

**DEVELOPMENT/AGING
ORAL PRESENTATION****WEDNESDAY NOVEMBER, 15****9.15AM - 9.30AM****O27****NEURAL CREST DEFICIT IN CRANIOFACIAL PRECURSORS OF DOWN SYNDROME MICE**RJ Roper¹, RH Reeves²¹Indiana University-Purdue University Indianapolis, Indianapolis, IN, United States, ²Johns Hopkins University School of Medicine, Baltimore, MD, United States

Trisomy 21 results in phenotypes collectively referred to as Down syndrome (DS) including facial dysmorphology, a distinguishing feature found in all DS individuals. Because several tissues affected in DS, including craniofacial skeleton, have a neural crest cell (NCC) component, it has been hypothesized that trisomy 21 causes a defect in NCC. Ts65Dn mice are trisomic for orthologs of about half of the genes found on human chromosome 21 and exhibit craniofacial abnormalities that specifically correlate to those seen in individuals with DS. To test whether trisomy affects NCC and contributes to the etiology of DS craniofacial dysmorphology, we crossed Ts65Dn mice to mice homozygous for the *Wnt1-lacZ* transgene that is expressed in NCC at embryonic day 9.5. At later somite stages, unbiased stereology revealed a significantly reduced NCC number and volume of the 1st branchial arch (BA1), the mandibular precursor, in Ts65Dn vs. euploid littermates. Fewer NCC were found between the neural tube and BA1 and fewer mitotic cells were found in the BA1 of Ts65Dn compared to euploid embryos suggesting deficits in NCC generation, migration, and/or proliferation. Additionally, a reduction in BA2 NCC in Ts65Dn embryos suggests a generalized NCC deficit is associated with trisomy. We have developed a neural tube culture assay to assess the intrinsic and extrinsic cellular differences in NCC caused by trisomy. This is the first direct demonstration that trisomy affects NCC and supports further investigations into the etiology of the NCC deficit.

**DEVELOPMENT/AGING
ORAL PRESENTATION****WEDNESDAY NOVEMBER, 15****9.30AM - 9.45AM****O28****IDENTIFICATION OF A FUNCTIONALLY REDUNDANT ROLE FOR THE HECT UBIQUITIN LIGASES ITCH AND WWP1 IN LUNG DEVELOPMENT**LE Matesic¹, K Huang², T Yamaguchi³, EH Bresnick², NG Copeland³, NA Jenkins³¹Department of Biological Sciences, University of South Carolina, Columbia, SC, United States, ²Department of Pharmacology, University of Wisconsin Medical School, Madison, WI, United States, ³National Cancer Institute at Frederick, Frederick, MD, United States

The nine mammalian members of the Nedd4 family of ubiquitin ligases are characterized by a similar overall structural organization comprised of an N-terminal, membrane-interacting C2 module, two to four WW domains, which determine target specificity, and a C-terminal HECT domain that confers ubiquitin ligase activity. *Wwp1* and *Itch* represent a distinct subset of this family as they both contain two tandem pairs of WW domains. Further, both *Itch* and *Wwp1* are ubiquitously expressed in the developing embryo and in adult tissues. While animals homozygous for a loss-of-function mutation in *Itch* develop a systemic, fatal autoimmune disease, *Wwp1*^{-/-} animals are viable and fertile, suggesting that there is functional redundancy among members of the Nedd4 family. When *Wwp1*^{-/-} mice were bred to *itchy* mice, all double homozygotes died within 72h of birth due to lung hemorrhage. Evidence of non-fatal hemorrhage was also found in the developing embryo in the ventricles of the brain, in the cardiac sac, and in the lungs. When the lungs of developing double homozygotes were characterized histologically and immunohistochemically, it appeared that lung development did not progress from the canalicular stage to the terminal sac stage, although type I and type II alveolar epithelial cells were present, similar to the phenotype reported for ES cell chimeric *Klf2*^{-/-} animals. A role for *Klf2* in lung development is also evidenced by the lethal lung hemorrhagic phenotype seen in *Foxf1*^{+/-} mice that stochastically express low levels of *Foxf1* and *Klf2*. Since *Klf2* has been reported to be an *in vitro* target for ubiquitination by *Wwp1*, it is likely that these molecules act in the same pathway during lung development.

