Supplementary Information

The International Mouse Phenotyping Consortium (IMPC): a functional catalogue of the mammalian genome that informs conservation

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Supplementary Methods

Viability data and mouse-to-human orthologue inferences

We used mouse viability data collected by the International Mouse Phenotypic Consortium (IMPC, www.mousephenotype.org) using a dedicated embryonic pipeline. The current dataset corresponds to Data Release (DR) 7.0., which includes data for 1,751 genes previously published (Dickinson et al. 2016, DR 4.0) as well as data collected since then. Viability data by the IMPC is analysed as defined in IMPRESS (the International Mouse Phenotyping Resource of Standardised Screens, https://www.mousephenotype.org/impress/), based on a minimum of 28 pups screened before weaning and ascertains absence of knockout (null) homozygote pups to classify the gene as essential. Thus, lethal lines are defined as those with an absence of null homozygous pups, or lacking a heartbeat, while subviable lines are those with fewer than 12.5% homozygous pups (half of the 25% expected; *P* < 0.05, binomial distribution). Viable mouse lines are those for which homozygote (null and wild type) and heterozygote pups are observed in normal Mendelian ratios. We filtered out genes for which sample size was insufficient (total pups < 28, *n* = 10 genes), hemizygous genes (*n* = 1 gene), as well as those with conflicting calls (genes that appear in more than one viability category, *n* = 29 genes), resulting in a set of 4,237 genes, of which 1,052 had a lethal phenotype, 383 a subviable phenotype, and 2,802 a viable phenotype (25%, 9% and 66%, respectively). We compared these results with those obtained in studies of human cell lines, in which gene essentiality was determined by observing cell proliferation (cell viability) after a gene was knockout, of a total of approximately 18,000 genes. We used the human cell datasets from Blomen et al. (2015), Hart et al. (2015) and Wang et al. (2015), involving 2, 5 and 4 human cell lines, respectively.

In order to compare mouse and human viability data sets based on IMPC mouse data and human cell studies, first we unified all human gene names in all data sets for the most recent HGNC approved symbol. To do this, we used the Multi-symbol Checker tool available through the HGNC website. All symbols from the literature (Hart et al. 2015, Wang et al. 2015) that mapped to more than one identifier (previous symbol, synonym) were discarded (~4% of the genes). When Ensembl identifiers as well as symbols were provided (Blomen et al. 2015), we obtained HGNC identifiers by accessing the Ensembl REST API using a custom script and the Multi-symbol Checker tool available through the HGNC website; conflicting identifiers were discarded. This resulted in a combined dataset comprising 18,862 genes. We then assigned the labels "essential" and "non-essential" to each cell line value, following the thresholds established in the original studies for each cell line. We then classified a gene as essential if it was essential for cell viability in more than 50% of the cell lines where the gene had been studied, when it had been studied in more than 50% of the cell lines (equivalent to ≥ 6 cell lines; 1,189 genes discarded). Second, we inferred mouse-to-human orthologues using custom scripts and the human-mouse orthologue file compiled by HCOP (Eyre et al. 2007), which we downloaded on 6 March 2018 from the HGNC website (HUGO Gene Nomenclature Committee; Gray et al. 2015). Orthologue predictions compiled by HCOP were obtained using 12 inference methods (eggNOG, Ensembl Compara, HGNC, HomoloGene, Inparanoid, NCBI Gene Orthology, OMA, OrthoDB, OrthoMCL, Panther, PhylomeDB and TreeFam). We kept inferences having a prediction based on 5 or more inference methods, which resulted in a dataset containing 4,087 mouse-to-human orthologues with IMPC viability information. This IMPC mouse-to-human orthologue viability dataset comprised 1,044 lethal, 380 subviable and 2,663 viable genes (26, 9 and 65%, respectively), thus resulting in nearly identical proportions to those obtained from the entire viability dataset. Of these, 4,028 genes had been analysed in the human cell studies. With these data, we obtained genes necessary for organism and cell viability (Table 1).

Obtaining mouse orthologues for genes in non-model species

In order to explore the opportunities for IMPC data to contribute to conservation and evolutionary biology studies of mammal species, we selected studies that explored functional adaptations in non-model species based on genomic data. The selected species included the African cheetah (*Acinonyx jubatus)*, the gorilla *(Gorilla gorilla)*, the grey wolf (*Canis lupus*), the giant and red pandas (*Ailuropoda melanoleuca* and *Ailurus fulgens*), the Iberian lynx (*Lynx* *pardinus*), the polar bear (*Ursus maritimus*), and the Tasmanian devil (*Sarcophilus harrisii*), and explored the opportunities for the IMPC to contribute functional knowledge.

