

1 **Short communication**

2 **Intracranial pressure changes during mouse development**

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35 **Abstract:**

36 During early stages of postnatal development, pressure from the growing brain as well as
37 cerebrospinal fluid, i.e. intracranial pressure (ICP), load the calvarial bones. It is likely that
38 such loading contributes to the peripheral bone formation at the sutural edges of calvarial
39 bones, especially shortly after birth when the brain is growing rapidly. The aim of this study
40 was to quantify ICP during mouse development. A custom pressure monitoring system was
41 developed and calibrated. It was then used to measure ICP in a total of seventy three wild
42 type mice at postnatal (P) day 3, 10, 20, 31 and 70. Retrospectively, the sample in each age
43 group with the closest ICP to the average value was scanned using micro-computed
44 tomography to estimate cranial growth. ICP increased from 1.33 ± 0.87 mmHg at P3 to
45 1.92 ± 0.78 mmHg at P10 and 3.60 ± 1.08 mmHg at P20. In older animals, ICP plateaued at
46 about 4 mmHg. There were statistically significant differences between the ICP at the P3 vs.
47 P20, and P10 vs. P20. In the samples that were scanned, intracranial volume and skull
48 length followed a similar pattern of increase up to P20 and then plateaued at older ages.
49 These data are consistent with the possibility of ICP being a contributing factor to bone
50 formation at the sutures during early stages of development. The data can be further used
51 for development and validation of computational models of skull growth.

52

53 **Key words:** Intracranial pressure, Skull, Suture, Biomechanics, Development

54 **Introduction:**

55 During early stages of postnatal development, intracranial pressure (ICP), from the growing
56 brain and cerebrospinal fluid load calvarial bones and sutures (Moss, 1954; Cohen, 1993;
57 Opperman, 2000; Herring, 2008). It is likely that such loading contributes as an epigenetic
58 factor to the peripheral bone formation at the edges of calvarial bones just after birth. Once
59 forceful mastication starts, muscles also load the craniofacial system and presumably
60 influence cranial growth (Nakata, 1981; Rafferty and Herring, 1999; Al Dayeh et al., 2013).
61 While there is a large ongoing effort to understand the genetic causes of various craniofacial
62 developmental disorders (e.g. Morriss-Kay and Wilkie, 2005; Richtsmeier and Flaherty,
63 2013; Cox et al., 2013), understanding epigenetic factors such as biomechanical loading
64 based on ICP during normal and abnormal development is crucial too. A broad
65 understanding of the various factors involved in the development of the craniofacial system
66 can in the long term enhance the treatment of various congenital diseases such as
67 craniosynostosis and Treacher Collins syndrome.

68

69 Quantifying ICP in infants is clearly challenging, but animal models can provide invaluable
70 insights. In particular, accurate invasive but accurate methods can be employed, rather than
71 non-invasive methods that are safer for children but inadequate for this study (Silasi et al.,
72 2009; Raboel et al., 2012; Murtha et al., 2012; Uldall et al., 2014). Mice are particularly
73 useful in that like other mammals, they have many similarities to humans in terms of calvarial
74 morphology and genome (Morriss-Kay and Wilkie, 2005), their genetics is well characterized
75 and there are models available to investigate the pathogenesis of various craniofacial
76 deformities. Despite a long-standing interest in skull development in mice (e.g. Fong et al.,
77 2003; Henderson, et al., 2005) and rats (e.g. Jones et al., 1987), to the best of our
78 knowledge, intracranial pressure during normal mouse development has not been quantified
79 previously. Such data can be used to enhance our understanding of the biomechanics of
80 normal calvarial growth and possibly, ultimately, management of related congenital

81 diseases. Therefore, the aims of this study were to develop a suitable ICP measurement
82 system and quantify ICP during wild type mouse development. To highlight morphological
83 changes during development one sample per age group was scanned and analysed.