**DEVELOPMENT/AGING
ORAL PRESENTATION****WEDNESDAY NOVEMBER, 15****9.45AM - 10.00AM****O29****IDENTIFICATION AND CHARACTERIZATION OF OREO, A MOUSE MODEL OF DOMINANT MICROCEPHALY AND HYPOPIGMENTATION**

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The neural crest is a population of multipotent stem cells that gives rise to the entire peripheral nervous system, enteric neurons, and melanoblasts of the integument. *Sox10* is a transcription factor essential for neural crest survival and differentiation, and *Sox10^{LacZ}* heterozygotes exhibit ventral spotting due to reduced numbers of melanoblasts. From a sensitized ENU screen to identify mouse mutants with disrupted neural crest development, we have identified a mutation called *Oreo* that exhibits synergistic spotting with *Sox10^{LacZ}*. *Oreo* heterozygotes have dorsal and ventral spotting that indicates a defect in neural crest-derived melanoblast development. Consistent with this adult phenotype, melanoblast marker expression is reduced in *Oreo/+* embryos. *Oreo* is essential for viability since *Oreo* homozygotes are embryonically lethal prior to E9.5 and most *Oreo* heterozygotes are perinatal lethal. Surviving *Oreo* heterozygotes have microcephaly, with brains that are 50% smaller than normal due to loss of midline cerebral cortex, cerebellum, and corpus callosum. We mapped the *Oreo* mutation and identified a mutation in a gene that encodes a component of the exon-junction complex (EJC), which regulates nonsense-mediated decay and RNA localization. Transgenic expression of BACs containing this gene rescued the hypopigmentation and lethality associated with *Oreo* heterozygotes. In addition, two independent gene-trap alleles exhibit hypopigmentation and lethality. This is the first report of a mammalian mutation in any EJC component and of a role for this complex in mammalian neural crest and brain development.

**DEVELOPMENT/AGING
ORAL PRESENTATION****WEDNESDAY NOVEMBER, 15****10.00AM -10.15AM****O30****ZIC2 IS REQUIRED FOR THE DEVELOPMENT OF THE PRECHORDAL PLATE**

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The zinc finger containing, putative transcription factor ZIC2 is associated with a defect of forebrain development, known as Holoprosencephaly (HPE), in both humans and mouse. Despite this association, the mechanism by which aberrant ZIC2 function causes HPE has remained unexplained. The zinc finger domain of all mammalian Zic genes is highly homologous with that of the *Gli* genes, which are transcriptional mediators of Shh signalling. Mutations in *Shh* itself and many other Hh pathway members cause HPE and it is therefore widely proposed that *Zic2* acts within the Shh pathway (as a substitute for, or in association with *Gli* genes) to cause HPE. We have investigated both the embryological cause of *Zic2*-associated HPE and the relationship between *Zic2* and the Shh pathway using mouse genetics. We show that *Zic2* does not interact genetically with *Shh* to produce HPE and that molecular defects able to account for the HPE phenotype are present in *Zic2* mutants before the onset of Shh signalling in the mouse embryo. Mutation of mouse *Zic2* causes HPE due to a defect in the maturation of the prechordal plate (PCP), a structure required for the division of the forebrain into left and right hemispheres. No other mouse mutants are known to act at the same stage of PCP development and the Ku allele of *Zic2* is a valuable model for investigating the cause of *Zic2*-associated HPE as well as the development of the PCP.

**DEVELOPMENT/AGING
ORAL PRESENTATION****WEDNESDAY NOVEMBER, 15****10.15AM - 10.30AM****O31****TUMOR REPRESSION IN DOWN SYNDROME**

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Inheritance of three copies of human chromosome 21 (Hsa21) results in Down syndrome (DS) which is associated with two contrary cancer-related phenotypes. Children with DS have a significantly increased risk for leukemia, especially the AMKL sub-type. Paradoxically, several epidemiological studies provide evidence supporting a decades old suggestion that people with DS have a reduced incidence of solid tumors. These studies are limited by the small number of individuals with both DS and a specific form of cancer, resulting in inconsistent conclusions. Further, epidemiological studies cannot provide insight into the genetic mechanisms that result in this protection. To directly test whether trisomy results in a decreased incidence of solid tumors, we analyzed *Apc^{Min}*-mediated tumors in the DS mouse models Ts65Dn, Ts1Rhr and Ms1Rhr. Ts65Dn, *Apc^{Min}* mice, which are trisomic for orthologs of about half of the genes on human chromosome 21, and Ts1Rhr, *Apc^{Min}* mice, which are trisomic for only 38 human chromosome 21 orthologs, both showed a significant reduction in tumor number compared to euploid. In contrast, Ms1Rhr mice, which have segmental monosomy for the same 38 genes triplicated in Ts1Rhr, had a 52% increase in tumor number. Tumor size was reduced in Ts65Dn but not in Ts1Rhr mice. Thus, trisomy for genes found on Hsa21 is protective against tumorigenesis in the small intestine, and a small subset of these genes is sufficient to provide protection in a dosage sensitive manner. Tumor incidence and progression in the various models suggests that full protection from tumorigenesis is a product of multiple genes. This protective effect differs from tumor suppression, which requires normal gene function to prevent cellular transformation. We refer to the genes involved in this protective effect as tumor repressor genes.