Quantitative Phenotyping

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After the completion of the genome sequence of the human and of many other species, it becomes critically important to understand the role of each gene in normal development and in disease. For mammalian systems, much of this will be worked out in the mouse. The mouse has a 99% overlap in recognizable genes with the human. The mouse can show many of the symptoms of human diseases. Mice are relatively easy to breed and house, and there are sophisticated biological techniques available for deliberately manipulating the genome.

With the intent of understanding the role of genes in human, an international consortium has recently completed the knockout of each of the ~20,000 genes in the mouse, one at a time, resulting in 20,000 unique strains of mice(1,2) . Most of these knockouts currently exist only as frozen embryonic stem cells. The present challenge facing the international community is the production of each of these genetically modified lines into live mice and then the phenotypic characterization of differences in each of the knockout lines. For mice

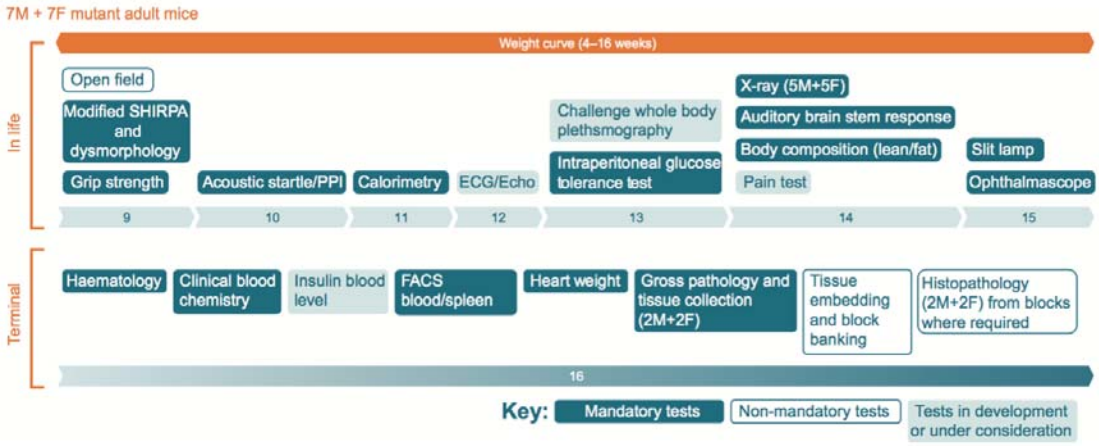

Figure 1 The International Mouse Phenotyping Consortium (IMPC) pipeline for phenotyping adult mice strains that have each had one of the ~20,000 encoding genes knockout out(3).

that reach adulthood, systematic protocols have been developed for the phenotyping of these mice as shown in Figure 1. Phenotyping for this purpose needs to consist of quantitative and statistically evaluable measures that can be achieved at hithroughput and minimal cost. By early 2016, 5,000 of these lines will have been phenotyped and funding is currently being arranged to complete the full task by 2021.

About 30% of these mouse knockout lines will be embryonic lethal. Nonetheless, it is important to understand in what way these critical genes are essential to viability of the

individual mouse. Phenotyping, in this case, will be carried out using three-dimensional imaging techniques and quantitative analysis of the mutant group compared with the control group to identify differences in anatomy (Fig. 2)(4). The computer analysis used is

