Identifying Human Disease Genes through Cross-Species Gene Mapping of Evolutionary Conserved Processes

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Abstract

Background: Understanding complex networks that modulate development in humans is hampered by genetic and phenotypic heterogeneity within and between populations. Here we present a method that exploits natural variation in highly diverse mouse genetic reference panels in which genetic and environmental factors can be tightly controlled. The aim of our study is to test a cross-species genetic mapping strategy, which compares data of gene mapping in human patients with functional data obtained by QTL mapping in recombinant inbred mouse strains in order to prioritize human disease candidate genes.

Methodology: We exploit evolutionary conservation of developmental phenotypes to discover gene variants that influence brain development in humans. We studied corpus callosum volume in a recombinant inbred mouse panel (C57BL/6J×DBA/2J, BXD strains) using high-field strength MRI technology. We aligned mouse mapping results for this neuro-anatomical phenotype with genetic data from patients with abnormal corpus callosum (ACC) development.

Principal Findings: From the 61 syndromes which involve an ACC, 51 human candidate genes have been identified. Through interval mapping, we identified a single significant QTL on mouse chromosome 7 for corpus callosum volume with a QTL peak located between 25.5 and 26.7 Mb. Comparing the genes in this mouse QTL region with those associated with human syndromes (involving ACC) and those covered by copy number variations (CNV) yielded a single overlap, namely HNRPU in humans and Hnrpul1 in mice. Further analysis of corpus callosum volume in BXD strains revealed that the corpus callosum was significantly larger in BXD mice with a B genotype at the Hnrpul1 locus than in BXD mice with a D genotype at Hnrpul1 (F=22.48, p<9.87*10⁻⁶).

Conclusion: This approach that exploits highly diverse mouse strains provides an efficient and effective translational bridge to study the etiology of human developmental disorders, such as autism and schizophrenia.

Introduction

The corpus callosum is the fibrous structure that connects both hemispheres of the cortex in all placental mammals [1,2]. In humans, this bridge is made up of more than 100 million axons of neocortical neurons routing information between the left and the right sides of the brain [3,4]. Improper development of the corpus callosum may manifest itself in infancy by feeding problems, delays in acquiring proper posture and the ability to walk, and impairments in hand-eye coordination, speech, and visual and auditory memory. In mild cases, symptoms such as repetitive speech, social awkwardness, rigid thinking, poor problem solving, and odd communication patterns may appear during elementary school years. During puberty, children with an abnormal corpus callosum (ACC) often fall behind in social understanding, social communication, comprehension of non-verbal language, problem solving, executive skills, recognition of emotions, self-awareness, and personal insight. Given the lack of specificity of symptoms of an ACC, it is critical to properly diagnose an ACC, which may either occur as an isolated clinical entity, as part of a syndrome (for reviews see: [1,2,5,6]) or in association with complex phenotypes such as autism or pontocerebellar hypoplasia [7,8]. Recent studies have put forward potential candidate genetic mechanisms underlying an ACC in humans, however, the question remains how these genes contribute to abnormal development of this brain region relevant to proper human functioning.

For example, developmental processes in mammals are presumed to be controlled by complex genetic networks often involving interaction of several loci and genes [9,10]. Unraveling the myriad interactions within these networks is a major task in clinical genetics, which faces the challenge of correctly interpreting the phenotypic manifestations of perturbed development in
relation to data on genome alterations. Since these fundamental developmental processes are among the evolutionary most conserved, we hypothesize that the underlying genetic networks and their interaction patterns are also highly conserved. Here, we present an approach to interpret human genomic data relating to perturbed developmental disease processes by making use of their evolutionary conservation through natural genetic variation within and across species. As a test case, we have analyzed a locus involved in the development of the corpus callosum in the human and the mouse brain.

