

plasia without typical hand anomalies in three inbred families was subsequently reported. We report a new family of at least four siblings and one first cousin affected with a lethal Desbuquois-like dysplasia with typical hand abnormalities. Further genomic analysis excludes linkage to chromosome 17q25.3 in this family.

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**SPLIT HAND-SPLIT FOOT MALFORMATION 3 AT 10Q24: CLINICAL AND LABORATORY DIAGNOSIS AND GENE SEARCH.** C.D. DeLozier,<sup>1,2</sup> R. Lyle,<sup>2</sup> U. Radhakrishna,<sup>2</sup> C. Schwartz,<sup>3</sup> S.E. Antonarakis,<sup>2</sup> J.L. Blouin,<sup>2</sup> Genetic Medicine Central California and UCSF-Fresno; <sup>2</sup>Department of Genetic Medicine and Development, University of Geneva, Switzerland; <sup>3</sup>JC Self Research Institute, Greenwood Genetics Center, Greenwood, SC. Split hand/split foot disorder (SHFM, also called ectrodactyly) is a clinically and genetically heterogeneous group of congenital limb malformations, which includes syndromic and nonsyndromic forms. The typical limb defect is a deep median cleft due to absence of the central rays, affecting from one to all four members, with feet more often and more significantly affected than hands. SHFM can, however, present with a range of malformations of highly variable severity even within a family. The inheritance pattern in most families is autosomal dominant, with variable penetrance. However, autosomal recessive and X-linked forms have also been described. Five SHFM loci have been mapped: SHFM1 (7q21), SHFM2 (Xq26), SHFM3 (10q24), SHFM4 (3q27), and SHFM5 (2q31). To date mutations in a specific gene (TP63) have been identified only for SHFM4. We have studied three large families with SHFM3, showing the classical autosomal dominant transmission and variable severity. After first mapping these families to 10q24, we have worked on defining the mechanism of gene disruption, which can be shown by pulsed-field gel electrophoresis to be caused by an approximately 500 kb DNA rearrangement. This region contains a number of candidate genes for SHFM3, but the gene or genes involved in the pathogenesis of SHFM3 are not yet known. We have developed a diagnostic test for SHFM3 that uses two different techniques, FISH and quantitative PCR, to show that SHFM3 is caused by a minimal 325 kb duplication containing only two genes (BTFC and POLL). The data presented provide improved methods for diagnosis and begin to elucidate the pathogenic mechanism of SHFM3, as at least 2 genes of 13 studied from the 10q24 region are overexpressed in SHFM3 patients as compared to controls.

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**INTRACELLULAR GLUTATHIONE LEVELS ARE LOW IN PERIPHERAL BLOOD CELLS IN PATIENTS WITH PRIMARY OR SECONDARY MITOCHONDRIAL DYSFUNCTION.** K.R. Atkuri,<sup>1</sup> L.A. Herzenberg,<sup>1</sup> G.M. Erns,<sup>2</sup> Department of Genetics and <sup>2</sup>Department of Pediatrics, Division of Medical Genetics, Stanford University, Stanford, CA.

