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Heterozygosity reduction in children whose parents are closely related

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ABSTRACT

In this study, the impact of consanguineous marriage and inbreeding on heterozygosity was evaluated by comparing levels of heterozygosity in children of closely related parents with those of unrelated parents. Compared to the average expected heterozygosity, the average observed heterozygosity was lower in the children with first-cousin parents. This was not the case in the children with unrelated parents, where an increase in the average observed heterozygosity was noted. Differences in the average heterozygosity between parents-children also observed between related and unrelated parents. First-cousin parents had higher average heterozygosity than the children compared to the unrelated parents where the average heterozygosity was lower than the children. Further investigation with bigger sample size and different populations will provide better understanding, however this study showed that a careful approach should be taken when dealing with parentage testing involving closely related parents.

1. Introduction

Consanguineous marriage is quite common in many regions of the world [1], especially between cousins in the region of South Asia [2]. Even though consanguineous marriages can result in an increase in genetic disorders among children [3], in a forensic context, inbreeding could cause reduction in heterozygosity [4] which is problematic in kinship testing, especially in motherless paternity analysis [5]. This is evident when using a limited number of Short Tandem Repeat (STR) markers in kinship testing, when trying to differentiate related and unrelated individuals [6]. Thus, the heterozygosity in children with related or unrelated parents in South Asian families was studied using autosomal STR markers to identify any changes in heterozygosity ratios.

2. Materials and methods

2.1. Sample collection

Buccal swab samples from twenty South Asian trios with self-proclaimed ethnicity for three generations were collected with informed consent. Ten of the trios were with first-cousin parents and the other ten with unrelated parents.

2.2. DNA profiling

Samples were extracted using Prep-n-Go™ Buffer (Applied Biosystems™) and amplified using VeriFiler™ Express PCR Amplification Kit (Applied Biosystems™), following an optimized reduced volume method [7]. Capillary electrophoresis was performed on the ABI Prism® 3500xL Genetic Analyzer (Applied Biosystems™) and alleles were determined using the GeneMapper® ID-X v1.6 software (Applied Biosystems™).

2.3. Statistical analysis

Statistical calculations for the relationships between tested trios were carried out using Converge™ software (Applied Biosystems™) [8]. Expected and observed heterozygosities in children's profiles were generated using FORSTAT, a web-based forensic and population genetics analysis tool [9].

3. Results and discussion

First-cousin relationships between the parents were verbally verified during sample collection. They were also statistically confirmed using a likelihood ratio (LR) threshold of 10 (probability >90 %). The same LR

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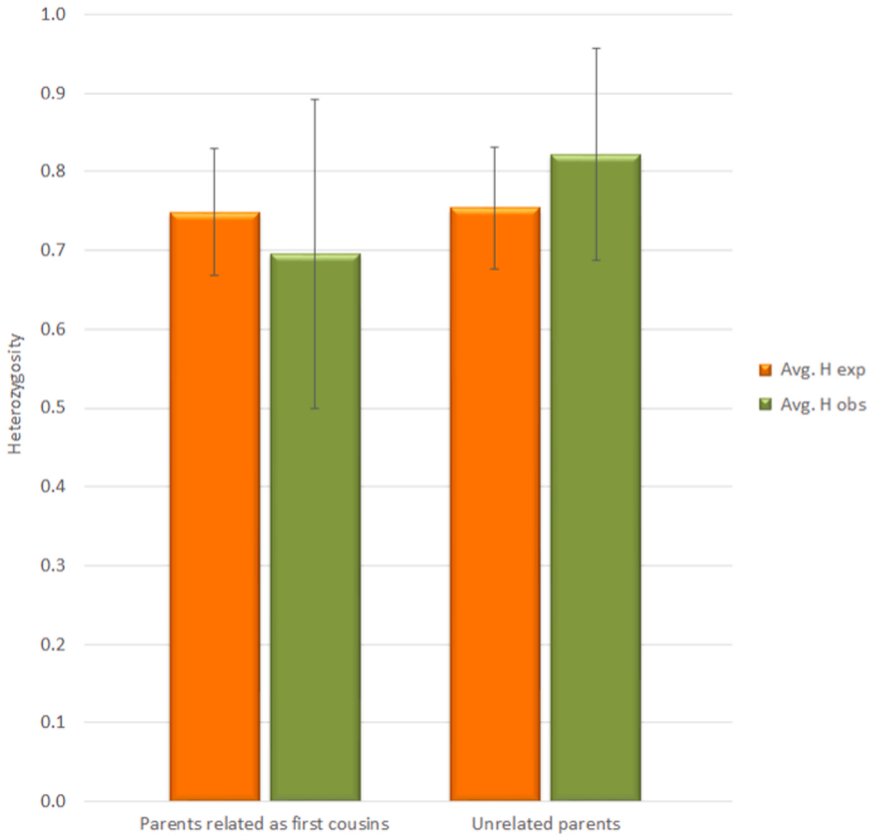

