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Data in Brief

Transcriptomic analyses of *Hand2* transgenic embryos

Noriko Funato [a,*], Hiroki Kokubo [b,c,d], Yumiko Saga [b,c]

[a] Department of Signal Gene Regulation, Research Center for Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan
[b] Division of Mammalian Development, National Institute of Genetics, Yata 1111, Mishima, Shizuoka 411-8540, Japan
[c] Department of Genetics, The Graduate University for Advanced Studies, Yata 1111, Mishima, Shizuoka 411-8540, Japan
[d] Department of Cardiovascular Physiology and Medicine, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minamiku, Hiroshima 734-8551, Japan

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ABSTRACT

In this article, we further provide the data generated for the previously published research article "Specification of jaw identity by the Hand2 transcription factor." To better understand the downstream genes of the basic helix-loop-helix transcription factor Hand2, we generated double-transgenic mice (*Hand2*^{NC}) by intercrossing *CAG-floxed CAT-Hand2* mice with *Wnt1-Cre* mice for conditional activation of *Hand2* expression in the neural crest. Altered expression of *Hand2* induces transformation of the upper jaw to the lower jaw in *Hand2*^{NC} mutant mice. This data article provides Tables detailing the differentially expressed genes between wild-type and *Hand2*^{NC} mutant embryos. The raw array data of our transcriptomes as generated using Affymetrix microarrays are available on the NCBI Gene Expression Omnibus (GEO) browser (Reference number GSE75805).

Specifications	
Organism/cell line/tissue	*Mus musculus*
Sex	N/A
Strain	A mixed C57BL/6J × ICR genetic background
Sequencer or array type	Affymetrix GeneChip Mouse Genome 430 2.0
Data format	Raw
Experimental factors	Wild-type and *Hand2* transgenic mice; Altered expression of *Hand2* in the neural crest
Experimental features	For the conditional activation of *Hand2*, *Wnt1-Cre* male mice were crossed with *CAG-CAT Hand2*$^{Tg/+}$ females, generating *Hand2*^{NC} mutant and age-matched littermates. Transcriptomes from *Hand2*^{NC} mutants and age-matched littermates were assayed on an Affymetrix GeneChip Mouse Genome 430 2.0 array to investigate the effect of Hand2 on global gene expression.
Consent	The data are publicly available and open for re-use given appropriate citation.
Sample source location	Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan

Abbreviations: bHLH, basic helix-loop-helix; CAG, the CMV early enhancer/chicken β actin; CAT, chloramphenicol acetyl transferase; aRNA, amplified RNA.

* Corresponding author.
E-mail addresses: noriko-funato@umin.ac.jp (N. Funato), hkokubo@hiroshima-u.ac.jp (H. Kokubo), ysaga@nig.ac.jp (Y. Saga).

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE75805

2. Experimental design

The neural crest plays a key role in craniofacial development. The basic helix-loop-helix (bHLH) transcription factor Hand2, is expressed in the branchial arch neural crest and is crucial for development of the cranial neural crest [1–5]. Mice lacking the *Hand2* branchial arch-specific enhancer (termed *Hand2*$^{BA/BA}$) develop a spectrum of craniofacial defects, including a hypoplastic mandible, abnormal middle ear ossicles, and a cleft palate [4,6]. Neural crest-specific ablation of *Hand2* demonstrated that Hand2 is also necessary for the differentiation of sympathetic and enteric neurons [7,8]. *Hand2* expression is observed in the mandibular process of the first branchial arch but not in the maxillary process [4,9]. Consistent with its expression pattern, the Hand2 sequence is conserved among jawed vertebrates but is less conserved among jawless vertebrates and invertebrates [9].

