

Cranial dysplasia in *Nf1* ob^{-/-} mice is a model of sphenoid wing dysplasia in Neurofibromatosis Type 1.

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INTRODUCTION: A large proportion of NF1 patients display skeletal abnormalities including alterations in bone size and shape, the presence of scoliosis, and a tendency to develop pseudoarthroses. Hypoplasia or absence of the greater wing of the sphenoid bone is the most common cranial anomaly identified with NF1(1). Dysplasia of the sphenoid wing is seen in 7-11% of NF1 patients and is always unilateral (2). To gain insights into the molecular basis and pathogenic processes underlying the clinical manifestations of NF1, mice have been generated in which the neurofibromin gene has been disrupted by targeted deletion. We have recently established a breeding colony of *Nf1ob*^{-/-} mice in which the *loxP*-flanked *Nf1* gene is conditionally deleted in bone-forming osteoblast cells by type 1 collagen promoter driven cre recombinase. When expanding the colony, it was observed that *Nf1ob*^{-/-} mice presented a cranial asymmetry which had not been noted or characterized in earlier work. Micro-CT of these animals show a progressive loss of craniofacial symmetry at the sphenoid bone and other cranial bones, in a manner similar to NF1 (3). In these animals, the face appears longer with a deviation of the nasal bones, often leading to malocclusion. Notably, this phenotype is presumably dependent upon conditional *Nf1* gene knockout in osteoblast cells.

METHODS: Founding animals were the generous gift of Dr. Florent Elefteriou, Vanderbilt University (4). All animal experiments were performed with the approval of the BIDMC Institutional Animal Care and Use Committee and conform to all applicable local, State, and Federal laws and regulations.

RESULTS: Progressive cranial dysplasia in *Nf1ob*^{-/-} mice: In the process of growing *Nf1ob*^{-/-} animals, unilateral bulging of the eye and malocclusion were noticed. The malocclusion was not severe enough to interfere with feeding. Micro-CT analysis at 16 weeks shows (figure 1, A) a marked cranial asymmetry (arrow #3) including the squamosal/temporal bone, sphenoid bone, and the frontal bone. The nasal and premaxilla bones are curved in the horizontal plane and twisted in the sagittal plane (arrow #1). Maxilla and lacrimal bones are thinned (arrow #2). Malocclusion is evident figure 1,B (arrow #4). Cranial sutures are also thickened relative to wild type (not shown). These characteristics are notably different from wild type mice and malocclusion has an incidence rate 8.9 per 10,000 C57BL6 mice (Jackson Labs, 2002). Micro-CT imaging of animals at 16 and 20 weeks of age indicate a progressive phenotype. The progressive overt phenotype of bulging eye and malocclusion was observed in 2 out of 4 individual males at 10 weeks, 3 out of 4 individual males at 16 weeks, 4 out of 4 individual males at 18 weeks and 3 of 3 males at 45 to 50 weeks (see D).

DISCUSSION: NF1 is an autosomal dominant disorder caused by deletion or mutation of the neurofibromin gene. NF1 is characterized by a predisposition to benign and malignant tumors of both the peripheral and central nervous system. There are a broad spectrum of craniofacial skeletal abnormalities among patients with NF1, with sphenoid wing dysplasia as the most severe and mild and local lesions of the sphenoid bone at the other end of the spectrum (5). Sphenoid wing dysplasia usually presents as a unilateral defect affecting an orbit and frontal bone (6,7). Sphenoid wing dysplasia in NF1 is progressive with dysplastic changes contributing to the distinct facial appearance in NF1 and resulting in serious medical problems (3,8). Defects have been described at the cellular level in the differentiation and function of both bone-forming osteoblasts and bone-resorbing osteoclasts however the cellular and molecular pathogenesis of the NF1 skeletal phenotype is not fully elucidated.

Cranial bone components are largely conserved between mouse and human however cranial bone morphology is very different. Mouse eyes are not set in walled-off sockets as such but in a channel formed by the zygomatic arch. The sphenoid bone does not form the back of an eye socket and each eye's visual axis is superior and lateral to the same axes in humans. Given these morphological differences the

similarities between human sphenoid wing dysplasia and *Nf1ob*^{-/-} mouse cranial dysplasia are remarkable.

There are currently no treatments known that block or slow the progression of sphenoid wing dysplasia in NF1. We have characterized a novel and relevant mouse model which recapitulates the phenotype of sphenoid wing dysplasia. Using this mouse model we are positioned to test the effects of currently approved drugs and diet on progression of the phenotype in the mouse model. The ultimate goal of these experiments is to identify currently approved treatments to treat NF1 patients with sphenoid wing dysplasia.

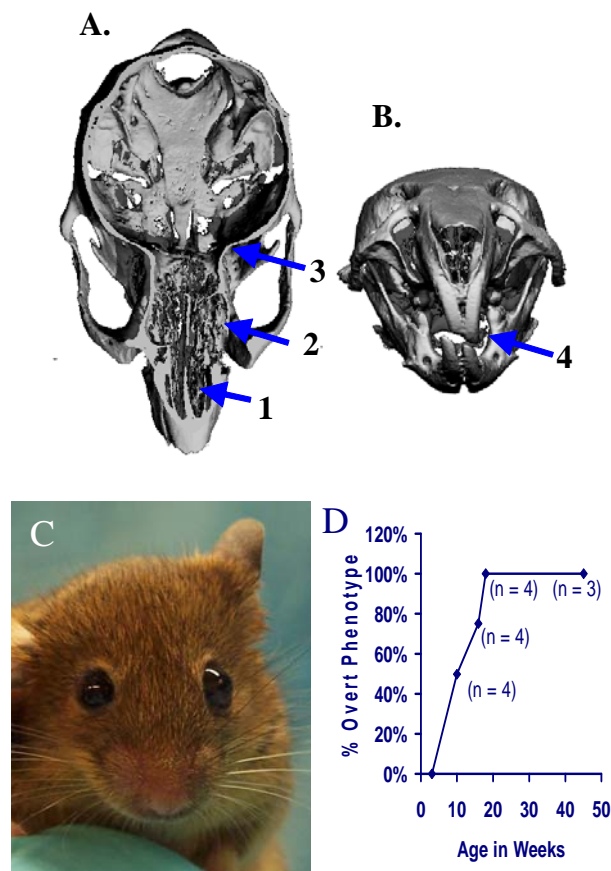


Figure 1. Micro-CT images of the skull of a 16 week old *Nf1ob*^{-/-} male mouse. Horizontal cutaway image at the level of the eye (A) and frontal view (B). Arrows indicate: curved nose and septum (1); thinned bone adjacent to the eye (2); asymmetry of the skull (3); malocclusion (4). Bulging eye is apparent in a 36 week old *Nf1ob*^{-/-} male mouse (C). Time-course of progressive overt phenotype (D).

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