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EXPRESSION SIGNATURE OF GENES ASSOCIATED WITH TELOMERE-TELOMERASE COMPLEX IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND ACUTE LEUKEMIA: TEP1 GENE IS SURPRISINGLY UPREGULATED IN PROGRESSION OF MDS AND IN LEUKEMIC CELLS

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Background. Knowledge of dynamics of telomere-telomerase complex brings important sign into molecular background of leukemogenesis. Misbalance initiated by erosion of telomeres may affect also expression level of genes enrolled in regulation of telomere length and telomerase activity. Thus, data on expression profiles of associated genes: hTERT encoding catalytic sub-unit of telomerase, the tankyrase (TNKS), TRF1 (Telomeric Repeat binding Factor 1), POT1 (Protection Of Telomeres 1), TEP1 encoding telomere associated protein, and myc may be valuable from the viewpoint of disease prognosis and monitoring of therapy effectiveness. *Aims.* To ascertain expression variations of genes involved in regulation of telomere-telomerase complex in patients with myelodysplastic syndromes (MDS), acute myelogenous leukemia (AML) from MDS, and primary untreated AML with the aim to evaluate their significance as prognostic factors of MDS evolution towards overt leukemia and markers of leukemic cells. *Methods.* The study was done on mononuclear bone marrow (BM) or peripheral blood (PB) samples from 42 patients with MDS, AML from MDS and untreated primary AML divided into subgroups according to the FAB criteria. Mononuclear cells of 14 healthy BM or PB progenitor cells healthy donors served as normal controls. RNA was extracted using modified method of Chomczynski. Relative expressions of hTERT, TNKS, TRF1, POT1, TEP1, and myc RNA were assayed by real-time RTPCR with specific Taq-Man probes in RotorGene 3000A (Corbett Research) in comparison to expression of the housekeeping gene. Results with the ratio higher than mean + 2 s .d. of healthy controls were postulated as cases with positive gene expression. Expression signatures were discussed together with telomere length, telomerase activity and clinical features: proportion of blast cells, results of the DFS analysis and also with individual patients risk score established for MDS according to the International Prognostic Scoring System (IPSS). *Results.* Notable increase of expression of hTERT, TEP1, and POT1 genes was observed in patients with advanced form of MDS (RAEB and RAEB-t) in contrast to insignificant changes of telomerase activity representing a later event in misbalance of telomere-telomerase complex. Significant correlation between individual values of POT1 gene expression and telomerase activity confirmed in MDS and AML patients (p=0.0079) supports role of the POT1 gene as positive molecular regulator of telomerase. On the other hand, no relationships were found between POT1 expression and the IPSS risk score of MDS patients on one side and portion of blast cells in BM/PB both in MDS and AML on the other side. *Summary/Conclusion.* We showed that hTERT and POT1 genes up-regulated already in early forms of MDS and its expression has increasing trend with disease progression. Significantly increased expression of these genes is also feature of mononuclear BM/PB cells of majority of patients at diagnosis of primary AML. These observations predestine POT1 and hTERT genes at least as additional prognostic factors of MDS and molecular markers of AML. High TEP1 expression in patients with advanced forms of MDS and AML indicates on its more active role in signaling of telomere-telomerase complex as it has been supposed.

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TEN NOVEL MUTATIONS IN THE HMBS GENE RESPONSIBLE FOR ACUTE INTERMITTENT PORPHYRIA