Results

Prioritizing candidate loci and genes

Following a procedure used in a recent study of patients with multiple congenital anomalies and mental retardation [11] we used a disease cohort-specific compilation of genes involved in syndromes involving an ACC to prioritize contributing loci, genes, and biological processes. For the 61 autosomal recessive and dominant, X-linked, metabolic, and chromosomal syndromes which involve an ACC, 51 human candidate genes have been identified (Table 1). Of these 19 (ARK, ATRX, DCX, EFNB1, EP300, FGFR1, FLNA, GLE3, HESX1, L1CAM, LARGE, MID1, PAFAH1B1, PAX6, PTCH, RELN, WHSC1, and ZFHX1B) fit into the Gene Ontology (GO) category of development (GO:0007275; p value<0.0001 and Bayes Factor 15). The more narrow category of neurogenesis (GO:0007399) includes 11 genes (DCX, EFNB1, EP300, FGMD, FLNA, HESX1, L1CAM, LARGE, PAFAH1B1, PAX6, and ZFHX1B; p<0.0001 and Bayes Factor 16).

Both the p values (assuming a normal distribution of likelihoods) and the Bayes factor, indicating the fold-likelihood that a model fits the data vs. the neutral null-hypothesis, indicate a highly significant association of these GO categories with an ACC [12]. Interestingly, the mouse locus also contains a small complex of genes that modulate neocortical development in close proximity on chromosome 7 in mouse. Thus, mapping of loci for an inherited defect in human patients is of the corpus callosum in relation to natural genetic variation. For this, we made use of evolutionary conservation of the development of the corpus callosum among mammals may provide us with a clue.

Gene mapping with crosses of inbred mouse strains

Unbiased phenotype-driven approaches in mice may also contribute to identification of genetic loci relevant to the development of the corpus callosum in humans. Analyses of mouse genetic reference populations (GRPs) allow for systematic identification of quantitative trait loci (QTL) using controlled genetic background and environmental conditions. For instance, a recombinant inbred (RI) panel which is generated from a cross between two inbred strains [29,30] (e.g., C57BL/6J×DBA/2] (BXD)) [31]; followed by an F1 intercross and 20 generations of inbreeding has proven to be a powerful instrument for studies of complex genetic traits on the basis of natural variation [29–31].

Here, we focused our analysis on the corpus callosum volume spanning a wide range (~0.5 to 17.6 mm$^3$) across individuals of the BXD panel (with known genotypes). Through interval mapping (using the online GeneNetwork system at www.genenet-work.org) we identified a single significant QTL on mouse chromosome 7 for corpus callosum volume with a QTL peak located between approximately 25.5 and 26.7 Mb (Figure 1A). The peak of the QTL-interval just reached genome-wide significance level with a log of odds (LOD) score of 2.8 (permutation likelihood ratio statistic, or LRS, threshold is computed of the genome-wide p value of 0.05 using 1000 permutations). This locus on chromosome 7 contains approximately 46 genes.

Comparing the genes in this QTL region with those associated with syndromes involving an ACC (Table 1) and those covered by CNVs yielded a single gene overlap, namely the HNRPU gene in humans and its homolog Hop11 in mice. Further analysis in BXD strains revealed that the corpus callosum was significantly larger in strains with a B genotype at the Hop11 locus (15.5±3.9 mm$^3$) compared to strains with a D genotype (10.85±2.65 mm$^3$) (ANOVA analysis: F=22.48, p<9.87*10$^{-9}$). The gene encoding HNRPU is located in the q44 region of human chromosome 1 and is included in the hemizygous 1q44 losses of 32 out of the 41 published cases showing an ACC on MRI (Figure 1B). Taken together, these suggest that humans and mice share a single locus encompassing one gene that may control corpus callosum development. Interestingly, the mouse locus also contains Zfp265 and Dopk, two highly polymorphic genes associated with cQTL in the neocortex (data not shown), suggesting that there may also be a small complex of genes that modulate neocortical development in close proximity on chromosome 7 in mouse. Under the proviso that an ACC is the outcome of a perturbation of neurodevelopment, it is conceivable that proper development of the corpus callosum is controlled by a gene involved in the processing of primary transcripts in the nucleus, which functions in the mouse as a quantitative trait locus.

