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Hemoglobin S haplotypes among sickle cell patients in the Puerto Rican population. C.R. Lopez[2], A. Rodriguez[2], E. Rivera-Caragol[2], P.J. Santiago-Borrero[2], and C.L. Cadilla[1]. University of Puerto Rico School of Medicine RCMI Human Molecular Genetics Unit and the Departments of Biochemistry[1] and Pediatrics, Sickle Cell Anemia Clinic, University Pediatric Hospital[2], PO BOX 365067, San Juan, P R 00936-5067

The Hb S mutation has been associated with four major haplotypes of the β-like gene cluster in the African continent, which include the following: haplotype #19 or Benin; #20 or Bantu, or Central African Republic (CAR); #3 or Senegal; and #17 or Cameroon. Neonatal screening in Puerto Rico in recent years has shown an overall incidence of abnormal hemoglobins of 2.24%, including 1.87% Hb S and 0.31% of Hb C. It has been postulated that certain unique mutant sites linked to the β gene cluster haplotypes might be responsible, at least partly, for the differences in the fetal globin gene expression and clinical severity observed among sickle cell patients. The purpose of our study was to identify the haplotypes present in Puerto Rican Hb S patients to better characterize sickle cell disease in Puerto Rico. Blood samples from twenty nine individuals with sickle cell disease and other sickling syndromes were collected at the Neonatal Hemoglobinopathy Screening Program Clinic. All blood samples were analyzed by cellulose acetate, citrate agar and isoelectric focusing electrophoresis. β-globin gene haplotypes were analyzed by allele-specific oligonucleotide (ASO) probe hybridization and/or by PCR RFLP. The predominant haplotypes for the forty eight β^s chromosomes analyzed were the haplotype #19 (33%), and haplotype #20 (33%). For the haplotype #3,31 mutation, 12.5% of the chromosomes presented the mutation. Mutations characteristic for haplotypes #10 and #11 were 8.34% of β^s chromosomes while 12.5% were mixed or other haplotypes. Comparing Puerto Rico with other countries, we have a prevalence of the Benin and Bantu haplotypes, differing with the United States prevalences in which more than 50% are Benin haplotypes. Our haplotype frequencies are similar to those found in a recent study from Cuba. The predominant haplotype in the SS population we studied (#19/20) is associated with a moderate clinical condition. In our studies a strict correlation with clinical severity and β^s haplotypes does not exist in all of the SS patients analyzed. We acknowledge support from NIH-RCMI G12-RR-03051 and the UPR School of Medicine.

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A new gene for dyslexia (DYX3) is located on chromosome 2. *H.A. Lubs[1,4], R. Raeymaekers[2], F.E. Toennessen[3], M. Pedersen[1], L. Tranebjaerg[1], T. Fagerheim[1].* 1) Dept. Medical Genetics, University Hospital of Tromso, Tromso, Norway; 2) Neurogen. Lab., university of Antwerp, Antwerp, Belgium; 3) Center for Reading Research, Stavanger, Norway; 4) Dept. Pediatrics, University of Miami, Miami, FL.

Dyslexia is usually defined as persistent difficulty in reading and spelling in the absence of any neurologic or other causes, in an individual with normal intelligence and adequate schooling. Difficulties in spelling and decoding may persist through adult life. The prevalence may be as high as 5-10%, but these figures probably include many types of learning disorders. Currently, there is no single test which permits a clear diagnosis of dyslexia. Over the last 15 years, possible localizations of genes for dyslexia have been reported on chromosome 15(DYX1), 6p21.3-23(DYX2), and 1p. We have investigated a large Norwegian family in which dyslexia is inherited as an autosomal dominant trait. A genome-wide search for linkage was initiated in 36 of the 80 family members and a region in 2p15-p16 was identified which cosegregated with dyslexia. Maximum lod scores of 3.54, 2.92 and 4.32 for three different diagnostic models were obtained. These results were confirmed by a non-parametric multipoint GENEHUNTER analysis in which the most likely placement of the gene was in a 4 cM interval between markers D2S2352 and D2S1337. Haplotype analysis showed that reading disability in this large kindred was due to another cause (most likely ADHD) in 2 children,. This is the first genome search that has been carried out in dyslexia. Isolation of DYX3 will give a new and exciting insight into the processes involved in reading and spelling.