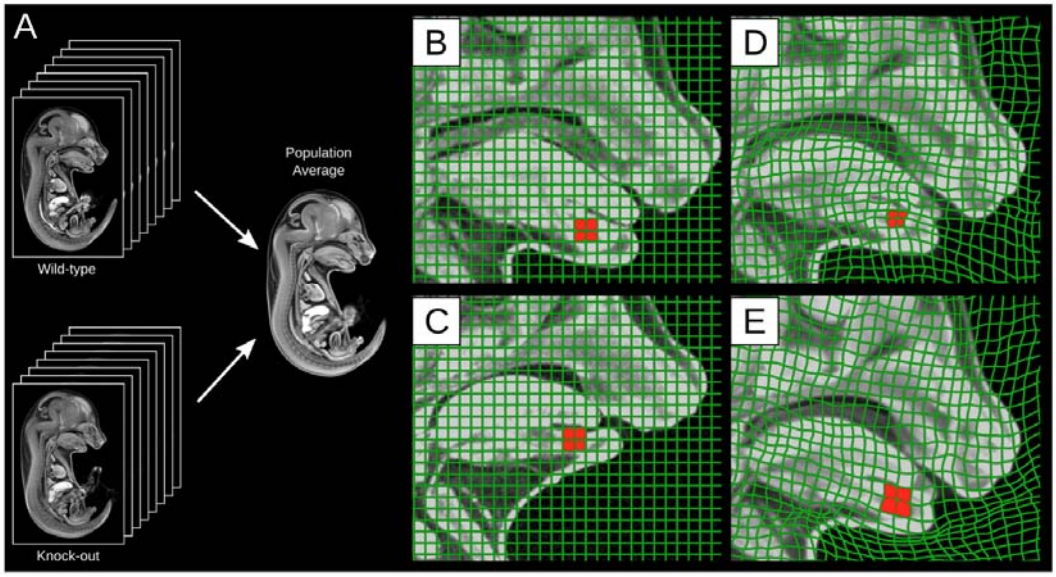

Figure 2 illustrates how registration enables voxel-based morphometry to identify statistically significant differences in anatomical volume between a mutant group and a wild type group of mice.

adapted from the analysis of both human and mouse brain imaging and is based on a process of multi-scale nonlinear registration followed by measures of statistically significant differences in neuroanatomy(5). These computer automated brain analysis techniques are now well established for the mouse brain and are used by a number of groups around the world interested in looking at the effect of gene changes on the structure (and function) of the mouse brain.

We have taken these techniques, adapted them to the analysis of embryos, at either E15.5 or earlier at E9.5. Three-dimensional embryo magnetic resonance imaging can be done at both of these time points (Fig.3). It turns out that better images can be obtained with optical projection tomography (OPT)(6,7) at E9.5 and with X-ray micro CT at E15.5 (Fig. 4). Both of these techniques are simpler than MRI and are more amenable to high throughput than is MRI. Nonetheless, the computer analysis techniques are essentially similar for any of the three-dimensional imaging data sets.

From initial experiments of embryonic lethal knockouts, we have shown that we recapitulate phenotypes where they have been reported in the literature and that we almost always find additional phenotypic variation that has not been realized without the full coverage of 3D imaging. In knockouts that have never been characterized, 3D imaging provides a very comprehensive view of differences between the knockout embryo and the wild type control.

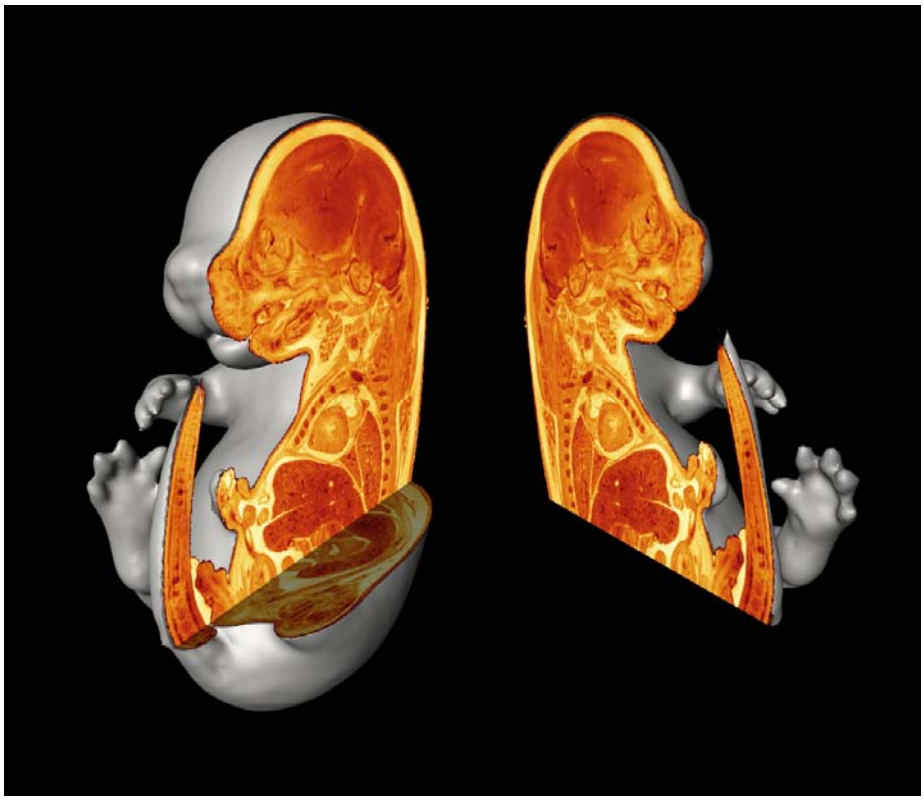

Figure 3 Three-dimensional MRI of a mouse embryo at E15.5. Image is taken with an FSE pulse sequence at 7T and an isotropic resolution of 25μm.

