**Title:**
Health and population effects of rare gene knockouts in adult humans with related parents *†*

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**Abstract:**
Examining complete gene knockouts within a viable organism can inform on gene function. We sequenced the exomes of 3,222 British Pakistani-heritage adults with high parental relatedness, discovering 1,111 rare-variant homozygous genotypes with predicted loss of gene function (knockouts) in 781 genes. We observed 13.7% fewer than expected homozygous knockout genotypes, implying an average load of 1.6 recessive-lethal-equivalent LOF variants per adult. Linking genetic data to lifelong health records, knockouts were not associated with clinical consultation or prescription rate. In this dataset we identified a healthy PRDM9 knockout mother, and performed phased genome sequencing on her, her child and controls, which showed meiotic recombination sites localised away from PRDM9-dependent hotspots. Thus, natural LOF variants inform upon essential genetic loci, and demonstrate PRDM9 redundancy in humans.

**Main Text:**

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Complete gene knockouts, typically caused by homozygous loss of function (LOF) genotypes, have helped identify
the function of many genes, predominantly through studies in model organisms and of severe Mendelian-inherited diseases in
humans. However, information on the consequences of knocking out most genes in humans is still missing. Naturally
occurring complete gene knockouts offer the opportunity to study the effects of lifelong germline gene inactivation in a living
human. A survey of LOF variants in adult humans demonstrated ~100 predicted LOF genotypes per individual, describing
around ~20 genes carrying homozygous predicted LOF alleles and hence likely completely inactivated(1). Almost all these
homozygous genotypes were at common variants with allele frequency >1%, in genes likely to have weak or neutral effects on
fitness and health(1). In contrast, rare predicted LOF genotypes were usually heterozygous and thus of uncertain overall
impact on gene function. A large exome sequencing aggregation study (ExAC), of predominantly outbred individuals,
identified 1,171 genes with homozygous predicted LOF genotypes in 60,706 individuals(2). Furthermore, 1,171 genes with
complete predicted LOF were identified in 104,220 Icelandic individuals(3), and modest enrichment for homozygous predicted
LOF genotypes shown in Finnish individuals(4). However, even in these large samples, homozygous predicted LOF
genotypes tend to be for variants at moderate (around 1%) allele frequency, and hence these approaches will not readily
assess knockouts in most genes, which are lacking such variants.

Here, we identify knockouts created by rare homozygous predicted loss of function (rhLOF) variants by exome
sequencing 3,222 Pakistani-heritage adults living in the UK who were ascertained as healthy, type 2 diabetic, or pregnant(5).
These individuals have a high rate of parental relatedness (often with parents who are first cousins) and thus a substantial
fraction of their autosomal genome occurs in long homozygous regions inferred to be identical by descent from a recent
common ancestor (autozygous). We link the genotype to healthcare and epidemiological records, with the aims of i)
describing the properties of, and assessing the health effects of, naturally occurring knockouts in an adult population, ii)
understanding the architecture of gene essentiality in the human genome, through the characterization of the population
genetics of LOF variants, and iii) studying in detail a knockout of the PRDM9 gene which plays a role in human meiotic
recombination(6).

On average, 5.6% of the coding genome was autozygous, much higher than that in outbred European heritage
populations (figs. 1A, S4). We identified, per subject, on average 140.3 non-reference predicted LOF genotypes comprising
16.1 rare (minor allele frequency <1%) heterozygotes, 0.34 rare homozygotes, 83.2 common heterozygotes and 40.6
common homozygotes. Nearly all rhLOF genotypes were found within autozygous segments (94.9%)(5), and the mean
number of rhLOF per individual was proportional to autozygosity (fig. 1B). Overall we identified 1,111 rhLOF genotypes at
847 variants (575 annotated as LOF in all GENCODE-basic transcripts) in 781 different protein-coding genes (fig. 1C)(5) in
821 individuals. Autozygous segments were observed across all exonic sites with a density distribution not significantly
different from random (5)(Shapiro-Wilks P=0.112). From these values we estimate that 41.5% of individuals with 6.25%
avtozygosity (expected mean for individual with first-cousin related but otherwise outbred parents) will have one or more
rhLOF genotypes (fig. 1B).

The majority of identified genes with rhLOF genotypes (422) had not been previously reported, although 167 had
been reported as containing homozgyous or compound heterozygous LOF genotypes in Iceland, and 299 in ExAC. In total,
107 rhLOF genes were common to all three datasets (5) suggesting a subset of genes either tolerant of LOF and/or with
higher rates of mutation. 89 rhLOF genotypes were homozygotes without observed heterozygotes, and we observed three
subjects each with 5 rhLOF genotypes. On the basis of these observations we predict that in 100,000 subjects with first-
cousin related parents of the same genetic ancestry we would expect at least one knockout in ~9,000 of the ~20,000 human
protein-coding genes (fig. S3)(5).

