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**Publication of population data of linearly inherited DNA markers in the International
Journal of Legal Medicine**

Walther Parson¹, Lutz Roewer²

¹ Institute of Legal Medicine, Innsbruck Medical University, Austria

² Institute of Legal Medicine and Forensic Sciences, Charité – Universitätsmedizin Berlin,
Germany

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Corresponding authors:

Y-Chromosomal polymorphisms:

Dr. Lutz Roewer

Institute of Legal Medicine and Forensic Sciences, Charité - Universitätsmedizin Berlin,
Germany

Tel.: 0049-30-450 525060; Fax: 0049-30-450 525912

Email: lutz.roewer@charite.de

Mitochondrial DNA:

Dr. Walther Parson

Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria

Tel.: 0043-512-9003 70640; Fax: 0043-512-9003-73640

E-mail: walther.parson@i-med.ac.at

Abstract

This manuscript extends on earlier recommendations of the editor of the *International Journal of Legal Medicine* on Short Tandem Repeat (STR) population data and provides details on specific criteria relevant for the analysis and publication of population studies on haploid DNA markers, i.e. Y-chromosomal polymorphisms and mitochondrial (mt)DNA. The proposed concept is based on review experience with the two forensic haploid markers databases YHRD and EMPOP, which are both endorsed by the International Society for Forensic Genetics. The intention is to provide guidance with the preparation of population studies and their results to improve the reviewing process and the quality of published data. We also suggest a minimal set of required information to be presented in the publication to increase understanding and use of the data. The outlined procedure has in part been elaborated with the editors of the journal *Forensic Science International Genetics*.

Introduction

In 1997 the editor of the *International Journal of Legal Medicine* has formulated guidelines for the submission of manuscripts on Short Tandem Repeat (STR) population data (Brinkmann, 1997). The current letter extends on the earlier recommendations and details on specific criteria relevant for the analysis and publication of population studies on haploid DNA markers, i.e. Y-chromosomal polymorphisms and mitochondrial (mt)DNA. The proposed concept is based on experience with review of Y-chromosomal and mtDNA data by aid of analysis tools available on YHRD (yhrd.org; (Willuweit and Roewer, 2007) and EMPOP (empop.org; (Parson and Dür, 2007)), which are both endorsed by the International Society for Forensic Genetics (isfg.org). The intention of this letter is to provide guidance with the preparation of population studies and their results to improve the reviewing process and the quality of published data. We also suggest a minimal set of required information to be presented in the publication to increase understanding and use of the data. The outlined procedure has in part been elaborated with the editors of the journal *Forensic Science International Genetics* (Carracedo et al. 2010).

The procedure of Y-STR/mtDNA data review is organized as follows:

Step 1) Prior to submission to the journal data table(s), one for each studied population, must be sent to YHRD/EMPOP (see details at <http://www.yhrd.org/Contribute> and <http://www.empop.org/modules/contribute>)

Step 2) Data review by YHRD/EMPOP, communication with author(s), provision of YHRD/EMPOP accession numbers if data quality is acceptable

Step 3) Authors submit manuscript and population data with reference to YHRD/EMPOP accession numbers

Step 4) Peer review and editorial decision

Preparation of the manuscript

Sampling

Sample collections arise from convenience sampling (blood banks, paternity testing, immigration cases, laboratory staff, etc) or targeted sampling (project specific sample recruitment). This may have implications for further use of the data in the forensic context and therefore this information is a requirement. Sample acquisition should be performed following the defined ethical considerations and under informed consent. This procedure should be stated explicitly. Sample numbers depend on the size of the investigated population. Urban and cosmopolitan population studies should involve at least 200 individuals whereas regional datasets should contain a minimum of 100 samples. Exceptions are valid for extremely small population sizes.

Description of the investigated populations

Haploid lineages show much more pronounced stratification compared to autosomal markers, which has consequences for their application in forensic genetics. It is therefore of utmost importance to carefully describe the sampled population correctly and in detail with respect to geographic origin and demographic background applying termini from molecular anthropology and population genetics. This includes the use of a correct ethonym (e.g. “Roma” instead of “Gypsy”, “Europeans” instead of “Caucasians” etc), the definition of the linguistic, and (if applicable) cultural groups (e.g. casts) and subgroups. The authors should follow the concept of metapopulations (as used in YHRD and EMPOP) and further ethnic

backgrounds should be indicated (e.g. 'North America, Idaho, Blackfoot Indians'). Both databases supply inventories of included populations that serve as guidance for the assignment of the relevant termini.

Presentation of the population data

Individual haplotypes have to be listed in the publication, usually as electronic supplement. Derived analytical data (statistical summaries) do not recompense for the presentation of the haplotypes. The haplotypes will also be included in the respective databases upon publication; note that contents between publication and database may differ due to continued database maintenance efforts.

Specific recommendations for Y-chromosomal STR systems (Y-STRs)

Population studies on new or known Y-STR markers are aimed to establish or to widen databases for biostatistical applications in a forensic genetic context. Generally spoken, such submissions are not hypothesis-driven and do not fulfil the requirements of an original article, but rather serve as an important documentary of locus- or haplotype-specific frequencies. Major population databases as the Y-STR haplotypes reference database (YHRD) endorse a policy of publication of local databases prior to its upload and refer to the published material as the original, quality proven source of information.