For the conditional activation of *Hand2* in the neural crest, we generated double-transgenic mice (*Hand2*^{NC}) by intercrossing *CAG-floxed CAT-Hand2* mice with *Wnt1-Cre* mice. When we examined the contribution of *Wnt1-Cre* to craniofacial development using *Rosa26* Reporter (*R26R*) mice, altered β-galactosidase staining was observed in the craniofacial region of *Hand2*^{NC} mutants (Fig. 1A). Conditional activation

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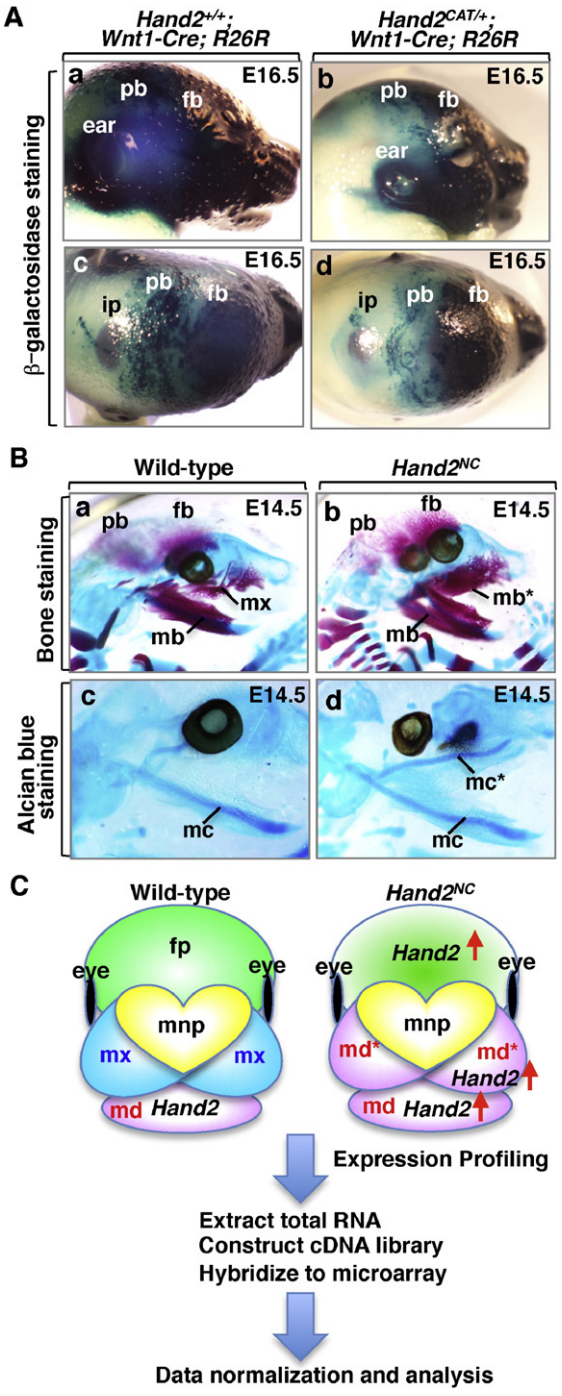

Fig. 1. Morphological transformation of the upper to the lower jaw in *Hand2*^{NC} mutants. A. Whole-mount β-galactosidase staining of E16.5 control (a, c) and *Hand2*$^{CAT/+}$*; Wnt1-Cre; R26R* (b, d) embryos. fb, frontal bone; pb, parietal bone; ip, interparietal bone. B. Alizarin red and alcian blue staining (a, b) and alcian blue staining (c, d) at E14.5 of wild-type (a, c) and *Hand2*^{NC} (b, d) embryos. The mutant maxillary bone (mx) is transformed to a duplicated mandibular bone (mb*) with duplicated Meckel's cartilage (mc*). C. Flow chart of the experimental design. The whole-body or heads collected from wild-type and *Hand2*^{NC} embryos were used for the microarray experiment. mc, Meckel's cartilage; mc*, duplicated Meckel's cartilage; mx, maxillary process; md, mandibular process; md*, duplicated mandibular process; fp, frontonasal process; mnp, medial nasal process. An asterisk indicates a duplicate.

of *Hand2* expression in the neural crest transforms the maxilla to the mandible (Fig. 4B), suggesting that Hand2 controls the specification of jaw identity [9]. Nested *Hand2* expression in the mandibular process is also necessary for palatogenesis [9]. The duplicated Meckel's cartilage in the maxillary region was confirmed by alcian blue staining of *Hand2*^{NC} mutants (Fig. 1B). The maxilla-to-mandible transformation of the first branchial arch in *Hand2*^{NC} mutants indicated that altered *Hand2* expression converts the genetic program from that of the maxillary to the mandibular process. To profile the mRNA levels from *Hand2*^{NC} embryos, we used an Affymetrix microarray platform (GeneChip Mouse Genome 430 2.0 Array) (Fig.1C). Our related work, which was recently published in Scientific Reports, used these molecular approaches to determine the downstream genes of Hand2 [9]. In this Data in Brief article, we provide additional information aiming to help other researchers interpret and use these datasets for their own research.