Discussion

Here we have presented an approach to identify genes that may control brain development associated with human disease. For this, we made use of evolutionary conservation of the development of the corpus callosum in relation to natural genetic variation. Thus, mapping of loci for an inherited defect in human patients is complemented by experimental data generated by crossing inbred mouse strains. Such crosses of inbred mice strains provide a tool for “genetic experiments” to test such hypotheses and select relevant human candidate genes from the many genes currently associated with syndromes involving an ACC. We hypothesize that comparing patterns of genetic control of conserved developmental processes among animal species may be fruitful for other, not only neurological, developmental processes. Very recent studies indicated that cross-species genome comparisons in relation to preserved phenotypes may also apply to other complex disorders, such as hypertension [32] and psychiatric disorders [33], and thus may open new roads for understanding disease etiology.

This conjecture prompts several ramifications. First, the development of the corpus callosum may be linked to the control of a transcriptional network composed of HNRPU and its putative downstream targets. Second, hemizygous losses of HNRPU may not be the sole genetic cause of an ACC. Several studies have
Table 1. Syndromes involving an abnormal corpus callosum (ACC).

| Syndrome                        | Locus   | Gene   | OMIM    | Syndrome                        | Locus   | Gene   | OMIM    |
|---------------------------------|---------|--------|---------|---------------------------------|---------|--------|---------|
| **Autosomal-dominant**           |         |        |         |                                 |         |        |         |
| Apert                           | 10q26   | FGFR2  | 101200  | Lissencephaly 3                 | 12q12-q14 | TUBA1A | 611603  |
| Basal cell nevus                | 9q22.3  | PTCH1  | 109400  | Rubinstein-Taybi                | 16p13.3 | CREBBP | 180849  |
| Greig cephalo-polysyndactyly    | 7p13    | GLI3   | 175700  | Septo-Optic dysplasia (SOD)     | 3p21.2-p21.1 | HESX1 | 182230  |
| Kallmann                        | 8p11.2-p11.1 | FGFR1 | 147950  | Sotos                           | 5q35    | NSD1   | 117550  |
| **Autosomal-recessive**         |         |        |         |                                 |         |        |         |
| Acrocallosal                    | 7p13    | GLI3   | 200990  | Lissencephaly 2                 | 7q22    | RELN   | 257320  |
| Andermann                       | 15q13-q14 | SLC12A6 | 218000  | Marden-Walker                   |         |        |         |
| Aniridia type II                | 11p13   | PAX6   | 106210  | Meckel-Gruber                   | 17q22-q23 |        | 249000  |
| Coffin-Siris                    | 7q32-q34 |        |        | Microcephalic                   |         |        |         |
| Dincoy                          |         |        |        | osteodysplastic primordial      |         |        |         |
| Frys                            |         |        |        | dwarfism (MOPD) type 1          |         |        |         |
| Fukuyama congenital muscular   | 9q31    | FCMD   | 253800  | MOPD type 3                     |         |        |         |
| dystrophy                       |         |        |        |                                 |         |        |         |
| Hydroethalohus                  | 11q24.2 | HYLS1  | 236680  | Mowat-Wilson                    | 2q22    | ZFHX1B | 235730  |
| Joubert                         | 9q34.3  |        | 213300  | Muscle-eye-brain disease        | 1p34-p33 | POMGNT1 | 253280  |
|                                 | 11p12-q13.3 |       | 608091  | Neu-Laxova                      |         |        |         |
|                                 | 8q21.1-q22.1 | TMEM67 | 610688  | Septooptic dysplasia            | 3p21.2-p21.1 | HESX1 | 182230  |
|                                 | 6q23.3  | AHI1   | 608629  | Toriello-Carey                  |         |        |         |
|                                 | 2q13    | NPHP1  | 609583  | Vici                            |         |        |         |
|                                 | 3q11.2  | ARL13B | 612291  | Walker-Warburg                  | 9q34.1  | POMT1  | 607423  |