To facilitate and streamline the reviewing and the editing process and to avoid redundancy within and between articles we propose an article format presenting the frequency data as follows:

Following the abstract (~ 100 words) the communication should be reduced to the following topics:

Introduction

- Description of the population: A short but detailed description of the population is essential (a map might be included here) as well as a description of the interest of that population for population genetics and forensic purposes. Previous population genetic studies should be referred as well as the geographic location, ethnicity, method of sampling, and characteristics of the population.

Material and Methods should be reduced to a skeleton of references describing standard methods and commercial kits; exceptions from this should be reported in an abbreviated form.

The statement on informed consent / approval of an ethical committee should be included here.

The accession numbers issued by the YHRD curators after review of the raw data should be included in this section (see <http://www.yhrd.org/Contribute> for details).

Results

- the Y-STR haplotype data (extended by Y-SNPs if possible) should be represented in an obligatory Table 1 published as ESM

- this table is a standard spreadsheet file (e.g. Microsoft Excel, the example file and the format description is available at <http://www.yhrd.org/Contribute>). The first 2 columns specify an identification number and the origin of the samples. For the latter we request a ternary identifier in the form “region, country [ethnic group]” e.g. “Berlin, Germany [German]”. The geographic background of the samples should be further detailed in an accompanying text or by maps.

- the following columns list the common Y-STR loci; the allele nomenclature should closely follow the updated and very detailed recommendations of the ISFG (International Society of Forensic Genetics) on the use of Y-STRs in forensic analysis (Gusmao et al. 2006); according to these recommendations the nomenclature of 10 widely used Y-STRs (“minimal haplotype or minHt) should not be altered (e.g. DYS389AB is still DYS389II in a forensic context)

- please note these additional format rules

(i) alleles at multiplied loci are separated by a comma e.g. “11,14”

(ii) intermediate alleles are indicated by a dot (e.g. 11.2)

(iii) “Null” alleles as resulting from molecular mechanisms (primer site mutation, deletion) and confirmed by appropriate techniques are indicated by a “0”

- to transcribe different published locus and allele nomenclatures to one widely accepted standard is of utmost importance (e.g. DYS461 is DYS461 according to Genbank nomenclature); if an ambiguous allele nomenclature is chosen, the transcription to other published nomenclatures should be made transparent (for example allele 29 at DYS389II could be called allele 16, if the repeat 13 of DYS389I is subtracted from DYS389II)

- locus-specific allele frequencies should not be reported with the exception of (i) the locus has never been described before or (ii) the allele frequency of a given allele or the frequency distribution curve as a whole points to an underlying mechanism which needs a further detailed description in the “Remarks” section
- the samples should be given unique identifiers rather than haplotype numbers to trace them back if additional information is published in the future
- biostatistical parameters to describe the population sample(s) may include: discrimination capacity, haplotype diversity, frequency of the most frequent haplotype(s)
- informative comparisons to relevant reference populations (e.g. from the YHRD) should be included; values as F_{st} , Φ_{st} or R_{st} and a significance test (*p values*) should be calculated, an MDS plot or STR network to illustrate genetic distances between populations could be included (tools to perform such analyses e.g. AMOVA are available at <http://www.yhrd.org/Analyse>); the outcome of these comparisons should be discussed in short

Other remarks

- here additional data, e.g. on observed mutations, haplogroup affiliation etc. can be reported in short
- if mutations are observed (affecting the repeat length, the flanking site or the number of amplified sequences) the repeat type should be verified, usually by sequencing, and mutated alleles should be reported to the YHRD as recommended by the ISFG (Gusmao et al. 2006)
- the father’s age distribution in the cases with and without a mutation should be reported
- if a mutation at one or more STR is caused by a known chromosomal rearrangement (e.g., DYS464) the observation should be related to the appropriate reference
- when haplotypes are extended by phylogenetically informative Y-SNPs (as recommended) the most recent nomenclature (Karafet et al. 2008) should be followed; the full panel of typed Y-SNPs, specified by “+” for the derived state, “-“ for the ancestral state at a given locus (“-1” for “not typed”) should be included in the Table 1 as an extension to each individual Y-STR haplotype. The last two columns of the Table 1 should include the final branch marker used for haplogroup assignment, e.g. M3 and the assigned branch name (Q1a3a). h

The following statement should conclude this topic: “This paper follows the recommendations of the ISFG on the use of Y-STRs in forensic analysis (Gusmao et al 2006) and the guidelines for publication of population data requested by the journal.”

A statement that the Y-STR population data have been submitted of to the YHRD should be included in the manuscript.

These rules apply in principle also to the communication of data on Y-STR mutation rates, although a combined submission of haplotype and mutation data are recommended (Gusmao et al. 2006). The minimum number of allele transmissions should be 100 per locus.