84

85 **Materials and Methods:**

86 A pressure monitoring system was developed to measure ICP in mice of 5 age groups. ICP
87 was recorded while animals were anaesthetised. Following the recording animals were
88 decapitated while still under anaesthesia. Then, the sample with closest ICP to the average
89 ICP for each age group was selected for morphological analysis.

90

91 **Pressure monitoring system:** A 22-gauge needle (outer diameter 0.70 mm; length 6 mm)
92 was connected via luer-lock to silicone tubing (outer diameter 4 mm; length 250 mm) which
93 was then connected to a differential pressure sensor (TruStability® Board Mount Pressure
94 Sensors: HSC Series, Honeywell, NJ, USA). The measurement range of the sensor was
95 ± 18.68 mmHg with total error band of 0.19 mmHg. The signal, i.e. changes in the voltage
96 due to external pressure at the tip of the needle, was acquired at 100 Hz using a custom
97 program written in LabVIEW 2013 (National Instruments Corp, Austin, TX, USA). The
98 pressure measurement system was calibrated using tubes with 50, 70, 100, 120 and 150
99 mm of water, with each test repeated five times.

100

101 **In vivo recording of ICP:** A total of seventy-three inbred wild type mice (*Mus musculus*,
102 C57BL/6J - Jackson Labs, Bar Harbor, Maine, USA) at postnatal (P) day 3 (2.23 ± 0.27 g), 10
103 (5.05 ± 1.1 g), 20 (9.06 ± 1.48 g), 31 (17.75 ± 1.91 g) and 70 (22.46 ± 4.01 g) were used. Sex
104 was not recorded for the younger groups, but a retrospective statistical analysis comparing
105 the ICP between males and females at P31 did not show a significant difference. The P70
106 mice were all female. All protocols were approved by the Institutional Animal Care and Use
107 Committees of the University of Washington and Seattle Children's Research Institute. Mice

108 were anesthetized using isoflurane in a non-rebreathing custom set-up. During testing heat
109 support was provided via a warm water pad. Once the animals did not respond to toe pinch,
110 a sagittal incision was made over the calvaria. The needle was inserted through the left
111 parietal bone ca. 2 mm lateral to the sagittal suture and 2 mm anterior to the lambdoid
112 suture. With care it was possible to penetrate the bone with the needle even in older
113 animals, but it was important not to enlarge the hole beyond its diameter. The needle was
114 inserted to a depth calculated to position it in the subarachnoid space, which is filled with
115 cerebrospinal fluid. No external pressure was applied to the skull once the needle had been
116 inserted. It was held in place until ICP reached a maximum (typically 1-2 min); when ICP
117 began to drop, or after several minutes if it did not drop, the needle was removed and the
118 maximum recorded pressure was reported. Once recording was completed, the animals
119 were decapitated while still under anaesthesia.

120

121 **Statistical analysis:** Statistical analysis was performed in SPSS (IBM SPSS, NY, USA).
122 One-way analysis of variance (ANOVA) with post-hoc Bonferroni and Tukey tests was
123 carried out, with Levene's test used to test for equal variances. The significance level was
124 set at $p < 0.05$.

125

126 **Ex vivo micro-computed tomography:** The specimen with measured ICP closest to the
127 average ICP value of each age group was scanned using an X-Tek HMX 160 micro-CT
128 scanner (XTek Systems Ltd, Hertfordshire, UK) with a voxel size of 0.01mm in x, y, and z
129 directions. AVIZO (FEI Visualization Sciences Group, Merignac Cedex, France) was used to
130 reconstruct three dimensional models. The scans were automatically aligned with respect to
131 each other in AVIZO based on minimization of the root mean square distance between the
132 nodes forming the triangulated surfaces of the skull (i.e., Procrustes method) using an
133 iterative closest point algorithm. Each skull surface was typically consisted of about 300,000
134 nodes. Skull length, width and intracranial volume (ICV) were measured using the software.