3. Materials and methods

3.1. Generation of Hand2-mutant mice

CAG-floxed CAT-Hand2 mice (*Hand2*$^{CAT/+}$; Stock No.RBRC01366, RIKEN) have been described previously [9]. We generated *Hand2*^{NC} mutant mice by intercrossing *Hand2*$^{CAT/+}$ transgenic mice with *Wnt1-Cre* mice [10] (Stock No. 7807, The Jackson Laboratory). *R26R* mice (Stock No. 6148, The Jackson Laboratory) have been described previously [11]. Wild-type littermates were used as controls. All the experimental animal procedures were reviewed and approved by the Institutional Animal Care and Use Committees of the Tokyo Medical and Dental University. All experiments were carried out in accordance with the approved guidelines.

3.2. Microarray study design

To determine the downstream genes of Hand2, we performed gene expression profiling on embryonic day (E) 11.5 *Hand2*^{NC} embryos ($n = 4$ per genotype) and E12.5 *Hand2*^{NC} heads ($n = 3$ per genotype). We routinely freeze our samples at −80 °C. Total RNA was extracted with TRIzol (Thermo Fisher Scientific), according to manufacturer's instructions, and was resuspended in nuclease-free water. Total RNA was then purified with the RNeasy Mini Kit (QIAGEN) according to the manufacturer's instructions. Replicate samples within the same genotype were grouped together. For each RNA sample, concentration and purity were measured using a NanoDrop ND-1000 spectrometer, and RNA quality was examined with the 2100 Bioanalyzer (Agilent Technologies). All RNA integrity number (RIN) scores were 10 (Fig. 2A).

3.3. RNA labeling and hybridization

The microarray analysis was performed by the KURABO Bio-Medical Department using the Affymetrix GeneChip Mouse Genome 430 2.0 Array. Biotin-modified amplified RNA (aRNA) was prepared from 250 ng total RNA according to the standard Affymetrix protocol (GeneChip 3′ IVT Express Kit User Manual, P/N 702646 Rev.1 Affymetrix). Following fragmentation to approximately 35–200 nt aRNA fragments with a peak at approximately 100–120 nt, 10 μg of aRNA were hybridized for 16 h at 45 °C on a GeneChip. The GeneChip was washed, stained in the Affymetrix Fluidics Station 450, and then scanned using the GeneChip Scanner 3000 (Affymetrix 00-0074).

3.4. Data normalization and analysis

The data were analyzed with the GeneChip Operating Software ver1.4 (MAS 5.0) using the default Affymetrix analysis settings and global scaling as the normalization method. The trimmed mean target intensity of each array was arbitrarily set to 500. Using this algorithm, present, marginal, or absent calls were obtained for each probe set in each array. For the comparative analysis of gene expression profiles, data assigned to absent calls were omitted. Scatter plots of normalized signal values are shown in Fig. 2B. The change in expression level for a transcript between a baseline and an experimental array was expressed