**Introduction:** Both primary and secondary mitochondrial respiratory chain dysfunction result in generation of abnormal levels of free radicals that deplete intracellular stores of glutathione. The glutathione system is the main natural defense mechanism that helps to combat oxidative damage. We report the intracellular reduced glutathione (iGSH) levels in patients with either primary mitochondrial disease or organic acidemias. **Methods:** Thirty-five subjects, classified as having either known electron transport chain defects (eg, specific complex deficiencies, pathogenic mtDNA mutations) ( $n = 17$ ), unknown mitochondrial disease ( $n = 9$ ), or organic acidemias ( $n = 9$ ) were studied. Peripheral blood buffy coats were treated with monochlorobimane (MCB), then stained for leukocyte subset markers and subjected to Hi-D fluorescence activated cell sorting (FACS). **Results:** *Primary mitochondrial disorders:* When compared to normal controls ( $n = 20$ ), iGSH levels in CD4 T cells (MCB ratio  $1.38 \pm 0.32$  v.  $1.66 \pm 0.27$  in controls,  $p = .004$ ), CD8 T cells (MCB ratio  $1.29 \pm 0.29$  v.  $1.52 \pm 0.21$  in controls,  $p = .001$ ), B cells (MCB ratio  $0.65 \pm 0.2$  v.  $0.98 \pm 0.2$  in controls,  $p = .03$ ), and monocytes (MCB ratio  $0.96 \pm 0.18$  v.  $1.25 \pm 0.2$  in controls,  $p = .04$ ) were significantly lower in mitochondrial disease subjects who were not on antioxidants. No significant difference in either T cell subtype was seen in those taking antioxidants when compared to normal controls. iGSH levels in neutrophils tended to be lower in patients that were not on antioxidants ( $p = .057$ ). *Organic acidemias:* When compared to normal controls ( $n = 20$ ), iGSH levels in CD4 T cells (MCB ratio  $1.12 \pm 0.15$  v.  $1.69 \pm 0.27$  in controls,  $p = .002$ ), CD8 T cells (MCB ratio  $1.04 \pm 0.20$  v.  $1.52 \pm 0.21$  in controls,  $p = .003$ ), neutrophils (MCB ratio  $0.69 \pm 0.24$  v.  $1.00 \pm 0.24$ ,  $p = .044$ ), and monocytes (MCB ratio  $0.90 \pm 0.28$  v.  $1.22 \pm 0.25$ ,  $p = .045$ ) were significantly lower in subjects with organic acidemias. No significant difference in iGSH levels was observed in B cells. **Conclusions:** T cells (CD4 and CD8 subsets), neutrophils, and monocytes may have lower iGSH levels in mitochondrial disorders and organic acidemias. In subjects taking antioxidants, there appears to be sparing of iGSH levels. The significance of the selective low iGSH levels in T cell subsets when compared to B cells is unclear. Given that T cell iGSH levels tend to be low in mitochondrial disorders and organic acidemias, it is reasonable to explore the therapeutic effect of N-acetylcysteine in these disorders.

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**MUTATIONAL ANALYSIS OF PATIENTS WITH NONKETOTIC HYPERGLYCEINEMIA AND THE IDENTIFICATION OF LARGE DELETIONS IN THE GLDC GENE USING A NOVEL DETECTION SYSTEM.** L.N. Puls, G. Scharer, J.L. Van Hove, University of Colorado Health Sciences Center, Aurora, CO.

Nonketotic hyperglycinemia (NKH) is an autosomal recessive disorder of glycine metabolism caused by a defect in the glycine cleavage system and characterized by neurological disease. Severity of presentation varies considerably and, to date, prognosis is entirely based on extent of symptoms. We present a molecular study of 35 patients diagnosed with NKH. Mutations in either the glycine decarboxylase (GLDC) or the aminotransferase (AMT) protein of the cleavage system were identified in all 35 patients, many of which were novel mutations. We propose here that prognostic outcome in NKH patients is rooted in the nature of the mutations in two key genes. Through molecular analysis of the AMT and GLDC genes in both patients and parents, we can utilize genotypic information to help predict severity of outcome. In concordance with this goal, we have also designed a method of deletion detection for exon 3 of GLDC. Deletions encompassing in this exon have been identified in many NKH patients. However, compound heterozygosity precludes these deletions from detection by direct sequencing. Our detection system is based on real-time, quantitative PCR and utilizes the pseudogene to GLDC as an internal control. This technique has allowed identification of 8 individuals possessing a large deletion in GLDC. Pedigree and Southern blot analysis verify these results. An increased ability to detect mutation and predict phenotype will aid in identifying patients who would benefit from available

treatment. Since decisions to abort or continue pregnancy may depend on predicted severity, molecular analysis will greatly aid in prenatal testing options as well.

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**DNA HYPOMETHYLATION AND NEURONAL SURVIVAL.** A. Thaker, L. Hutnick, G. Fan, Department of Human Genetics, UCLA, Los Angeles, CA. DNA cytosine methylation in vertebrate animals is a major epigenetic modification involved in gene regulation, genomic imprinting, and X-inactivation during development. To understand the role of DNA methylation in CNS development, specific conditional knockout mice for the maintenance enzyme DNA methyltransferase 1 (Dnmt1) were previously generated using the cre/loxP system. Linking cre recombinase to the Emx-1 promoter restricted the deletion to pallial cortical precursors that give rise to the neocortex and hippocampus. Cre/LoxP-mediated Dnmt1 deletion occurred in precursor cells between E9–12, resulting in hypomethylated postmitotic cortical and hippocampal neurons during migration. Massive neuronal degeneration was evident upon gross observation with a dramatic reduction of cortical and hippocampal structures. TUNEL staining suggests that hypomethylated cortical neurons and precursor cells undergo apoptotic cell death at around E14. It has also been previously reported that Dnmt1 deletion in primary fibroblasts results in cell death via a p53-mediated pathway. This study sought to investigate if a similar, p53-dependent mechanism is involved in the cortical and hippocampal cell death observed in the aforementioned Dnmt1 conditional knockouts. Western blot analysis failed to show induction of an active, serine-phosphorylated (S15) form of p53 in E15 mutant cell lysate. However, preliminary data from quantitative, real-time PCR reveal that there is an induction of p53 transcript and its downstream product p21 in mutant cDNA at E13.5. Additional investigation is required to determine which active, phosphorylated form of p53 is up-regulated in Dnmt1-deficient apoptotic neurons and which additional downstream targets of p53 are involved.