We retrieved mouse orthologues for the giant panda, the grey wolf, the western gorilla, the Tasmanian devil, the polar bear, and the Iberian lynx using MetaPhOrs (Pryszcz et al. 2011), applying an Orthology Consistency Score (CS) cut-off of 0.5 (ftp://phylomedb.org/metaphors/release-201601/orthologs, last accessed 06 September 2017) (Supplementary Table S4). MetaPhOrs provided mouse orthologues that we converted to Ensembl protein identifiers using the MetaPhOrs cross-reference file, ext2meta.txt.gz, and then to MGI (Mouse Genome Informatics) identifiers by means of the BiomaRt R package (Durinck et al. 2009). MetaPhOrs is a publicly available repository of phylogeny-based orthology and paralogy predictions computed using seven homology prediction services (COG, eggNOG, Ensembl Compara, Fungal Orthogroups, OrthoMCL, PhylomeDB and TreeFam) and based on 705,123 phylogenetic trees for 829 genomes. We used these data sets to retrieve the number of IMPC phenotypes that could be associated to these species via their mouse orthologues (Figure 2).

We then obtained specific sets of genes of interest from the different species, including infertility genes in the African cheetah (Dobrynin et al. 2015), loss-of-function (LoF) genes and disease genes in gorillas (Xue et al. 2015), positively selected genes in the polar bear (Liu et al. 2014), wolf genes potentially under selection (42K SNP array, Schweizer et al. 2016a), wolf genes with nonsynonymous mutations significantly correlated with environmental variables (resequencing data of 1,040 loci, Schweizer et al. 2006b), and giant and red panda genes involved in convergent evolution to a bamboo-rich diet (Hu et al. 2017). We inferred orthologous genes using Ensembl BioMart (Ensembl release 92) to obtain ENSMUSG (mouse) identifiers. When BioMart provided no orthologue, we used the Ensembl website, which contains additional information. We used the ENSMUSG identifiers to obtain MGI identifiers in the MGI database. Ensembl mouse gene identifiers (ENSMUSGXXXXX) or MGI identifiers (MGI:XXXXX) can both be used to query the IMPC (www.mousephenotype.org) and MGI (http://www.informatics.jax.org/) databases to obtain phenotype data.

Supplementary Tables

Supplementary Table S1 Test for the differences in the distribution of gorilla LoF alleles in the 3 viability categories obtained for IMPC mice (alternative hypothesis, H_1) at the significance level of 0.05 (A). A test in accordance to 2 viability categories was also performed (B). Gbb, mountain gorillas; Gbg, eastern lowland gorillas; Ggg, western lowland gorillas.

A) χ² goodness-of-fit test

B) Exact binomial test

Supplementary Table S2 Exact binomial test for the differences in the distribution of gorilla LoF alleles in the 2 viability categories obtained for human cell lines $(H₁)$ at the significance level of 0.05. See Table S1 for gorilla population abbreviations.

Supplementary Table S3 Human (a) and mouse orthologues (b) of gorilla genes with homozygous LoF alleles and their association to essentiality based on human cell studies (a) or IMPC and MGI data (b), used in Figure 1.

(A) Human orthologues

(B) Mouse orthologues

** Includes young adult phenotypes, viability and fertility data ** Includes young adult phenotypes, viability and fertility data

References not in the main text

- Durinck S, Spellman PT, Birney E, Huber W (2009) Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. Nature Protocols, 4, 1184.
- Eyre TA, Wright MW, Lush MJ, Bruford EA (2007) HCOP: a searchable database of human orthology predictions. Briefings in Bioinformatics, 8, 2-5.
- Gray KA, Yates B, Seal RL, Wright MW, Bruford EA (2015) Genenames.org: the HGNC resources in 2015. Nucleic Acids Research, 43, D1079-D1085.
- Pryszcz LP, Huerta-Cepas J, Gabaldon T (2011) MetaPhOrs: orthology and paralogy predictions from multiple phylogenetic evidence using a consistency-based confidence score. Nucleic Acids Research, 39, e32.