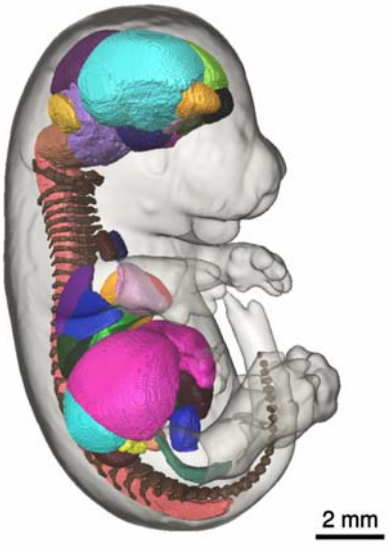

Figure 4 An average wild type embryo atlas from 35 three-dimensional microCT images that has been manually segmented to identify specific organs of interest for volumetric determination.

One additional challenge in the imaging and analysis of embryos is that significant differences can occur between the mutant and its control simply due to growth delay in the mutant. Additionally, because we only know the embryonic age of an embryo to ± 0.5 days and embryo development is happening very rapidly over a single day, comparisons can show differences which are merely the result of growth delays. To correct this, we have developed a four-dimensional atlas of 3D embryo anatomy developing over time as a reference data set. Then, individual mutant embryos can be compared with this 4D data set and both anatomical differences can be identified and growth delays can be recognized (Fig. 5)(8). These analysis techniques provide powerful methods for handling three-dimensional data sets with associated time variation.

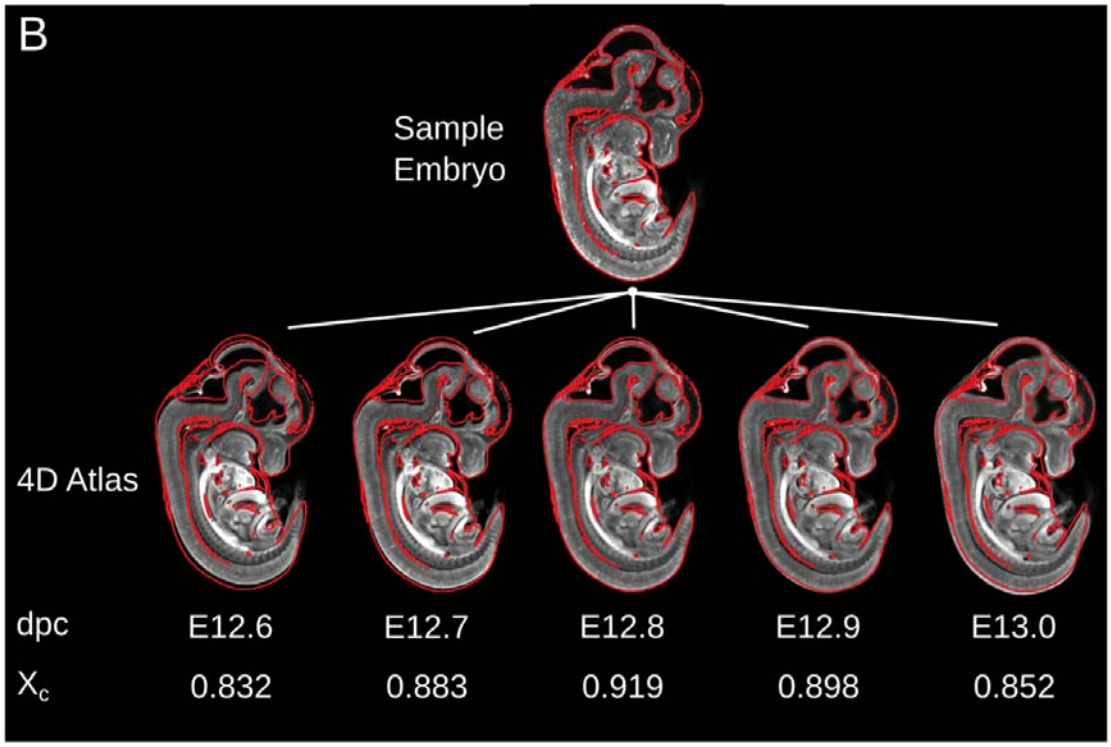

Figure 5 A single embryo is compared with a four-dimensional atlas plus time of development, enabling the apparent age to be identified to a resolution of ± 0.1 days or 2½ hours.

In summary, while MRI will continue to be used as the imaging method of choice for mouse brain phenotyping, embryos will be imaged with CT and OPT in the case of embryonic lethals. Nonetheless, the analysis methods for obtaining quantitative phenotypic differences based on anatomy will be similar for all types of three-dimensional imaging. Completion of the phenotypic task will give a primary phenotypic analysis of the role of every gene in the mammal and its contribution to normal development and an indication of its implication in disease models.

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