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**DISCORDANT MONOZYGOTIC TWINS WITH WILDERVANCK SYNDROME: A PROPOSED MODE OF INHERITANCE.** N. Vatanavicharn,<sup>1</sup> J.M. Graham Jr,<sup>1</sup> K. Dawson,<sup>1</sup> J. Kohlase,<sup>2</sup> Department of Pediatrics/Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; <sup>2</sup>Institute of Human Genetics and Anthropology, Freiburg University, Germany.

Wildervanck syndrome or cervico-oculo-acoustic syndrome is a recognizable malformation syndrome with major clinical features including Klippel-Feil sequence, Duane retraction syndrome, hypoplastic thumbs, and sensorineural hearing loss. Affected individuals with these findings must be differentiated from other malformation syndromes, including Townes-Brocks syndrome, Okhiro syndrome, Goldenhar syndrome, and MURCS association. Most previous reported cases with Wildervanck syndrome have been sporadic and female, although a few males with the condition have been described. Several modes of inheritance have been proposed, but X-linked inheritance appears most likely. We report an 18-year-old female with Wildervanck syndrome and her unaffected monozygotic twin sister. She was the product of a twin pregnancy. She and her twin sister were born to her 23-year-old G1 mother and 25-year-old father after an uncomplicated pregnancy. The neonatal course was uneventful. She was previously diagnosed with Goldenhar syndrome based on the clinical features of a right epibulbar dermoid, small and dysplastic ears with bilateral preauricular tags, mild facial hypoplasia of the right, butterfly vertebrae. She has profound sensorineural hearing loss on the right and mild-moderate mixed hearing loss on the left. She has poor vision on the right, but left eyesight is normal. Development of gross motor skills was normal, although she had difficulty with balance. Her speech was delayed. Skeletal anomalies include hypoplastic thumbs with ankylosis, progressive scoliosis, congenital fusion in the lower cervical spine C5 and C6, and butterfly vertebrae in the upper thoracic spine. She also has Duane retraction syndrome. She had been clinically diagnosed with Okhiro syndrome, but complete SALL4 gene sequencing was not able to identify a mutation, and molecular analysis confirmed monozygosity. We propose that this female has Wildervanck syndrome with unaffected identical twin sister. Skewed X inactivation might explain discordance in the monozygotic twins, suggesting X-linked dominant inheritance. Review of the literature on Okhiro syndrome and Wildervanck syndrome will be included.

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**EXPANDING THE PHENOTYPE OF MOSAIC TRISOMY 20.** M.J.H. Willis,<sup>1</sup> L.M. Bird,<sup>1,3</sup> M. Dell'Aquila,<sup>1,2</sup> M.C. Jones,<sup>1,3</sup> Department of Pediatrics, <sup>2</sup>Department of Medicine, University of California, San Diego; <sup>3</sup>Children's Hospital San Diego.

Mosaic trisomy 20 is one of the more common cytogenetic abnormalities found on amniocentesis or chorionic villus sampling. Studies have shown that outcome is normal in 90–93% of prenatally diagnosed cases. There are, however, reports in the literature of children with mosaic trisomy 20 described as having an assortment of dysmorphic features and varying levels of developmental delay. Unfortunately, the literature has not defined a specific phenotype for this entity. Here we report three patients diagnosed prenatally with mosaic trisomy 20. Over a number of years of follow-up it has become apparent that there are some striking similarities among the three. Comparison between our patients and the literature cases suggests a more consistent phenotype than has previously been suggested. Recurring features include spinal abnormalities (including spinal stenosis, vertebral fusion, and kyphosis), hypotonia, lifelong constipation and possible colonic aganglionosis, sloped shoulders, and significant learning disabilities despite normal intelligence. These findings may be overlooked on routine history and physical exam or assumed to be standard pediatric problems. It is not our intention to suggest that there is a distinctive face for this entity but to suggest that a subtle phenotype does exist. We have attempted to identify a set of findings for which any child diagnosed with mosaic trisomy 20 should be assessed or followed even in the presence of an apparently normal physical exam at birth.