Fig. 1. Heterozygosity in children with related and unrelated parents.

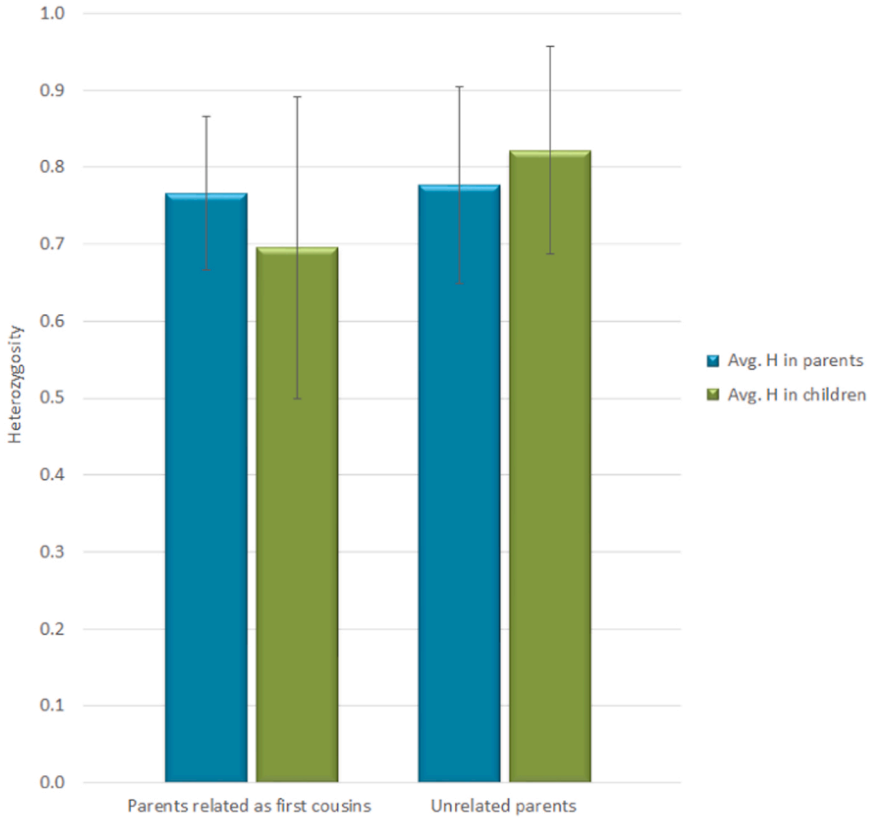

Fig. 2. Comparison of heterozygosity between parents and children.

threshold was also used for confirmation of unrelated parents. Parentage was confirmed with combined paternity/maternity index greater than 10,000 (probability >99.99 %). All these statistical analyses were carried out using the developed DNA profiles with 23 autosomal STR markers.