|                                 | 4p15.3  | CC2D2A | 61285   |                                 | 14q24.3 | POMT2  | 607439  |
|                                 | 16q12.2 | RPGRIP1L | 611560 |                                 | 19q13.3 | FKRP   | 606596  |
|                                 | 12q21.3 | CEP290 | 610188  |                                 | 22q12.3-q13.1 | LARGE | 603590  |
| Lowry-Wood                      |         |        | 226960  |                                 |         |        |         |
| Lyon                            |         |        | 225740  |                                 |         |        |         |
| **X-linked**                    |         |        |         |                                 |         |        |         |
| Aicardi                         | Xp22    |        | 304050  | X-linked lissencephaly          | Xq22.3-q23 | DCX  | 300067  |
| ATR-X                           | Xq13    | ATRX   | 301040  | Lissencephaly X-linked 2        | Xp22.13 | ARX   | 300215  |
| Aquedectal stenosis/ hydrocephalus (MASA syndrome; X linked) or Hydrocephalus due to congenital stenosis of aqueduct of Sylvius | | | | | | | |
| Xq28                            | L1CAM   |        | 307000  | Lujan-Fryns                     | Xq13    |        | 309520  |
| Microphthalmia with linear skin defects | Xp22.31 |        | 309801  |                                 |         |        |         |
| Optitz                          | Xp22    |        | 300000  |                                 |         |        |         |
| Optitz-Kaveggia                 | Xq13    |        | 305450  |                                 |         |        |         |
| Oro-facial digital type 1       | Xp22.3-p22.2 | CXORF5 | 311200 |                                 |         |        |         |
| Craniofrontonasal               | Xq12    | EFNB1  | 304110  | Periventricular heterotopia     | Xq28    |        | 300049  |
| Lenz microphthalmia             | Xq27-q28 |        | 309800  | Proud                           | Xp22.13 | ARX   | 300004  |
| **Metabolic disorders**         |         |        |         |                                 |         |        |         |
| Fumarase deficiency             | 1q42.1  | FH     | 606812  | Smith-Lemli-Opitz               | 11q12-q13 | DHCRI7 | 270400  |
| Glycine encephalopathy          | 9p22    | GCSFP  | 606812  |                                 |         |        |         |
| PDH deficiency                  | Xp22    | PDHA1  | 312170  | Zellweger                       | 6q23-q24 | PEX3  | 214100  |
| ACC with ectodermal dysplasia   |         |        |         | Miller Dieker                   | 225040  |        | 247200  |
| (hypohidrotic)                  |         |        |         | Lissencephaly                   | 17p13.3 |        |         |
| Dellemann syndrome              | 164180  |        |         | Ocular motor apraxia            | 2q13    |        | 257550  |
| (Oculocerebrocutaneous)         |         |        |         | (Cogan-syndrome)                |         |        |         |

Interspecies Convergence Mapping of Disease Genes
Table 1. Cont.

| Syndrome               | Locus   | Gene    | OMIM   | Syndrome        | Locus   | Gene    | OMIM   |
|------------------------|---------|---------|--------|-----------------|---------|---------|--------|
| Lissencephaly type I   | 17p13.3 | LIS1    | 607432 | Optiz GBBB      | 22q11.2 | 601545  | 145410 |
| Lissencephaly type III | 17p13   | PAFAH1B1| 601545 | Wolf-Hirschhorn | 4p16.3  | WHSC1   | 194190 |

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Figure 1. Genetic mapping of corpus callosum volume in the BXD mouse genetic reference panel. Likelihood ratio (LRS; ordinate) of the association or linkage between differences in corpus callosum volume and natural genetic variation as a function of position in the mouse genome (chromosome number and coordinates in megabases given; abscissa). Note the single genome-wide significant peak in mouse chromosome 7 (A). Overlapping deletions in genomic region 1q44 in patients with an ACC (B). Black bars and arrows indicate hemizygoously deleted regions. Grey bars and arrows indicate hemizygous deletions found in subject with a normal corpus callosum. Vertical lines indicate the region that is hemizygous in 32 out of 41 cases, and overlaps with the mouse QTL.