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Background. Acute intermittent porphyria (AIP) is an autosomal dominant disorder caused by a partial deficiency of hydroxymethylbilane synthase (HMBS), the third enzyme in the heme biosynthetic pathway. Clinical features of the disease are intermittent attacks of neurological dysfunction, including abdominal pain and neuropsychiatric symptoms. Most of the affected individuals remain asymptomatic throughout their life but 10-20% presenting severe acute attacks. Diagnosis of AIP is often difficult if based on urinary overproduction of porphyrin precursors ALA and PBG only. The erythrocyte HMBS activity is not extensively available and not always informative because of the overlap between the normal and carriers range. The molecular analysis of HMBS gene represents the most reliable diagnostic tool for AIP. The human HMBS gene maps to chromosome 11q24.1-q24.2 with a total of 15 exons. Two distinct promoters direct housekeeping and erythroid specific mRNAs by alternative splicing. So far, more than 210 different mutations have been identified world-wide in the HMBS gene as responsible for AIP, showing a high genetic heterogeneity. Most of the reported mutations have been detected only in single families, however a prevalence of specific mutations in different geographic areas has been reported. Only preliminary data are available for the Italian population. *Aims and Patients*. In this study we searched for molecular defects in HMBS gene, in order to identify the most common HMBS mutations in Italian subjects affected by AIP. We investigated twelve unrelated patients and their relatives. The diagnosis was based on clinical manifestations, elevated urinary excretion and reduced erythrocyte HMBS activity. *Methods.* The promoters, the entire coding region and the intron-exon boundaries of HMBS gene has been amplified by polymerase chain reaction and submitted to direct automated sequencing. Restriction fragment length polymorphisms, poly-acrylamide gel electrophoresis and XL PCR were performed to confirm the presence of putative mutations. *Results.* Twelve different molecular defects in HMBS gene have been identified. Two missense mutations (77 G>A and 962G>A) previously reported and ten mutations are new findings: five deletion, one insertion, one splicing defect, one nonsense and two missense. The 447-467del21bp causes the loss in frame of seven aminoacids in the exon 9 and the13890bp deletion causes the loss of the entire HMBS gene. The 181delG, the 418-419delAA, the 468-470delAA and 652insG mutations cause frameshift and protein truncation at aminoacids 96, 208, 207 and 249 respectively. The loss of exon 6 is due to the IVS5-1G>A splicing defect; the nonsense mutation (940C>T) in exon 15 is responsible for creation of a stop codon at aminoacid 314; two missense mutation (242T>C and 1075 G>A) in exon 6 and 15 result in a Leu 81Pro and Asp359Asn amino acid substitution respectively. *Summary.* These results allowed the identification of ten novel HMBS mutations. In a previous work, we have identified other 11 new molecular defects for a total of 21 new different mutations restricted to the Italian population. This study confirmed the high heterogeneity of molecular abnormalities responsible for AIP phenotype and the presence of clusters of mutations in particular geographic areas.

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ASSOCIATION OF HUMAN PLATELET ALLOANTIGENS 1, HPA2, HPA,3 HPA4, AND HPA5 ALLELES AND GENOTYPES WITH SICKLE CELL ANEMIA

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Background. Insofar as sickle cell anaemia (SCA) was described as a hypercoagulable state where occlusive vascular complications (OVC) and progression to stroke are frequently seen, polymorphisms of human platelet alloantigen (HPA) were reported as risk factors for several vascular anomalies, including stroke. With the exception of a lone report documenting association of HPA-5b with SCA OVC, studies on potential association of HPA1 through HPA5 with SCA are lacking. *Aims.* This study investigated the prevalence of HPA1, HPA2, HPA3, HPA4, and HPA5 alleles and genotypes among Bahraini SCA patients and control subjects. Linkage disequilibrium analysis will be used to investigate the disease association of the these polymorphisms. Method. This was a case control study. Study subjects comprised 135 SCA patients (mean age 15.8±9.8) and 187 healthy controls (mean age 27.8±15.1); all were Bahraini nationals. Mutation analysis was assessed by PCR-SSP analysis. Statistical analysis was performed on SPSS v. 13.0 statistics software, significance being set at $p < 0.05$. *Results.* The distribution of HPA2 (p=0.225) and HPA4 (p=0.075) genotypes were comparable between SCA patients and controls. In contrast, higher frequencies of HPA1a (Pc < 0.001), HPA3a (p=0.007) were found among controls, while HPA3b (p=0.034) and HPA5a (P < 0.001) alleles were more frequent in patients. Whereas HPA 3a/3a (p=0.036; RR = 0.463) and HPA 5b/5b (p<0.001; RR = 0.182) were more prevalent among controls, HPA 1b/1b (p<0.001; RR = 19.958), HPA 3b/3b (p=0.042; RR = 1.734), and HPA 5a/5b (p < 0.001; RR = 3.073) were significantly higher among SCA patients. Significant linkage disequilibrium were noted between HPA alleles, with the strongest occurring between HPA1b and HPA5a (_ = 0.119; p< 0.001). *Summary/Conclusion.* Differential association of HPA polymorphism with