The heterozygosity results showed that the average observed heterozygosity among children with first-cousin parents was 69.57 % (σ: 19.65 %) but the average expected heterozygosity was higher at 74.83 % (σ: 8.06 %). This was the opposite in children with unrelated parents, where the average observed heterozygosity was 82.17 % (σ: 13.47 %) compared to the average expected heterozygosity of 74.83 % (σ: 7.77 %) (Fig. 1).

These results were also supported by the average heterozygosity observed among the parents. The average heterozygosity among unrelated parents was 77.72 % (σ: 12.76 %), increased to 82.17 % (σ: 13.47 %) among the children, while the average heterozygosity among first-cousin parents was 76.63 % (σ: 10.01 %), reduced to 69.57 % (σ: 19.65 %) among the children (Fig. 2). The highest reduction was in a child with only 13 heterozygotes (56.52 %) while the parents had 17 (73.91 %) and 19 (82.61 %) heterozygotes. Conversely, no reduction in heterozygosity was observed in children with unrelated parents.

4. Conclusion

This study demonstrates that there is a reduction in heterozygosity in children whose parents are closely related. A further study with larger data set and different populations will provide a better understanding of this phenomenon. However, this study indicates that additional familial information should be gathered when test samples are collected, especially for duo parentage testing with closely related parents. Any potential mutation in this situation should be treated carefully and supplementary markers should be added to produce a conclusive result.

Conflict of interest

None.

References

[1] H. Hamamy, Consanguineous marriages, J. Community Genet. 3 (2012) 185–192, https://doi.org/10.1007/s12687-011-0072-y.
[2] F.Z.A. Khan, S.B. Mazhar, Current trends of consanguineous marriages and its association with socio–demographic variables in Pakistan, Int. J. Reprod. Contracept. Obstet. Gynecol. 7 (5) (2018) 1699–1705, https://doi.org/10.18203/2320-1770.ijrcog20181898.
[3] S. Temtamy, M. Aglan, Consanguinity and genetic disorders in Egypt, Middle East J. Med. Genet. 1 (2012) 12–17, https://doi.org/10.1097/01.MXE.0000407744.14663.d8.
[4] J.R. Powell, B.R. Evans, How much does inbreeding reduce heterozygosity? Empirical results from *Aedes aegypti*, Am. J. Trop. Med. Hyg. 96 (1) (2017) 157–158, https://doi.org/10.4269/ajtmh.16-0693.
[5] N. von Wurmb-Schwark, V. Mályusz, E. Simeoni, E. Lignitz, M. Poetsch, Possible pitfalls in motherless paternity analysis with related putative fathers, Forensic Sci. Int. 159 (2006) 92–97, https://doi.org/10.1016/j.forsciint.2005.07.015.
[6] R. Fimmers, M. Baur, U. Rabold, E. Seifried, C. Seidl, STR–profiling for the differentiation between related and unrelated individuals in cases of citizen rights, Forensic Sci. Int. Genet. Suppl. Ser. 1 (2008) 510–513, https://doi.org/10.1016/j.fsigss.2008.01.002.
[7] J. Perry, T. Munshi, T. Haizel, S. Iyavoo, Validation of reduced volume VeriFiler™ Express PCR amplification kit for buccal swab samples extracted using Prep-n-Go™ buffer, J. Forensic Sci. 67 (2022) 1971–1978, https://doi.org/10.1111/1556-4029.15084.
[8] J.G. Shewale, D. Wang, C. Zhong, S. Gopinath, J. Mulero, A. Razdan, S. Vijaychander, J. Deng, P. McClurg, C. Dallett, A. Minn, From sample collection to report generation – a new integrated workflow solution for paternity and relationship testing, Forensic Sci. Int. Genet. Suppl. Ser. 4 (2013) e232–e233, https://doi.org/10.1016/j.fsigss.2013.10.119.
[9] P.G. Ristow, M.E. D'Amato, Forensic statistics analysis toolbox (FORSTAT): a streamlined workflow for forensic statistics, Forensic Sci. Int. Genet. Suppl. Ser. 6 (2017) e52–e54, https://doi.org/10.1016/j.fsigss.2017.09.006.