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demonstrated that hemizygosity for HNRPU is not sufficient to cause an ACC [14,15,34]. However, any mutation affecting proper functioning of the HNRPU gene product and its interaction with other proteins may lead to an ACC. These mutations are not necessarily limited to the HNRPU gene product, but may include genes encoding downstream targets. Such genes may either have been identified in syndromes involving an ACC or may be covered by the CNVs found in sporadic patients with an ACC, or have as yet not been identified. This offers a potential explanation for the phenotypic diversity of patients with hemizygous losses in 1q44. It also suggests an explanation for the heterogeneity of loci and genes potentially involving an ACC. Given the clinical importance of an ACC, and the many ramifications of this hypothesis, functional experimental tests appear worthwhile. Furthermore, this approach of integrating mouse genetic mapping data of evolutionary conserved phenotypes may also prove useful for a wide variety of complex human diseases, such as congenital heart disease, eating disorders, and autism spectrum disorders.

Materials and Methods

Prioritizing candidate loci and genes

To systematically determine genes or CNVs that were specifically found among patients with an ACC, or in syndromes involving an ACC, we analyzed our data using the Gene Annotation Tool to Help Explain Relationships (GATHER) developed by Chang and Nevins [12]. The algorithms embedded herein allow to determine significance of association with regards to shared biological processes (using Gene Ontology: http://www.geneontology.org/GO.doc.shtml), chromosomal locations or biochemical pathways (using KEGG: http://www.genome.jp/kegg/). The algorithms generate p values (assuming a normal distribution of likelihoods) and a Bayes factor, indicating the fold-likelihood that a model fits the data vs. the neutral null-hypothesis, to indicate significance of association of GO categories with an ACC [12].

Gene mapping with crosses of inbred mouse strains

To study genetic factors that contribute to differences in brain structure we focused on a subset of fully inbred BXD RI strains, where each of the strains contains a unique genetic pattern of the genomes from the maternal and paternal strains. Age-matched pairs (male and female) of mice belonging to 11 inbred strains (56–64 days of age) were obtained directly from the Jackson Laboratory (www.jax.org): C57BL/6J (B6), DBA/2J (D2), and the following nine BXD recombinant inbred strains—BXD1, BXD6, BXD15, BXD16, BXD24, BXD28, BXD29, BXD34, and BXD40. We intentionally studied age-matched male-female pairs from different litters. To ensure that the low levels of within-strain variance are not simply due to a common litter effect, we chose that same strain mice coming from different litters. (This is analogous to the situation of monozygotic human twins raised in different environments.)

To study structural variation of the brain in this family of strains we used high-resolution magnetic resonance microscopy (MRM) imaging techniques [35] of actively stained brain specimens, followed by semi-automated segmentation of the brain images [36–39] into 33 major regions—gray matter nuclei, white matter fibers, and ventricular space.