We observed a lower density of annotated rare LOF variants within autozygous tracts, where they are
homozygous, compared to outside autozygous tracts, where they are typically heterozygous, indicative of direct negative
selection on a fraction of homozygotes (fig. 2A). We matched each of the 16,708 rare annotated LOF (heterozygous and
homozygous) variants to a randomly selected synonymous variant of the same allele frequency, and observed 842 rare
LOF variants with => 1 homozygous genotype versus an average of 975.5 rare synonymous variants with => 1
homozygote, indicating a deficit of 13.7% (95% confidence interval 8-20%) of variants with rhLOF genotypes (fig. 2B)(5).
We attribute this deficit to some rhLOF genotypes resulting in early lethality or severe disease and thus being incompatible
with our selection criteria as healthier adults, although our data does not inform whether these are due to fewer high-
penetrance, or more low-penetrance variants. This deficit is higher than in the Icelandic population (6.4%)(3), consistent
with that analysis being biased towards more common variants already subject to selection.

We then combined the calculated deficit rate with the observed number of heterozygous annotated LOF variants,
integrating across allele frequencies, to obtain a direct estimate of the recessive lethal load per person. This suggests that
a typical individual from the population we sampled carries 1.6 recessive annotated LOF lethal-equivalent variants in the
heterozygous state(5). This is similar to previous estimates of the lethal load calculated by correlating the number of
miscarriages, stillbirths and infant mortalities with the level of autozygosity (fig. 2C)(7, 8), and also similar to measurements
in other species(9). Using epidemiological data from 13,586 mothers from the same Born In Bradford birth cohort studied in
our genetic analysis, we estimated 0.5 lethal equivalents resulting in miscarriage, stillbirth or infant mortality per individual
in our population(5). The difference between our two estimates can be accounted for by the fact that the first includes
embryonic lethals, whereas the second only involves deaths after a registered pregnancy, suggesting that there are twice
as many recessive mutations that are embryonically lethal as those that result in fetal or infant death. Controlling for other
effects by comparing to synonymous mutations, we see a significant but moderate decrease (RA/B jackknife test P=0.04) in
the rhLOF mutational load in our Pakistani heritage population dataset compared to outbred populations from the 1000
We examined 215 genes with rhLOF in our dataset that have an exact 1:1 mouse:human gene ortholog. From mouse gene knockout data there were 52 genes where a lethal mouse phenotype had been reported on at least one genetic background(10). Whether or not the mouse ortholog knockout is lethal is not associated with alteration of protein function, duplication or changes in gene expression(5). Genes containing rhLOF showed 50% fewer molecular interactions compared to all genes in the STRING interactome dataset (Kruskal-Wallis P=3.4 x 10⁻⁵), predominantly driven by the Binding Interaction class (Kruskal-Wallis P=9.3 x 10⁻¹¹). We saw a similar reduction in the Icelandic data (table S4), in contrast to both known pathogenic LOF variants and pathogenic gain-of-function (GOF) variants reported in Orphanet, which showed increased overall molecular interactions (P=1.1x10⁻⁶, 2x10⁻¹² respectively)(5). Furthermore rhLOF genes that are drug targets have 11.4% phase I to approval rate versus 6.7% for all target-indication pairs (chi-squared P=0.046), although we observed no difference in the proportion of genes known or predicted to be druggable targets(11) for rhLOF genes (15%) compared to all genes (13%, P=0.098)(5).

In subjects from the Born In Bradford study, where full health record data was available, we observed 54 rhLOF genotypes in 52 individuals in Online Mendelian Inheritance in Man (OMIM) confirmed recessive disease genes. Our expectation was that these would be enriched for false positive observations(7). After a quality control analysis of the sequence-based genotype calls(5), we inspected the annotation of these variants(7). We considered 16 of 54 rhLOF genotypes to be possible genome annotation errors (i.e. incorrectly described as LOF) (5)(table S2). Only six of the remaining 38 rhLOF subjects had definite lifetime primary health record diagnoses recorded consistent with the OMIM phenotype, with a further three genotypes suggestively compatible (table S3). We suggest that the remaining 29 are due to a combination of incomplete penetrance(12–16), late onset of disease (i.e. not yet having occurred), individuals with mild symptoms not seeking medical attention, unrecognised technical issues with sequencing or annotation (e.g. tissue specific alternative splicing), or dubious evidence to support the gene-phenotype assignment (in table S3 we assess the available evidence for these possibilities).

We next assessed electronic health records in the Born In Bradford adults, focusing on the time since study recruitment(5). Drug prescription rate and clinical staff consultation rate have previously been shown to correlate strongly with health status(17). We compared individuals with one or more rhLOF (n=638) to individuals without rhLOF (n=1524), and found no association with prescription rate (logistic regression, OR 1.001, 95% CI 0.988 - 1.014) or consultation rate (OR 1.017, 95% CI 0.996 - 1.038), nor any associations in rhLOF subgroups (5).