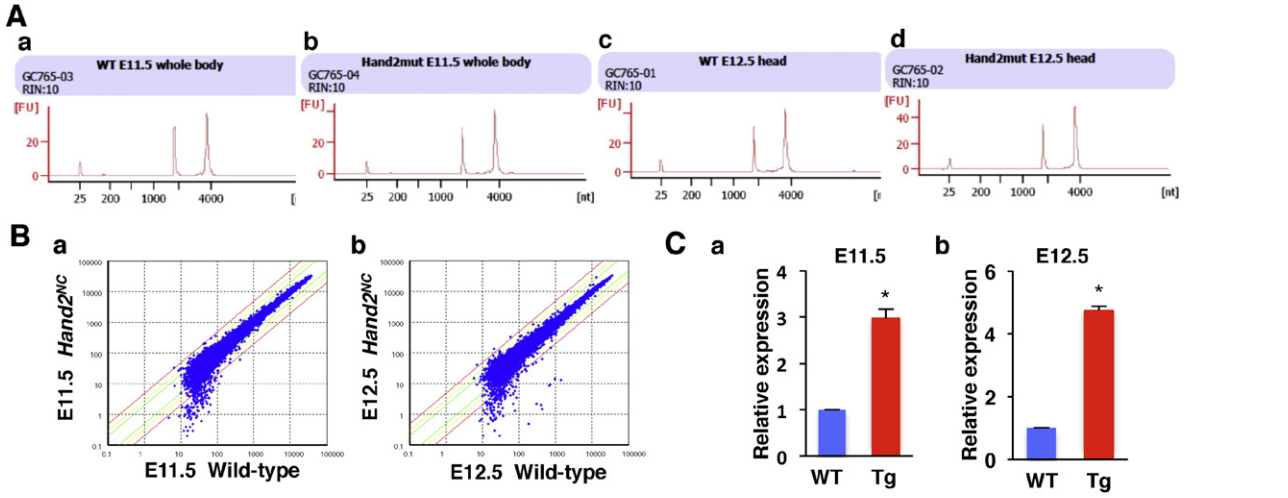

Fig. 2. RNA quality control. A. RNA quality was measured using the Agilent 2100 Bioanalyzer for wild-type (a, c) and *Hand2*^{NC} mutant (b, d) samples at E11.5 (a, b) and E12.5 (c, d). The RNA Integrity Number (RIN; value assigned from 0 to 10) and histograms are shown. B. Scatter plots showing the correlation of signal values between two samples from E11.5 (a) and E12.5 (b) embryos. Data assigned to absent calls were omitted. C. qPCR analysis of the *Hand2* transcript levels from wild-type (WT) and *Hand2*^{NC} (Tg) embryos at E11.5 (a) and E12.5 (b). *Hand2* expression was upregulated in the *Hand2*^{NC} embryos. The experimental data were analyzed using two-tailed Student's *t*-tests and were expressed as the mean ± standard error of the mean (SEM). *P*-values less than 0.05 were considered as significant.

as the $\log_2$ ratio. A signal log ratio of 1 is the same as a fold change of 2. Upon gene expression profiling on *Hand2*^{NC} embryos, 17 genes, including the *Hand2* gene, were found to be upregulated and 51 genes were downregulated, with a two-fold change cut-off in E11.5 *Hand2*^{NC} embryos (Table A.1) [9]. In E12.5 *Hand2*^{NC} heads, 61 genes were upregulated, and 84 genes were downregulated, with a two-fold change cut-off change (Table B.1) [9]. The ontology analyses of the altered genes have been previously reported [9].

3.5. Alcian blue and β-galactosidase staining

Alcian blue staining and whole-mount β-galactosidase staining were performed as described previously [12].

3.6. Quantitative polymerase chain reaction (qPCR)

qPCR was performed as previously described [13]. All data were normalized to *Actb* (β-actin) expression. qPCR assays were performed in triplicate in three independent experiments. The primer sequences used for amplification were as follows: *Hand2* forward: 5′-CCTCTTTCACGTCGGTCTTC-3′, *Hand2* reverse: 5′-AAGATCAAGACA CTGCGCCT-3′; and *Actb* forward: 5′-ATGGAGGGGAATACAGCCC-3′, *Actb* reverse: 5′-TTCTTTGCAGCTCCTTCGTT-3′.

4. Results and discussion

Gene expression changes between genetic cohorts were validated by real-time qPCR (Fig. 2C). Consistently, qPCR analyses confirmed the upregulation of *Hand2* (Fig. 2C). To verify the microarray data, 20 genes were selected and whole-mount *in situ* hybridization was performed, and the results were found consistent with the microarray findings (Figs. 3 and 4 of [9]). Thus, we conclude that the overall reproducibility of the microarray gene expression data sets is very good.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2016.06.015.

Conflicts of interest

The authors declare no competing financial interests.

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