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New point mutation in the *RET* oncogene among an African American kindred with MEN II-A in Puerto Rico. C.L. Cadilla[1], G. Vázquez[2], A. Alcántara[2], J.Y. Renta[1], F. Aguiló[2]. Depts. of Biochemistry[1] & Endocrinology[2], Univ. of Puerto Rico, School of Medicine, San Juan, P.R. 00936

MEN II A was described by Sipple 3 decades ago. Since then its autosomal dominant nature has been well established, usually presenting first with medullary thyroid carcinoma (MTC), whose C cells produce calcitonin. Hyperparathyroidism and pheochromocytomas complete the triad. Most reports are among White Caucasian patients, especially in Europe. Our experience suggests that MEN II A is uncommon among African Americans. For the past 22 years we have studied the kindred whose propositus (II-1) had MTC and whose mother (I-4) had the complete triad when recalled for medical check up. She had 12 children, 6 of which were diagnosed with MTC via elevated TCT levels. Seven out of 12 have undergone MTC removal and Hyperparathyroidism, while Pheochromocytomas have been identified in 3 out of 12. We wanted to established whether the same cysteine-rich region of exons 10-11 in the *RET* oncogene were responsible, or whether this African American family had mutations in other exons, such as exon 15. By DNA sequencing of PCR products of the exon 10 and 11 regions of the *RET* gene in this kindred we were able to identify one previously unknown exon 10 mutation, an Asp→Asn mutation in codon 624. Most of the previously reported ret gene mutations associated with MEN II A are located in the amino acid 609-635 region, of the *ret* protein, including the mutation found in this kindred. This extracellular region of the *ret* protein has been implicated in >90% of the studies of affected MEN II A families. MEN II A kindreds of African American origin might have unusual point mutations in the *RET* gene. This could have clinical relevance in screening young members of such families in order to prompt early therapeutic intervention. We acknowledge financial support from the NIH-RCMI-G12RR03051, NIH-MBRS S06GM08224 and the UPR School of Medicine.

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The Fragile X syndrome in patients with mental retardation of unknown cause in Mexico. A. González-del Angel[1,2], S. Vidal[1], Y. Saldaña[1], V. Del Castillo[1], L. Orozco[1]. Departamento de Genética, Instituto Nacional de Pediatría[1], Programa de Biomedicina Molecular, CINVESTAV-IPN[2], México.

The Fragile X syndrome (Fra-X) is characterized by mental retardation, macroorchidism and characteristic facial features, it is associated with the folate sensitive fragile site FRAXA at Xq27.3. The disease results from lack of expression of the FMR1 gene and in the vast majority of cases the mutation is an expansion of a CGG trinucleotide repeat localized in the 5' untranslated region. In the National Institute of Pediatrics in Mexico City, there are many patients who are considered to have mental retardation of unknown cause (MRUC) after a multidisciplinary and exhaustive study. Some of them have clinical manifestations of Fra-X, although in 25 years, diagnosis has been confirmed with cytogenetic study in only five cases. This may be was due to a low frequency of Fra-X in our population or to a misdiagnosis because molecular techniques were not previously available.

The purpose of this paper was to establish the frequency of Fra-X in a group of patients with MRUC by the molecular analysis of the FMR-1 gene.

Forty pediatric patients of both sexes and their first-degree relatives were included. The CGG trinucleotides were analyzed with PCR and Southern blot. Two of our patients had an expansion of the trinucleotides and it was interesting that one of them had not somatic alterations of the disease and mental retardation was the unique clinical manifestation of Fra-X. We demonstrated by Southern blot that both mothers had a premutation and the brother of one of the index cases was also affected. In our cases two patients had a family history and clinical manifestations which strongly suggested Fra-X but it was not corroborated with this molecular study. Our results confirm that the molecular analysis of the FMR-1 gene is necessary for a correct diagnosis of Fra-X and suggest that this disease is the cause of mental retardation in 5% of the patients considered to have MRUC in our population. Partial supported by CONACYT: A. González-del Angel 90158.

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Mutation analysis of the fragile histidine triad gene transcripts of primary tumors and unaffected tissue using restriction nuclease fingerprinting and sequencing. M. Kaelbling. Univ. of Mississippi Medical Center, Jackson, MS.

The fragile histidine triad gene (FHIT) is a putative tumor suppressor gene (TSG) that spans the fragile chromosomal site FRA3B at 3p14.2. To elucidate the role of this TSG in tumorigenesis, the transcripts of 81 primary neoplasias of various tumor types and 10 benign tumors (leiomyoma uteri) are being compared to those of constitutive tissue of the same patients. Total RNA is extracted from all samples and reverse-transcribed into cDNA. Exons 3–10 including the translated exons 5–9 are PCR-amplified. The transcripts of each tumor/constitutive panel are first quantified and analyzed for size differences of > 50 bp in agarose gels. In one of 71 panels analyzed (1/71), the tumor FHIT was underexpressed compared to actin while 6/71 yielded size differences within the panel. Next, the transcripts are compared by restriction endonuclease fingerprinting (REF); using specific conditions, this modified SSCP technique allows detection of any nucleotide change. Of 45 tumor panels analyzed by REF, 39 contained mutations: 5/5 breast, 9/9 colon, 2/3 endometrial, 4/5 kidney, 1/1 liver, 3/3 lung, 3/3 ovarian, 3/4 skin, 4/4 stomach, 1/1 Wilms' and 4/7 leiomyoma uteri. Samples that revealed mutations are being sequenced. At least one breast cancer had lost the entire exon 4 and 11bp of the 5' end of exon 10, the same deletion as was reported missing in a lung cancer cell line. Combined results to date suggest that FHIT is nonrandomly mutated in breast as well as colon cancer and, most likely, in many other tumor types. Future work will include an analysis of underexpressed transcripts for transcriptional inactivation by hypermethylation.

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Molecular analysis of human *Jagged1* gene in an Indian family with Alagille syndrome. P.S. Lai, F.S.H. Cheah, M.H. Liew, M.M. Aw and S.H. Quak. Department of Paediatrics, National University of Singapore, Singapore 119074.

Alagille syndrome (AGS), a multisystem developmental disorder, is characterized by intrahepatic cholestasis and abnormalities of heart, eye and vertebrae and a characteristic facial appearance. The AGS phenotype has been associated with mutations in the *Jagged1* gene (*JAG1*) which encodes a ligand in the Notch signalling pathway that is important in cell-fate determination in a number of tissues.

We report an Indian family with a two year old male proband presenting severe AGS with marked hypercholesterolemia while the mother presented only characteristic facial phenotype but normal liver and kidney function. Mutation screening of all 26 exons of *JAG1* identified a 2841insA mutation in exon 21 (codon 824) in the patient. This mutation is predicted to cause a premature termination of translation of protein within the same codon. The mutated termination codon occurs within the EGF-like repeats domain of the JAG1 protein. This domain is highly conserved between *Drosophila*, rat and human, and is thought to be one of the regions which play a critical role in ligand-receptor interactions. Direct sequencing of genomic DNA from the mother of the patient failed to reveal the presence of the 2841insA mutation. As the 2841insA mutation abolishes a cutting site for *Bsm*I, this restriction enzyme was also used to confirm the absence of this mutation in the mother. Our data show that that factors not attributable to the specific 2841insA mutation must play a role in determination of the subclinical AGS phenotype seen in the mother.