135

136 **Results:**

137 **Sensor calibration:** As the needle was gradually moved down the tube of water, voltage
138 gradually increased, plateauing at the bottom of the tube. Similarly, upon removal from the
139 water, voltage decreased to its baseline value (Fig 1A). Calibration of the sensor at various
140 heights of water (each repeated five times) showed that the corresponding voltage changes
141 were stable, repeatable and linear (Fig 1B). Note the error bars corresponding to one
142 standard deviation (of five repeats) are shown in Fig 1B. These values were in the range of
143 0.004-0.01 V. These calibration data were used to convert the voltage changes during ICP
144 measurement to mmHg.

145

146 **ICP Measurements:** ICP was 1.33 ± 0.87 mmHg at P3, increasing to 1.92 ± 0.78 mmHg at
147 P10, 3.60 ± 1.08 mmHg at P20, 3.81 ± 1.14 mmHg at P31 and 4.11 ± 0.83 mmHg at P70.
148 There were statistically significant differences between P3 vs. P20, P31, P70, and P10 vs.
149 P20, P31, P70, but not between P20, P31 and P70 (Fig 2).

150

151 **Morphological changes:** Skull length was 13 mm in the P3 skull and 17, 19, 20 and 22 mm
152 at P10, P20, P31 and P70 respectively. Skull width increased to a lesser extent from 8 mm
153 at P3 to 11 mm at P70. ICV increased from 240 mm^3 at P3 to 339, 462, 474 and 504 mm^3 at
154 P10, P20, P31 and P70 respectively (Fig 3).

155

156 **Discussion:**

157 Intracranial pressure may be an important factor contributing to calvarial bone formation at
158 the cranial sutures during early postnatal stages of development. In this study ICP during
159 mouse development was quantified at several postnatal ages in a relatively large number of
160 specimens (10-20 at each age). These ages were chosen to capture various stages of
161 development from just after birth (P3) to juvenile (P10 and P20) to early adulthood (P30 and

162 P70 - see e.g. Hill et al., 2008; Flurkey et al., 2007). Notably, the majority of volumetric brain
163 growth in mice occurs by P20 with lesser increase during P30-P80 (e.g. Zhang et al., 2005).
164 Despite the initial calibration test and repeatability of the results, it is important to compare
165 the ICP data with the existing literature. While no data exist on ICP during mouse
166 development, several studies have quantified ICP in adult mice. Oshio et al. (2004) reported
167 ICP of 6.99 ± 1.03 mmHg in the lateral ventricle (P56-70; n=6), Feiler et al. (2010) reported
168 ICP of 5.0 ± 0.5 mmHg in the epidural space (body weight 23-25 g; n=6), and Yang et al.
169 (2008) reported ICP of 4.33 ± 0.62 mmHg (P70; n=7). Our location, chosen to correspond
170 with our ongoing biomechanical studies on the frontoparietal region (e.g. Moazen et al.,
171 2015), is close to that examined by Yang et al. (2008) i.e. 1 mm posterior to the coronal
172 suture and 1 mm lateral to the sagittal suture. ICP recorded for P70 mice in this study was
173 4.11 ± 0.83 mmHg (n=13), well within the range of data reported by Yang et al. (2008). This is
174 reassuring, as it validates the ability of our sensor to produce reasonable and reliable data
175 for the younger ages in the present study.

176

177 The data obtained showed that ICP increases from about 1.3 mmHg in P3 to 3.6 mmHg in
178 P20 to a limit of approximately 4 mmHg in mice older than P20. This finding is similar to that
179 of Mooney et al. (1998) who measured epidural ICP during rabbit development and reported
180 an increase in ICP from 3.24 ± 0.36 mmHg at P25 (n=28) to 5.68 ± 0.38 mmHg at P42 (n=21).
181 Our morphological measurements are also in agreement with literature (e.g. Zhang et al.,
182 2005; Aggarwal et al., 2009; Chuang et al., 2011). For example, our ICVs of 240, 461 and
183 504 mm^3 at P3, P20 and P70 are comparable to the values of 200, 400 and 430 mm^3
184 reported by Chuang et al. (2011) in C57BL/6 mice at the same ages.

