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INTRODUCTION: Noonan syndrome (NS) is an autosomal-dominant disorder that presents with a characteristic face, short stature, skeletal anomalies, and congenital heart defects. Protein-tyrosine phosphatase nonreceptor-type 11 (*PTPN11*), encoding SHP-2, mutation was the first reported gene involved and accounted for 31% to 60% of cases of NS. The *KRAS* gene was the second reported gene and was recently identified in a small number of patients with NS.
OBJECTIVE: Our goal was to perform mutational analysis of *PTPN11* and *KRAS* genes in children with NS.
METHODS: In this study we screened for mutation of the *PTPN11* and *KRAS* genes in 73 Taiwanese patients with NS. The mutation analysis of the 15 coding exons and exon/intron boundaries was performed by polymerase chain reaction and direct sequencing of the *PTPN11* gene. The mutation analysis of 5 coding exons and exon/intron boundaries was performed by polymerase chain reaction and direct sequencing of the *KRAS* gene. We identified 12 different missense *PTPN11* mutations in 15 (21%) patients with NS and 2 different missense *KRAS* (V14I and I36M) mutations in 2 (3%) patients with NS. These *PTPN11* gene mutations were clustered in exon 3 ($n = 6$) encoding the N-SH2 domain and 13 ($n = 5$) encoding the PTP domain.
CONCLUSIONS: This study provides support that *PTPN11* and *KRAS* mutations are responsible for NS in Taiwanese patients.

SCREENING OF MUTATIONS IN THE *NPHS2* GENE IN GREEK PATIENTS WITH AUTOSOMAL-RECESSIVE STEROID-RESISTANT NEPHROTIC SYNDROME

Submitted by Spyridon Megremis

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INTRODUCTION: Mutations in the *NPHS2* gene, encoding podocin, are a major cause of autosomal-recessive steroid-resistant nephrotic syndrome (SRNS) in childhood and have been observed in 6.4% to 30% of sporadic and 20% to 40% of familial cases.
OBJECTIVE: We investigated mutations in the coding region of the *NPHS2* gene in Greek patients with SRNS and identified a novel A295T mutation.
METHODS: The study included 16 child patients with SRNS (14 families); 11 cases were sporadic, and 5 (from 3 families) were familial. All 8 exons of *NPHS2*, including intron boundaries, were screened for sequence variations by using denaturing gradient gel electrophoresis followed by specific characterization using direct DNA sequencing.
RESULTS: The results revealed 2 pathogenic genotypes in 2 patients with sporadic SRNS (R138Q/R138Q and R229Q/A295T). In addition, 3 previously described *NPHS2* intronic polymorphisms (IVS3–46C→T, IVS3–21C→T, and IVS7+7A→G), 1 thus-far-unreported intronic variant (IVS3–17C→T), and 4 known silent mutations (G34G, S96S, A318A, and L346L) were detected in sporadic and familial cases as well as in healthy controls.
CONCLUSIONS: These findings indicate that *NPHS2* mutations are not a frequent cause of familial SRNS in Greek patients. Among patients with sporadic SRNS, the genotypes R138Q/R138Q and R229Q/A295T account for an allelic frequency of 18.2%. The R138Q mutation is well characterized. The novel mutation, A295T (883G→A), is predicted in silico to cause a structural alteration in the cytoplasmic domain of podocin (see the PolyPhen database at http://genetics.bwh.harvard.edu/pph). This is the first report of *NPHS2* mutations in the Greek population and the first description of the A295T amino acid substitution.

CLINICAL STUDIES AND ANALYSIS OF THE RETT SYNDROME GENE (*MECP2*) IN CHILDREN WITH MENTAL RETARDATION IN THE GREEK POPULATION

Submitted by Stavroula Psoni

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INTRODUCTION: Mutations in the methyl CpG-binding protein 2 (*MECP2*) gene are responsible for 70% to 95% of cases of Rett syndrome (RS), an X-linked dominant neurodevelopmental disorder that mostly affects girls. Classical RS is characterized by normal early development followed by psychomotor regression and gradual onset of microcephaly, although variable atypical forms have also been observed. *MECP2* has also been implicated in a variety of other mental retardation (MR) phenotypes, including X-linked MR, fragile X syndrome–like and Angelman syndrome (AS)–like phenotypes.
OBJECTIVE: Our goals were to evaluate the incidence and spectrum of *MECP2* mutations in children with RS

and atypical MR and to correlate the phenotype and genotype.