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Characterization of the Hermansky-Pudlak Syndrome in the Puerto Rican population. A.E. Maldonado-Valentin[1], P.J. Santiago-Borrero[2], A. González[3], R.A. Spritz[4], J. Oh[4] and C. Cadilla[1] Depts. of [1]Biochemistry, [2]Pediatrics and [3]Pathology; UPR School of Medicine, San Juan, PR 00936 and [4]Dept. of Medical Genetics, University of Colorado, CO

Hermansky-Pudlak Syndrome (HPS) is an autosomal recessive disorder consisting of a triad of tyrosinase-positive albinism, an accumulation of ceroid in tissues and a bleeding tendency due to storage pool deficient platelets. HPS is frequently found in the Puerto Rican population and a village of the Swiss Alps. Dense bodies analysis is considered the best way to diagnose this condition, because the majority of the HPS patients have a disminished number of granules on platelets, if they are not completely absent. In 1996 HPS gene was identified by linkage disequilibrium mapping and positional cloning. A 16 bp duplication leads to a frameshift mutation on exon 15 in the Puerto Rican patients. Frameshift mutations have also been found in Swiss, Irish and Japanese HPS patients. We have screened a total of 63 Puerto Rican patients for the 16 bp duplication and identified 30 (47.6%) positive homozygotes, 29 (46.0%) negative homozygotes and 4 (6.3%) heterozygotes. Based on the molecular and clinical data, we selected 8 duplication-negative patients whose bleeding times were higher than 15 minutes to perform exon screening looking for other mutations in the HPS gene. We also gathered additional clinical data available and patient family history. None of these patients had mutations in the HPS gene when they were screened by non radioactive SSCP and DNA sequencing. Electron Microscope analysis of platelets from some of the duplication-negative patients revealed a wide spectrum of dense bodies abundancies ranging between 2 to 8 dense bodies. We are currently screening these patients to find a second HPS gene in the Puerto Rican population. We acknowledge support from NIH RCMI-G12 RR 0305, NIH-R01-AR-39892, and UPR School of Medicine.

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The reason of non-specific background of methodologies for random mutation identification: χ-structures formation in purified PCR products solutions. A.A. Neschastnova, M.G. Yakubovskaya, Z. Lipatova, V.I. Popenko, G.A. Belitsky. Lab. Carcinogen Screening Methods, Cancer Research Center of RAMS, 1 - Engelgard Institute of Molecular Biology, Moscow, Russia.

Chemical or Enzyme Mismatch Cleavage represent the methodologies directed to point random mutation detection. They are based on the formation of heteroduplexes consisting of one wild-type and one mutant strand from PCR-products. During mutation detection analysis, high nonspecific background prompted a detailed investigation of the DNA used in the study. The phenomenon of diverse DNA structures of double size of PCR product approximately and of higher molecular weight appearing in concentrated solutions of purified DNA fragments was demonstrated for 5 PCR products differing both by size and sequence (various genes PCR products from 118 bp to 1249 bp). The present investigation on the model of p53 cDNA 1249 bp fragment confirm that the diverse DNA structures are formed by double stranded DNA fragments via S1-nuclease analysis: treated by the enzyme DNA fragments retain the ability to form higher molecular weight structures. Using the number of following purification procedures it was demonstrated, that the phenomenon is due to interaction of purified ds DNA fragments and is not connected with the any contamination of standard PCR products. The electroellution of twice the size DNA structures from agarose gel followed by electron microscopy reveals the presence of χ-structure in this fraction of DNA. In our investigation the ability of DNA to interact with formation of Holliday junction, the intermediate structures of the recombination process, without participation of cell proteins was demonstrated as it own phisico-chemical characteristic. Our data are significant for optimization of methodologies of CMC or EMC or other analysis using concentrated PCR product solutions via preventing of formation of complex DNA structures.