IMPROVING DIAGOSTIC SKILLS FOR INHERITED THROMBOCYTOPENIAS

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The project was aimed at identifying tools and strategies for a correct diagnosis of inherited thrombocytopenias, a heterogeneous group of diseases at both clinical and molecular levels. In addition to diagnose known forms, it is fundamental to clone genes responsible for a series of novel nosological entities, which are relatively frequent being recognized in almost 50% of the families affected.

The results obtained are described according to the specific aims of the project as follows:

- *Identification and etiologic characterization of inherited thrombocytopenias not yet described*
  Two new genes, each responsible for novel forms of autosomal dominant thrombocytopenias, have been localized by linkage analysis on chromosome 10p and 17q. While the first gene called TCH2 is still being characterized as new families linked to 10p are recognized, within the candidate region 17q a novel mutation has been identified in ITGB3, a gene encoding for a subunit of the fibrinogen receptor.

- *Identification of genes responsible for inherited thrombocytopenias that have been described previously but whose etiology is still unknown*
  We have identified a family with a suspected diagnosis of "gray platelet syndrome". The clinical and morphological platelet features were characterized. A positional cloning strategy is being carried out to identify the gene. A few putative candidate regions are further being studied as new family members are enrolled to define the localization of the gene.

- *Characterization of the mutations causing inherited thrombocytopenia in Italy*
  We identified mutations of the c-MPL gene and clonal chromosomal anomalies in five families with congenital amegakaryocytic thrombocytopenia. Moreover, we have extended the database of Italian Registry of MYH9-Related Disease (MYH9RD) to 82 unrelated families, in 80 of which the diagnosis has been confirmed by molecular genetic testing. The screening of mutation allowed us to identify six novel mutations, extending the limited spectrum of mutations identified so far in the MYH9 gene. In order to validate an immunofluorescence test revealing the presence of MYH9 aggregates in patients' neutrophils as a suitable tool in differential diagnosis, we sequenced the entire MYH9 gene in 36 patients with the clinical features of MYH9RD but with a normal distribution of the protein. Since no mutation was detected, we concluded that neutrophil inclusions of MYH9 are a pathognomonic sign of the disorder and that at least another gene is responsible for a phenotype similar to MYH9RD. Moreover, we have excluded mutations of the cytochrome C (CYCS) gene in 70 patients with features similar to those observed in patients with a defective CYCS.

- *Identification of genotype/phenotype correlations in patients affected by diseases with known etiology and wide phenotypic variability*
  In 108 MYH9RD patients from 50 unrelated families we identified a significant correlation between phenotype and genotype at least for the most four common mutations affecting 70% of patients. Thus, the risk of developing kidney failure, cataracts, deafness, and severe bleeding tendency may be predicted. Since some drugs modify the clinical course of kidney damage, patients recognized at risk of renal failure could undergo treatments to prevent or postpone the dysfunction. The genotype and phenotype correlation was also performed in CAMT patients. In this study we did not confirm previous reports and suggested that hematopoietic stem cell transplantation should not be postponed even in those patients whose c-mpl mutations may predict residual activity of c-MPL.

Papers published within the project

De Candia E, Pecci A, Ciabattoni G, Decristofaro R, Rutella S, Yao-Wu Z, Lazzareschi I, Landolfi R, Coughlin S, Balduini CL. Defective platelet responsiveness to thrombin and protease-activated receptors agonists in a novel case of gray platelet syndrome: correlation between the platelet defect and the a-granule content in the patient and four relatives. *J Thromb Haemost* 2007;5:551-9.

Gresele P, Falcinelli E, Giannini S, D'Adamo P, D'Eustacchio A, Corazzi T, Mezzasoma AM, Di Bari F, Guglielmini G, Noris P, Balduini CL, Savoia A. Dominant inheritance of a novel integrin b3 mutation associated with a hereditary macrothrombocytopenia with platelet dysfunction in two Italian families. *Hematologica* 2009;94:663-9.

Maserati E, Panarello C, Morerio C, Valli R, Pressato B, Patitucci F, Tassano E, Di Cesare-Merlone A, Cugno C, Balduini CL, Lo Curto F, Dufour C, Locatelli F, Pasquali F. Clonal chromosome anomalies and propensity to myeloid malignancies in congenital amegakaryocytic thrombocytopenia. *Haematologica* 2008;93:1271-3.

Pecci A, Panza E, Pujol-Moix N, Klersy C, Di Bari F, Bozzi V, Gresele P, Lethagen S, Fabris F, Dufour C, Granata A, Doubek M, Pecoraro C, Koivisto PA, Heller PG, Iolascon A, Alvisi P, Schwabe D, De Candia E, Rocca B, Russo U, Ramenghi U, Noris P, Seri M, Balduini CL, Savoia A. Position of NMMHC-IIA mutations predicts the natural history of MYH9-related disease. *Hum Mutat* 2008;29:409-17.

Pecci A, Granata A, Fiore CE, Balduini CL. Renin-angiotensin system blockade is effective in reducing proteinuria of patients with progressive nephropathy caused by MYH9 mutations (Fechtner-Epstein syndrome). *Nephrol Dial Transplant* 2008;23:2690-2.

Savoia A, Del Vecchio M, Totaro A, Perrotta S, Amendola G, Moretti A, Zelante L, Iolascon A. An autosomal dominant thrombocytopenia gene maps to chromosomal region 10p. *Am J Hum Genet* 1999;65:1401-5.

Savoia A, Dufour C, Locatelli F, Noris P, Ambaglio C, Rosti V, Zecca M, Ferrari S, di Bari F, Corcione A, Di Stazio M, Seri M, Balduini CL. Congenital amegakaryocytic thrombocytopenia: clinical and biological consequences of five novel mutations. *Haematologica* 2007;92:1186-93.

Savoia A, Balduini CL. MYH9-Related Disorders. 2008. In: GeneReviews at GeneTests: Medical Genetics Information Resource. Copyright, University of Washington, Seattle, 1997-2008. Available at http://www.genetests.org

Savoia A, Noris P, Perrotta S, Punzo F, De Rocco D, Oostra BA, Balduini CL. Absence of CYCS mutations in a large Italian cohort of patients with inherited thrombocytopenias of unknown origin. *Platelets* 2009;20:72-3.