METHODS: Exons 3 and 4 of the *MECP2* gene were analyzed by using denaturing gradient gel electrophoresis, sequencing, and gap polymerase chain reaction for (1) 124 children with FXS-like symptoms (102 boys, 22 girls) and 41 children with AS-like symptoms (14 boys, 27 girls) who tested negative for gene variation at the FXS and AS loci, respectively, (2) 23 girls with classical RS and 25 girls with atypical RS, and (3) 11 boys who were referred with possible RS. Statistical analysis (*t* and nonparametrical tests) included correlation of RS clinical severity score (Kerr, 2001) with *MECP2* mutations and frequency of *MECP2* mutations in the various patient categories.

RESULTS: Mutations were detected in 78.3% of classical and 20% of atypical RS cases, respectively. One boy carried the p.R106W mutation, and another boy showed a large rearrangement that required further characterization. Among AS- and FXS-like cases, 7.3% and 2.4% had *MECP2* mutations, respectively, including an X-linked MR case.

CONCLUSIONS: *MECP2* gene analysis provides an appropriate diagnostic tool for RS and contributes additional information for research into MR.

Hematology and Oncology

ASSESSMENT OF BONE MINERAL DENSITY AND MARKERS OF BONE TURNOVER IN CHILDREN UNDERGOING LONG-TERM ORAL ANTICOAGULANT THERAPY

Submitted by Maria Avgeri

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INTRODUCTION: Oral anticoagulants antagonize vitamin K action and potentially impair the carboxylation of osteocalcin, a protein that is essential for normal bone matrix formation.

OBJECTIVE: Our aim was to evaluate bone mineral density (BMD) and bone-turnover markers in 23 children who were undergoing long-term oral anticoagulant therapy (median age: 4 years) and 25 age- and gender-matched controls.

METHODS: BMD (characterized as a *z* score) of the lumbar spine was assessed by using dual energy radiograph absorptiometry. Osteoblast (bone alkaline phosphatase, osteocalcin, and amino-terminal procollagen 1 extension peptide) and osteoclast (urinary calcium and deoxypyridinoline and serum cross-linked C telopeptide) activity markers were measured. Vitamin D (25-hydroxy vitamin D, parathyroid hormone, whole and ionized calcium, phosphorus, and magnesium) and vitamin K (factors II, VII, IX, and X, protein C, protein S, and undercarboxylated osteocalcin [Glu-Oc]) statuses were determined.

RESULTS: Patients presented with higher levels of Glu-Oc, parathyroid hormone, and bone-resorption markers and lower levels of bone-formation markers and 25-hydroxy vitamin D; 52% of them showed signs of osteopenia ($-1.0 >$ BMD *z* score > -2.5). Statistical analysis demonstrated that anticoagulant therapy was an independent predictor of alterations in Glu-OC, osteocalcin, bone alkaline phosphatase, amino-terminal procollagen 1 extension peptide, and serum cross-linked C telopeptide levels.

CONCLUSIONS: Long-term use of coumarin derivatives may cause osteopenia in children with the risk of developing osteoporosis later in life.

IN VITRO ASSESSMENT OF MESENCHYMAL STROMAL CELL CHARACTERISTICS: IMPLICATIONS FOR THEIR CLINICAL USE

Submitted by Helen Dimitriou

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INTRODUCTION: Bone marrow (BM) stroma represents a source of progenitor stromal cells, termed mesenchymal stromal cells (MSCs), which are multipotent and can differentiate into cartilage, bone, and adipose tissue. Several questions have arisen regarding their long-term expansion and their safety before use.

OBJECTIVE: Our goal was to assess the long-term expansion and safety of MSCs in clinical practice.

METHODS: MSCs from BM of children with benign hematologic disorders and solid tumors without BM involvement were isolated and cultured for 10 consecutive passages (P). Immunophenotypic and functional characteristics, apoptosis, and the expression of cell cycle regulatory genes (*p53*, *p16*, and *Rb*) and signal transduction genes (*H-Ras*) involved in oncogenesis were assessed.

RESULTS: MSCs expressed mesenchymal-related surface antigens, >85% from P1. They had the ability to differentiate into osteocytes, adipocytes, and chondrocytes (reverse-transcription polymerase chain reaction). Colony forming units (fibroblast) ranged from 40.71 ± 4.3 at P1 to 15.5 ± 6.7 at P10. Their doubling time was 2.01 ± 0.14 days at P1 and 3.5 ± 1.19 days at P9. A low

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