Imaging was performed at the Duke Center for In Vivo Microscopy. All experiments were conducted in accordance with NIH guidelines, using protocols approved by the Duke University Institutional Animal Care and Use Review Committee under IACUC protocol number A123-07-04. The Duke animal program has AAALAC accreditation number 363; since 1976; NIH/PHS assurance number A3195-01, current through 2013. Mice were anesthetized with 100 mg/kg pentobarbital (i.p.) and then fixed by transcardial perfusion, first with a flush of 0.9% saline and gadoteridol contrast agent—ProHance (Bracco Diagnostics, Princeton, NJ) (10:1, v/v), followed by a mixture of 10% formalin and ProHance (10:1, v/v). Whole heads were stored overnight in formalin, and then trimmed to remove the lower jaw and muscle. Brains were scanned within the cranial vault to avoid distortions or damage to the tissue during excision from the cranium. The fixed specimens were imaged using a 9.4 T (400 MHz) vertical bore Oxford magnet with a GE EXCITE console (Epic 11.0). A 14-mm diameter solenoid RF coil was used for the ex-vivo, in-situ mouse brains. We used a 3D spin warp sequence with the readout gradient applied along the long (anterior–posterior) axis of the brain. The multispectral data consisted of a T1- and a T2-weighted imaging protocols. The T1-weighted sequence was acquired with an echo time (TE) of 5.1 ms, repetition time (TR) 50 ms, 62.5 kHz bandwidth, field of view (FOV) of 11×11×22 mm. A T2 multiecho sequence was acquired with a Carr Purcell Meiboom Gill sequence using the same FOV and bandwidth, with TR of 400 ms and echo spacing of 7 ms (16 echoes). To produce data heavily dependent on T2 differences the 16 echoes were MEFIC processed, i.e. Fourier transformed along the echo time line [40]. Asymmetric sampling of k-space with dynamic adjustment of receiver gain, and partial zero filling of k-space were used to achieve an image matrix size of 1024×512×512, resulting in an isotropic 21.5 μm resolution, in 2 h 7 min for the T1-weighted dataset. A matrix of 512×512×256 with isotropic resolution of 43 μm was generated for the T2-weighted data with total acquisition time of 4 h, 20 min.

We used the T1-weighted images and an atlas of the C57BL/6 mouse brain [36] as a basis for an atlas based segmentation. The T1 images were downsampled to 43 microns in order to decrease memory and computational demands. IRTK [41] was used to perform a suite of affine and nonrigid (free-form) registration between the atlas and the query datasets. The atlas labels were subjected to the same transformation that maximizes the normalized mutual information among the atlas and query images provided label sets for each query image. The resulting labels where manually corrected where necessary, using both the T1 and the T2 weighted scans, to improve the results based on local alignment.

The volumes of the segmented brain regions were calculated using MATLAB (MathWorks, Natick, MA). Strain averages for volumes of 35 independent and compound regions were entered into GeneNetwork (GN) [www.genenetwork.org]. Existing software tools within GN, including web QTL [42] were used for mapping of quantitative trait loci (QTLs) contributing to corpus callosum volume. Since only two genotypes can be investigated in these strains (B/B and D/D), all effects are analyzed under an additive model.

Author Contributions

Conceived and designed the experiments: MP AB RW MK. Performed the experiments: MP AB. Analyzed the data: MP AB RW MK. Contributed reagents/materials/analysis tools: MP AB RW. Wrote the paper: MP AB RW MK.
References

1. Richards LJ, Plachez C, Ren T (2004) Mechanisms regulating the development of the corpus callosum and its agenesis in mouse and human. Clin Genet 66: 276–289.

2. Donahoe AL, Richards LJ (2009) Understanding the mechanisms of callosum development through the use of transgenic mouse models. Semin Pediatr Neurol 16: 127–142.

3. Gazzaniga MS (2005) Forty-five years of split-brain research and still going strong. Nat Rev Neurosci 6: 655–659.

4. Dornow KW, Gazzaniga MS (2008) Neuroimaging techniques offer new perspectives on callosal transfer and interhemispheric communication. Cortex 44: 1023–1029.

5. Schöll-Apaxal CC, Wagner K, Bilder M, Erü-Wagner B, Heinrich U, et al. (2008) A 17 Mb deletion in 14q12 causes severe mental retardation, mild facial dysmorphism and Rett-like features. Am J Med Genet A 146A: 1994–1998.

6. Nagamani SC, Erez A, Lin Y, Li X, Ou Z, Chinault C, et al. (2009) Interstitial deletion of chromosome 15q21.2q21.3: a comparison with previously described cases. Eur J Med Genet 51: 639–645.

7. Masured-Paulet A, Callier P, Thawnin-Robinet C, Chouchane M, Mejean N, et al. (2009) Multiple cytvs of the corpus callosum and psychomotor delay in a patient with a 3.1 Mb 15q24.1q24.2 interstitial deletion identified by array-CGH. Am J Med Genet A 194A: 1504–1510.

