Exploring the (epi)genetic architecture of an Italian cohort of stillbirths with anomalies of the brainstem and heart conduction system

Edoardo Errichiello | Mauro Lecca | Agnese Spennacchio | Nicolò Mele | Graziella Alfonsi | Patrizia Leonardi | Giulia Ottaviani

Unit of Medical Genetics, Department of Molecular Medicine, University of Pavia, Pavia, Italy; Laboratory of Cytogenomics, IRCCS Mondino Foundation, Pavia, Italy; SUID and SIDS Italia Onlus, Turin, Italy | Medical Genetics Unit, Department of Molecular Medicine, University of Pavia, Pavia, Italy | Medical Genetics Unit, Department of Molecular Medicine, University of Pavia, Pavia, Italy | Lino Rossi Research Center, Anatomic Pathology, Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milan, Italy | Lino Rossi Research Center, Anatomic Pathology, Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milan, Italy | Lino Rossi Research Center, Anatomic Pathology, Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milan, Italy

Aims

To investigate the (epi)genetic background of a single-center Italian cohort of 42 stillbirths (mean gestational age: 38 weeks, range: 28-42 weeks) all with peculiar brainstem abnormalities documented histopathologically; concomitant heart conduction system anomalies were observed in 23 cases.

Methods

DNA samples, extracted from formalin-fixed paraffin-embedded brainstems and matched control tissues, underwent whole-exome sequencing (WES) including mitochondrial genome - and mitochondrial DNA (mtDNA) haplogroup assessment, LINE-1 (Long interspersed element-1) methylation analysis, and absolute mtDNA copy number quantification.

Results

WES identified variants in disease candidate genes enriched for pathways related to neurodevelopment, such as “Huntington's disease” and “Amyotrophic Lateral Sclerosis”, and cardiac function, namely “Cardiac Conduction”, “Ion Channel Transport”, “Hypertrophic Cardiomyopathy”, and “Dilated Cardiomyopathy”. Other relevant pathways were “JAK-STAT signaling”, “Circadian entrainment”, and “IL-6 signaling”.
LINE-1 methylation analysis showed a trend toward hypomethylation in brainstems compared with controls (68% vs 73%, p=0.0314), suggesting “confined” loss of methylation.
Absolute mtDNA copy number analysis showed an increase in mtDNA copies in affected tissues compared with the normal counterpart (3.23:1; p=0.005).

Conclusions

These findings support the suitability of WES as a first-tier genetic test for stillbirth workup on “difficult” archival samples and shed new light on molecular pathways underlying stillbirth associated with prominent brainstem anomalies and its intriguing overlap with sudden unexpected death in infancy and childhood, representing distinct etiological entities on a genotypic/phenotypic continuum from fetal life to childhood. Furthermore, our study reinforces previous evidence for the involvement of abnormal

mitochondrial DNA content and methylation in stillbirth, with potential implications as early biomarkers.