INVESTIGATION OF GENETIC AND EPIGENETIC MECHANISMS UNDERLYING BECKWITH-WIEDEMANN SYNDROME (BWS) ON A LARGE COHORT OF ITALIAN PATIENTS

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Beckwith-Wiedemann Syndrome (BWS, OMIM 130650) is a congenital disorder with an incidence of 1:15.000, characterized by overgrowth, a variety of developmental anomalies and increased risk of pediatric tumors. Clinical diagnosis ranges with a continuum of clinical signs from complete BWS syndrome to incomplete BWS till to Isolated Hemihypertrophia (HI) The genetic basis is complex and involves genetic/epigenetic alterations of imprinted growth regulatory genes in the 11p15 region. Genetic defects include 11p15 chromosomal rearrangements (2-3%), segmental chromosome 11 paternal UPD (20%) and mutations of the CDKN1C gene. Epigenetic alterations affect either IC1 (~2-7%) associated with H19 and IGF2 or IC2 associated with KCNQ10T1, which rules the expression of KCNQ and CDKN1C (~ 50%). Familial microdeletions associated with methylation within IC1 have been demonstrated by our group to be a rare mechanism involved in the syndrome.

The two Working Unit (WU), WU1 and WU2, jointly collected a wide cohort of patients including 270 complete BWS, 98 incomplete BWS and 104 HI. All genetic defects were found in the complete BWS cohort confirming the clinical diagnosis in about 66% of the patients, while only about 16% of cases were identified to carry a defect at IC2/IC1 within the remaining two groups, so attesting that other unknown mechanisms may be causative for incomplete BWS or IH. Concerning genotype-phenotype correlation our study substantially confirms what reported in the literature, mainly revealing the occurrence of overgrowth and omphalocele, associated with IC2 defects. Tumours were developed in at least 26 patients, with a major frequency among patients with IH, 11/104, roughly 10%, and complete BWS, 13/207 corresponding to 4,8%, while only 2 were developed by incomplete BWS cases. Most were Wilms tumors, but one hepatoblastoma, two neuroblastomas, two pancreatoblastomas and one very precocious mammary fibroma were detected. Unfortunately tumor tissues have been most often unavailable.

As the analysis of methylation defects and of uniparental disomy needs the use of multiple techniques, WU2 validated the MS-MLPA technique as a useful tool to detect 11p15 number and methylation anomalies.

In order to reveal methylation defects at genomic regions different from 11p15, WU1 and WU2 investigated the cohort of BWS patients both without and with a known genetic defect. One ICR normally methylated on the paternal allele, GTL2-IG (14q32), and eight maternally methylated ICRs, PLAGL1 (6q24), IGF2R (6q25.3), GRB10 (7p21), MEST (7q32.2), SNRPN

(15q11), PEG3 (19q13), GNAS (20q13.32) and NESPAS (20q13.32), were investigated. Methylation analysis of the above 11 ICRs showed that hypomethylation affecting multiple imprinted loci was restricted to 17 patients with hypomethylation of the KCNQ1OT1 ICR, and involved only maternally methylated loci. Both partial and complete hypomethylation was demonstrated in these cases, suggesting a postzygotic origin of a mosaic imprinting error. The study was carried out with the contribution of other European groups and has been reported.

Furthermore WU3 and WU1 developed a strategy to investigate fetuses with ultrasonographic indication (omphalocele occurrence) for BWS testing. Among 20 cases four revealed hypomethylation of IC2 and one had a mutation of CDKN1C, thus proving that this feature can be considered a II trimester good indicator of BWS in prenatal diagnosis.

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