Determination of parental origin of Gestational Trophoblastic Disease using MS-MLPA

Sara Cardoso [1,2*], Jorge Ferreira [3], Rui Silva Santos [3], Paula Lopes [3], Carla Pinto [4], Marta Rodrigues [5], Maria João Pinho [6], Liliana Capela [6], Sofia Dória [6], Carmen Jerónimo [2,7], Carla Bartosch [2,3,5]

[1] Clinical Pathology Department, Portuguese Oncology Institute of Porto (IPOP), Rua Doutor António Bernardino de Almeida 351, 4200-072 Porto, Portugal
[2] Cancer Biology & Epigenetics Group, Research Center of IPO Porto (CI-IPOP) / CI-IPOP@ RISE (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto)/Porto Comprehensive Cancer Center Raquel Seruca (Porto.CCC), Rua Doutor António Bernardino de Almeida 351, 4200-072 Porto, Portugal
[3] Pathology Department, IPOP, Rua Doutor António Bernardino de Almeida 351, 4200-072 Porto, Portugal
[4] Genetics Department, IPOP, Rua Doutor António Bernardino de Almeida 351, 4200-072 Porto, Portugal
[5] Pathology Department, Centro Hospitalar Universitário São João, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal
[6] Department of Genetics, Faculty of Medicine, University of Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal
[7] Department of Pathology and Molecular Immunology, ICBAS-School of Medicine and Biomedical Sciences, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
** sara.cardoso@ipoporto.min-saude.pt*

Background: Gestational trophoblastic disease (GTD) is a rare condition marked by abnormal proliferation of placental trophoblastic cells and encompasses a heterogeneous group of disorders with distinct clinical, imaging, and histological features, each requiring tailored therapeutic approaches. Although GTD generally has a good prognosis, early diagnosis, targeted treatment, and continuous surveillance are essential for high cure rates. Given the overlapping phenotypic features of GTD entities, histopathological evaluation can be subjective, highlighting the need for molecular diagnostic tests to complement morphological assessments. **Aim:** To determine the parental origin of 44 products of conception (POCs) by assessing methylation levels in chromosomal region 11p15.5. **Methods:** Forty-four cases, including 11 complete hydatidiform moles (CHM), 11 partial hydatidiform moles (PHM), and non-molar POCs (controls), were studied. DNA was extracted from formalin-fixed paraffin-embedded FFPE tissue samples, and MS-MLPA was conducted, followed by capillary electrophoresis. MS-MLPA, targeting differentially methylated regions (DMRs) such as H19DMR and KvDMR, enabled parental origin determination. **Results:** Parental origin was accurately established in 40 of the 44 samples. Four samples (2 CHM, 1 NM69 (non-molar triploid gestation), 1 NM47 (non-molar gestation with chromosomal trisomy)) could not be reliably analyzed using MS-MLPA. The mean final methylation ratio (FMR) values in CHM, PHM, NM69, and NM47 were calculated using Joergensen's modified formula, showing distinct methylation profiles across the groups [4]. **Conclusions:** Based on the methylation status of the DRMs studied, it was possible to identify the proportion of alleles inherited from the father and/or mother, with each being inversely methylated according to parental origin [4,5]. MS-MLPA proved effective in determining parental origin without requiring parental samples, which is especially useful in cases involving egg donor pregnancies where DNA genotyping may be unreliable [4,5,6]. MS-MLPA is therefore a valuable diagnostic tool for GTD; however, it should ideally be used alongside other diagnostic methods to ensure a more comprehensive analysis.

Keywords: Differentially methylated regions (DMRs); Gestational trophoblastic disease (GTD); Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA); Parental origin.

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