8. Audureau J, Lepretre F, Cuisinier JM, Goldenberg A, Dobbel B, et al. (2006) Deletion 15q21.2q21.32 involving TCF4 in a boy diagnosed by CGH-array. Eur J Med Genet 51: 172–177.

9. Najm J, Horn D, Wimpfinger L, Golden JA, Chizhikov VV, et al. (2008) Mutations of CASK cause an X-linked brain malformation phenotype with microcephaly and hypospadias of the brainstem and cerebellum. Nat Genet 40: 1065–1067.

10. Van Bever Y, Yeom L, Laridon A, Reynolds E, van Luijk R, et al. (2005) Clinical report of a pure subtelomeric lqter deletion in a boy with mental retardation and multiple anomalies adds further evidence for a specific phenotype. Am J Med Genet A 135: 91–95.

11. Poot M, Kroeis HY, V D Wijse SE, Elemej NV, Roos M, et al. (2007) Four patients with speech delay, seizures and variable corpus callosum abnormalities and the controversy about the candidate genes located in 1q44. Cytogenet Genome Res 127: 5–8.

12. Plomin R, McClearn GE, Gora-Maslak G, Neiderhiser JM (1991) Use of recombinant inbred strains to detect quantitative trait loci associated with behavior. Behav Genet 21: 99–116.

13. Peil J, Dale AM, Johnson GA (2008) Automated segmentation of the actively stained mouse brain using multi-spectral MR microscopy. Neuroimage 37: 82–89.

14. Koutnikova H, Laakso M, Lu L, Combe R, Paananen J, et al. (2009) Identiﬁcation of the UBP1 locus as a critical blood pressure determinant using a combination of mouse and human genetics. PLoS Genet 5: e1000591.

15. van Bever Y, Zoon M, van den Anstel HK, Hochstenbach R (2010) Recurrent copy number changes in mentally retarded children harbouring neuroimaging ﬁndings in a series of 41 patients. Am J Med Genet A 146A: 2501–2511.

16. Badea A, Sharief AA, Dale AM, Johnson GA (2008) Genetic dissection of the mouse brain. Magn. Res. Med 56: 717–725.

17. Koutnikova H, Laakso M, Lu L, Combe R, Paananen J, et al. (2009) Automated segmentation of the actively stained mouse brain using multi-spectral MR microscopy. Neuroimage 41: 689–690.

18. Visser P, van der Meulen E, Laridon A, Reynolds E, van Luijk R, et al. (2005) Clinical and molecular characteristics of 1qter microdeletion syndrome: is there a recognizable syndrome? Clin Dysmorphol 13: 103–106.

19. Laaleni SR, Sahoo T, Sanders ME, Peters SU, Beijani BA (2006) Coarctation of the aorta and mild to moderate developmental delay in a child with a de novo deletion of chromosome 15q21.2q22.2. BMC Med Genet 7: 8.

20. Tempesta S, Sollima D, Ghezzo S, Polini V, Sinagaglia B, et al. (2008) Mild mental retardation in a child with a de novo interstitial deletion of 15q21.2q21.1: a comparison with previously described cases. Eur J Med Genet 51: 639–645.

21. Poot M, Kroeis HY, V D Wijse SE, Elemej NV, Roos M, et al. (2007) Dandy-Walker complex in a boy with a 5 Mb deletion of region 1q44 due to a paternal t(1;20)(q44;q13.33). Am J Med Genet A 143A: 1038–1041.

22. Plomin R, Mclearn GE, Gora-Maslak G, Neiderhiser JM (1991) Use of recombinant inbred strains to detect quantitative trait loci associated with behavior. Behav Genet 21: 99–116.

23. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

24. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

25. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

26. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

27. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

28. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

29. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

30. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

31. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

32. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

33. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

34. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

35. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

36. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

37. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

38. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

39. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.

40. Johnson GA, Shariat AA, Badea A, Brandenburg J, Cofer G, et al. (2007) High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. Neuroimage 37: 82–89.