185

186 ICP, ICV and skull length measurements followed a very similar pattern, with a sharp
187 increase from P3 to P20 and then a plateau. In fact, the majority of bone deposition at the
188 cranial sutures occurs by P20. However, while none of the sutures fully fuse, except for

189 posterior frontal at about P10, most sutures narrow down to micrometer gaps at P20.
190 Nonetheless, intrinsic mechanical properties of the bone (approximately 4, 6 and 10 GPa at
191 P10, P20 and P70 respectively) and its thickness (approximately 30, 50 and 150 μm at P10,
192 P20 and P70 respectively) continue to increase (Moazen et al., 2015).

193

194 These data together highlight that, development of the brain, intracranial volume, intracranial
195 pressure and also perhaps bone mechanical properties are coupled. These changes occur
196 synchronously until the brain approximates adult size at P20, whereupon ICV and ICP
197 plateau, while bone elastic properties increasingly rigidify the skull (Moss, 1954). While the
198 data do not speak directly to the issue of whether ICP influences bone apposition at the
199 sutural margins (or is influenced by that apposition), they do suggest that the growth of the
200 neurocapsular matrix is not a response to overly high ICP, but rather that ICP rises when the
201 cranium slows its volumetric growth.

202

203 There were several limitations in this study. Firstly, it was not possible for us to visualize the
204 insertion of the needle into the skull, nor its final position. Therefore, we cannot be confident
205 that needle was in the subarachnoid space in all cases. However, an atlas of the developing
206 mouse (Aggrawal et al., 2009) was used to plan the needle insertion at various ages, and
207 the single operator (MM) was careful to insert the needle to the pre-identified depths to reach
208 the subarachnoid space. Secondly, animals were anesthetized using isoflurane, and this
209 might have had an impact on ICP (see e.g. Campkin, 1984; Scheller et al., 1987).

210 Nonetheless, the same procedure was applied to all animals, so the pattern of recorded ICP
211 in this study should remain valid. Finally, we cannot eliminate the possibility of a sex
212 difference in ICP because of missing data. However, at P31 there was no apparent effect of
213 sex.

214

215 In summary, this study quantified the changes in intracranial pressure during postnatal
216 development of the mouse. The results showed that ICP increases from about 1.3 mmHg at
217 P3 to 4 mmHg at P31, where it plateaus. These data can be used in computational models
218 of skull growth, allowing the strain patterns in the bone and sutures to be quantified.

219

220 **Conflict of interest**

221 The authors confirm that there is no conflict of interest in this manuscript.

222

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339 **Figures legend**

340

341 **Fig 1:** (A) Testing of the pressure sensor with varying heights of water. The needle was
342 slowly inserted to the bottom of a tube of water, held there for 10-25 sec, and then slowly
343 removed. (B) Calibration of the pressure sensor showed the response was linear. Small
344 brackets indicate the SD of measurements.

345

346 **Fig 2:** Changes in intracranial pressure during wild type mouse development (means and
347 SDs). The shaded areas indicate ICP data for all samples in the corresponding age group.
348 Asterisks show statistically significant differences.

349

350 **Fig 3:** (A) A P70 mouse, highlighting the sagittal and coronal planes used for length and
351 width comparisons. (B) Sagittal and (C) coronal sections of one animal per age. Note the
352 P10 skull became slightly deformed following the ICP measurement and prior to micro-CT
353 scanning. (D) Skull length, width and (E) intracranial volume at P3, P10, P20, P31 and P70.

