous testing n (%)
Excluded	14/76 (18.4)	30/75 (40.0) *
Not excluded	60/76 (78.9)	41/75 (54.7)
Inconclusive	2/76 (2.6)	4/75 (5.3)

* $P < 0.01$, vs in-person testing.

Analysis of DNA parentage service users by race

The majority of Hong Kong residents (about 95 %) are of Southern Chinese ethnicity.⁶ This is reflected in the racial characteristics of the alleged fathers using each service (Table 2). The proportion of mixed race children analysed with either the in-person (5/76, 6.6 %) or the anonymous

testing service (8/75, 10.7%) was not significantly different.

Table 2. Ethnicity of alleged fathers using DNA parentage test services

Ethnicity of alleged father	In-person testing n (%)	Anonymous testing n (%)
Chinese	74/76 (97.4)	69/75 (92.0) *
Other Asian	1/76 (1.3)	1/75 (1.3)
Non-Asian	1/76 (1.3)	5/75 (6.7)

* $P < 0.01$, vs Chinese father in-person testing; $P < 0.001$, vs non-Asian father in-person testing.

Analysis of inconclusive parentage tests

The number of inconclusive test results is larger with the anonymous testing service than with the in-person test service. This could influence the significance of the data interpretation. The larger number of inconclusive tests is not due to a higher frequency of mutated loci observed with one or other of the test services or the inability of the anonymous donors to collect their own tissue samples effectively (Table 3).

Table 3. Analysis of inconclusive cases

Number	In-person testing	Number	Anonymous testing
1	Mutation at D3S1358	1	Mutation at D5S818
2	Mutation at D8S1179	2	Insufficient sample for analysis
		3	CPI < 100 after 12 STR loci tested. Refer to client for further instruction
		4	Mother's sample was not provided for analysis. Observed exclusions could not be confirmed

DISCUSSION

The in-person test is generally performed when the result is required for legal proceedings, such as a claim for child maintenance. In contrast, the anonymous test is used simply for personal knowledge. Before either test is administered, the parents do not know the outcome, thus the expected frequency of men excluded and non-excluded from paternity should be similar. The in-person service is approximately 75% more expensive than the anonymous test. This could result in bias due to discrimination based on economic status, where low-income individuals/families select the less expensive anonymous test. Previous studies show that low-income is associated with increased number of sexual partners for males and females^{7,8} and reduced frequency of condom use.⁷ However, no data on income were collected from the test subjects upon application for testing, so the significance of this effect is unknown. It is likely that the anonymous test is selected for its convenience and the

assurance of anonymity over any economic considerations.

The onus for participating in parentage testing generally falls on the alleged father and helps explain the observed frequency of exclusions. Alleged fathers who strongly suspect that they are not the biological father tend to apply for in-person testing with the suitability of the test report for use in a legal setting, whilst alleged fathers who are merely satisfying a nagging doubt over paternity, select first for the anonymous test. That some 40% of these men are subsequently excluded from paternity may indicate the presence of a large population of children in Hong Kong whose parentage is in doubt. The average exclusion rate in non-US parentage testing centres is 30.5%, which includes both in-person and anonymous testing.⁹ Given the wealth and development of Hong Kong of China and its embrace of western values, this may further suggest a widespread breakdown of traditional conservative attitudes to the family in Hong Kong of China in recent years, that is supported in other data, such as increases in divorces,¹⁰ number of terminated pregnancies,¹¹ acceptability of pre-marital sex and co-habitation by young persons¹² and rate of infection with HIV and other sexually transmitted diseases in young adults.¹³ Conversely, it may simply reflect a previously unrecognised aspect of "traditional" Chinese society. Illegitimate children are more susceptible to child abuse, neglect and poor cognitive development.¹⁴

To our knowledge, this is the first report describing an apparent anomaly in parentage testing data based on the nature of the test service in Hong Kong of China and provides evidence for the existence of a potentially large and previously unrecognised group of children of doubtful parentage. The social consequences of this phenomenon in a closely knit homogeneous society such as Hong Kong warrants further attention from relevant government departments and non-governmental social welfare agencies in order to limit potentially adverse social consequences in the future.

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