One of our subjects was a healthy adult mother with a predicted rare homozygous LOF mutation in PRDM9, which we confirmed experimentally(5)(fig. S7A, S7B). PRDM9 is the major known determinant of the genomic locations of meiotic recombination events in humans and mice through its DNA binding site zinc finger domain (6, 18, 19). We excluded that this rhLOF was from a somatic loss of heterozygosity event on the basis that this subject is heterozygous, not homozygous, on phases of whole genome sequencing and identified 39 candidate crossovers(5). Using similar methods we saw that 18.5% of crossovers observed in the PRDM9 knockout duo (confidence interval: 1% - 42%) occurred in linkage disequilibrium based hotspots versus 75.7% in the control duo (confidence interval 57%-89% consistent with a previously published estimate of an average of 60% of crossovers occurring at hotspots(18))(5).

PRDM9 knockout mice demonstrate abnormal location of recombination hotspots with enrichment at gene promoters and enhancers, and also fail to properly repair double-stranded breaks and are infertile (both sexes sterile)(21, 22). Dogs, which lack Prdm9, retain recombination hotspots which unlike humans or knockout mice occur in high GC content regions(23). It has been speculated that dog recombination is controlled by an ancestral mammalian mechanism, and that PRDM9 competes and usurps these sites when active in non-canids(23, 24). However we did not see an increased overlap in our PRDM9-knockout duo crossover intervals with promoters and their flanking regions or enrichment in GC content, compared to the control duo(5). Thus the healthy and fertile PRDM9-deficient adult human suggests differences from both mice and dogs, and supports the possibility of alternative mechanisms of localizing human meiotic crossovers(25, 26).

Together these data suggest that apparent rhLOF genotypes identified by exome or genome sequencing from adult populations require caution interpretation. Although this class of variants has the greatest predicted effect on protein function, loss of most proteins is relatively harmless to the individual, and even in previously annotated disease genes predicted rare LOF homozygotes may not always be as clinically relevant as often considered. This becomes of increasing importance now that exome and genome sequencing is rapidly expanding into healthier adults. We anticipate that further efforts to identify naturally occurring human knockouts, whether in bottlenecked populations, or more efficiently as here in

Genomes Project, although this is less than that caused by the historic bottleneck in the Finnish population (FIN in fig. 2D)(5).
subjects with related parents, will yield both new data relevant to clinical interpretation, and new biological insights, as exemplified by our investigation here of a \textit{PRDM9} deficient healthy and fertile woman.
REFERENCES AND NOTES


5. Supplementary Materials.


23. E. Axelsson et al., Death of PRDM9 coincides with stabilization of the recombination landscape in the dog genome.


34. A. McKenna et al., The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 20, 1297–1303 (2010).


45. R. Do et al., No evidence that selection has been less effective at removing deleterious mutations in Europeans than in Africans. *Nat. Genet.* 47, 126–131 (2015).


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RD declares his interests as a founder and non-executive director of Congenica Ltd., that he owns stock in Illumina Inc. from previous consulting and is a scientific advisory board member of Dovetail Inc. MSL and KG are employees of 10X Genomics Inc.

Data reported in the paper are presented in the Supplementary Materials, and are available under a Data Access Agreement at the European Genotype-phenome Archive (www.ebi.ac.uk/ega) under accession numbers EGAS00001000462, EGAS00001000511, EGAS00001000567, EGAS00001000717 and EGAS00001001301.

SUPPLEMENTARY MATERIALS
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Fig. 1. Discovery and annotation of rhLOF variants. (A) Autozygous segment numbers and length for Pakistani heritage subjects in the UK, and 1000 Genomes project European (CEPH Utah residents with ancestry from northern and western Europe; CEU) individuals. (B) Autozygosity and rhLOF in 3,222 individuals. Count of number of individuals (left Y axis, blue columns) binned by fraction of autozygous genome (X axis, showing values from 0.00 to 0.12), with mean number of rhLOF genotypes per individual (right Y axis, orange circles). (C) Distribution of LOF variants by allele frequency, heterozygous or homozygous genotype, predicted protein consequence, and whether predicted for a full or partial set of GENCODE Basic transcripts for the gene.
Fig. 2.
Population genetic analysis of rhLOF variants.
(A) Comparison of number of LOF variants per unit length in autozygous regions (LOF A) with expected rate from non-autozygous sections (LOF NA) showing suppression of rhLOFs (t-test). A similar analysis of synonymous (Syn) variants shows no significant differences. (B) Observed number of variants with homozygote genotypes in 16,708 rare LOF variants (orange circle) versus a frequency matched subsampling of synonymous variants (blue violin plot). (C) Quantification of the recessive lethal load carried on average by a single individual. Direct subsampling estimate for rhLOF variants from current study (orange circle); epidemiological estimates from correlating infant mortality rates to estimated autozygosity in current and published data (blue circles); direct estimate from large Hutterite pedigree (green circle). 95% confidence intervals as black bars. (D) Relative number of derived LOF alleles that are frequent in one population and not another (under neutrality the expectation is 1.0), calculated for 1000 Genomes Project populations and the current Birmingham/Bradford Pakistani heritage population (BB), compared to the CEU population. Error bars represent ±1 (black) or 2 (grey) standard errors, and significant differences (R_{AB} jackknife test) versus CEU population are highlighted in orange circles.