Specific recommendations for mtDNA population data

MtDNA population studies are aimed to establish or to widen databases for biostatistical applications in a forensic genetic context. In a similar way as outlined for YHRD the mtDNA database EMPOP endorses a policy of publication of local databases prior to its upload and refers to the published material as the original source of information. In contrast to Y-STR data, that are generally established under well defined conditions (commercial kits, allelic ladders, international positive controls), mtDNA data rely on the scientific and technical expertise of the individual laboratory. Therefore and due to the complexity of mtDNA data, it has been demonstrated that quality control plays a more important role compared to other genetic markers (Röhl et al. 2001). The following criteria help improving the quality of mtDNA studies:

Subjects

It is imperative that the authors detail where the investigated samples originate from (see chapter “Description of the investigated polymorphism” above for further details).

Methods

The methods of DNA extraction, the selected amplification and sequencing primers and the version of the sequencing chemistry play an important role for the footprint of the sequence raw data (Parson, 2007) and should be clearly defined.

The analysis of the individual hypervariable segments HVS-I and HVS-II within the mtDNA control region (CR) is no more state of the art. First, independent amplification and sequencing of multiple mtDNA segments increase the risk of sample mix-up (artificial recombination, (Parson and Bandelt 2007)). Second, many useful polymorphisms residing at CR positions outside HVS-I/HVS-II are missed, which results in decreased discrimination power and the loss of relevant haplogroup-specific sites. It has been shown that sequencing of the entire CR substantially adds to our knowledge of the mtDNA phylogeny and variation and provides a sound basis for forensic mtDNA analysis. Streamlined and optimized protocols for amplification and sequence analysis of the entire CR are presented in (Parson and Bandelt 2007) and references therein.

Single-stranded sequence data have shown to harbour an unacceptable rate of phantom mutations resulting in artificial mtDNA haplotypes. The consensus haplotype reported for a sample must originate from at least full double-stranded sequence information. Although this was demanded earlier, it has not yet become routine practice. This is most importantly true for samples exhibiting length heteroplasmy, which require additional sequencing in order to achieve confirmed base call assignment. In particular we recommend sequencing length heteroplasmic regions with additional primers to unambiguously define the dominant variant as suggested in Bandelt and Parson (2008) and Forster et al (2010) and references therein.

The statement on informed consent / approval of an ethical committee should be included here.

The accession numbers issued by the EMPOP curators after review of the raw data should be included in this section (see <http://www.empop.org/modules/contribute> for details).

Results

- The manual transcription of mtDNA profiles into tables of publications constitutes the highest risk of introducing errors. IT-based conversion of the data is recommended. In any case, examination of the compiled data should be performed with scrutiny. This involves confirmation of the population data in the table by an independent scientist and/or double reading of the data under the four-eye principle.
- It is important to present and evaluate population data on the basis of the established mtDNA phylogeny. Alignment and annotation of mtDNA sequences should be performed according to the recommendations by the ISFG (Bär et al 2000), refined guidelines for the notation of haplotypes with more than one possible alignment are available in (Bandelt and Parson 2008).
- Haplotypes should be assigned to mtDNA haplogroups using the relevant literature (a continuously updated mtDNA tree is provided by (van Oven and Kayser 2009; www.phylotree.org). Especially for samples with unclear haplogroup affiliation it has proven useful to confirm their assignment using coding region information.

- The “phylogenetic check” is a very probative means for the evaluation of the data as yet unobserved variation becomes apparent and can be inspected by means of the raw data. This can be performed using the relevant literature or the NETWORK software provided via the EMPOP database (www.empop.org; Parson and Dür 2007).
- All electropherograms that are necessary to reconstruct the submitted data should be stored by the authors and provided upon particular request (check for questionable base calls).

Format of result tables (ESM)

- The representation of mtDNA variation in matrix-based “dot-tables” is error prone due to the fact that columns and rows can be mistakenly re-arranged during the publication process. Also these tables are usually difficult to read.
- It is required that mtDNA haplotypes are reported one by one in difference-based format (with respect to the rCRS) using a unique identifier as sample information that can further be used to make reference to a sequence.
- Also the interpretation range of the haplotypes needs to be specified. This range usually complies with the sequenced fragment, primer sequences disregarded (e.g. 16024 - 576 for the entire control region), but can also be restricted to smaller regions.
- EMP-format: EMPOP offers software for quality control of mtDNA sequences which requires a standard format to enable solid performance. Specific information on the file-format and an example file can be found on the EMPOP website (see Tools and Download sections at www.empop.org). This format can also be used for publication of the data as ESM in the journal.

Statistical parameters

- The report of frequency values of single mtDNA polymorphisms is meaningless as transitions and transversions are linked within haplotypes. Values to describe the population sample biostatistically should include: number of haplotypes, number of unique haplotypes, haplotype diversity, random match probability, frequency of the most frequent haplotype(s).

- Informative comparisons to relevant populations should include values as Φ_{st} or R_{st} and a significance test (*p values*)

General issues

- A statement that the mtDNA population data have been submitted to the EMPOP database should be included in the manuscript.
- The following statement should conclude this topic: “This paper follows the recommendations of the ISFG on the use of mtDNA in forensic analysis and the guidelines for publication of population data requested by the journal.”

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