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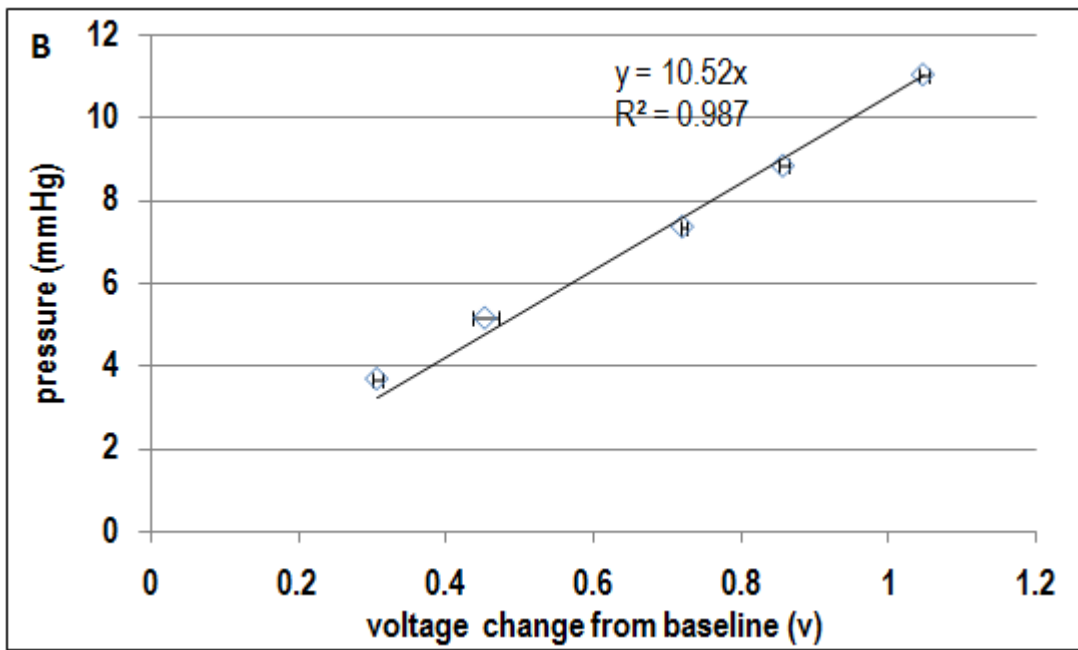
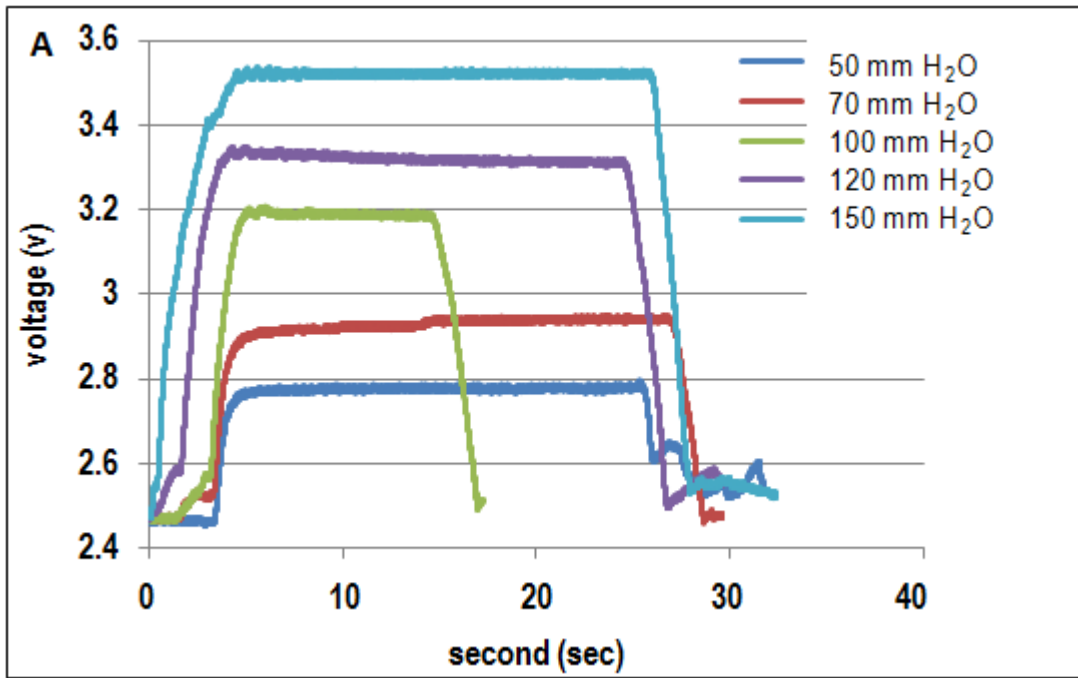
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366 Fig 1



