

Abnormal erythrocyte morphology in *CDKL5* Rett syndrome

Joussef Hayek ¹, Lucia Ciccoli ², Eugenio Paccagnini ³, Mariangela Gentile ³, Silvia Leoncini ^{1,2}, Cinzia Signorini ², Gloria Zollo ^{1,2}, Alessio Cortelazzo ^{1,4}, Claudio De Felice ⁵

¹ Child Neuropsychiatry Unit, University Hospital (AOUS), Policlinico "S. M. alle Scotte", Siena, Italy; ² Department of Molecular and Developmental Medicine, University of Siena Siena, Italy; ³ Department of Life Sciences, University of Siena, Siena, Italy; ⁴ Department of Medical Biotechnologies, University of Siena, Siena, Italy; ⁵ Neonatal Intensive Care Unit, University Hospital, AOUS, Siena, Italy

An abnormal erythrocyte (RBCs) shape was described by our group in *MECP2*-mutated RTT (*MECP2*-RTT) patients and appears to be mainly modulated by oxidative stress (OS). In particular, leptocytes were found to be the predominant altered erythrocyte shapes in typical RTT (1). Aim of the present study was to assess RBCs morphology in *CDKL5*-mutated RTT patients. A total of n=15 *CDKL5*-mutated RTT (*CDKL5*-RTT) patients, n=15 *MECP2*-RTT patients, as well as n=15 healthy controls of comparable age participated in the study. Peripheral blood was collected in heparinized tubes for Scanning Electron Microscopy (SEM) analysis of erythrocytes. Erythrocytes were prepared as previously described (1). Altered RBCs shapes were evaluated by counting ≥ 800 cells (50 erythrocytes for each different SEM field at a magnification of $\times 3000$) from all groups of subjects.

A total of 36.2 % of RBCs with altered shapes was evidenced in peripheral blood from *CDKL5*-RTT patients vs. 96.2 % from *MECP2*-RTT vs. 19 % from healthy subjects (ANOVA, $P < 0.05$). In particular, abnormally shaped RBCs feature mainly knizocytes (RBCs with two or three concavities, separated by a ridge) in *CDKL5*-RTT (54.9 % of all altered shapes). Knizocytes are reported in haemolytic and acute or chronic liver disease, and neonatal period. *In vitro* exposure of RBCs to high concentration of primaquine has been reported to convert the majority of cells to knizocytes (3), thus reinforcing the key role of the redox homeostasis in the maintenance of a normal RBCs morphology.

In conclusion, an unexplained relative knizocytosis was observed for the first time in *CDKL5*-RTT.

References

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