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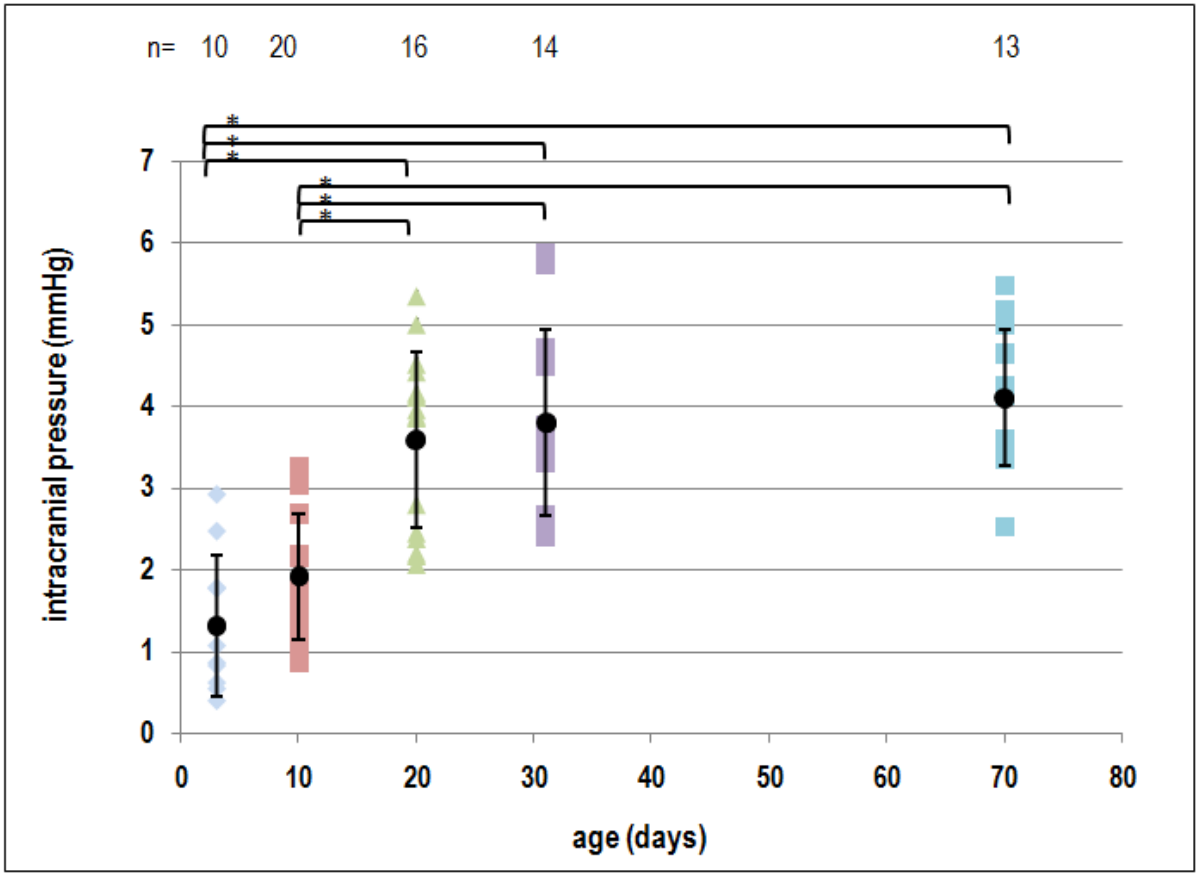
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373 Fig 2



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