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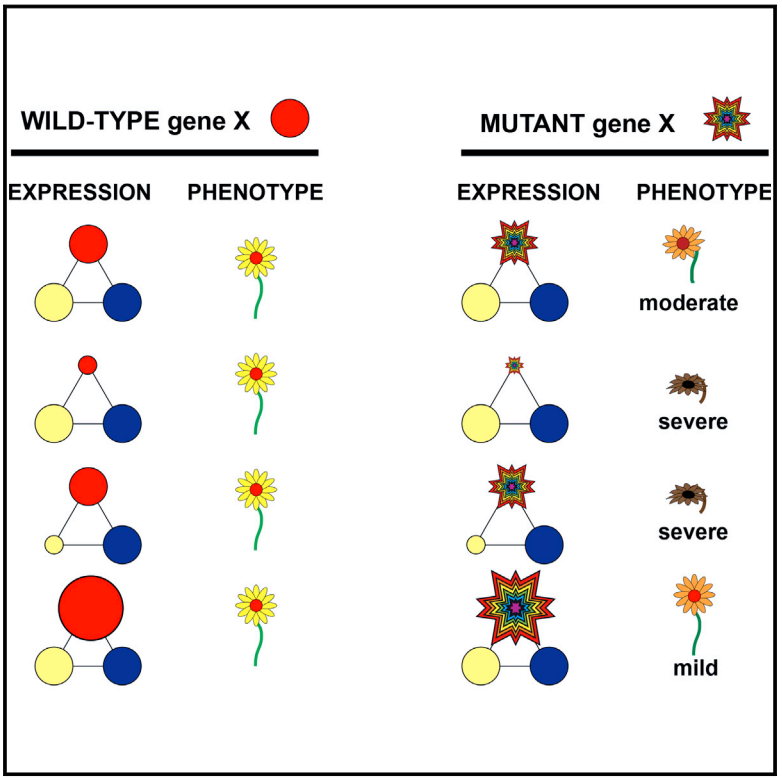
Natural Variation in Gene Expression Modulates the Severity of Mutant Phenotypes

Graphical Abstract



Highlights

- Comparison of loss-of-function phenotypes of 1,400 genes in two *C. elegans* isolates
- ~20% of genes have different loss-of-function phenotypes in two individuals
- Differences in severity of mutant phenotypes predictable from expression

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In Brief

How does genetic background affect the severity of a mutation? A large-scale comparison between two *C. elegans* isolates indicates that the level of expression of a gene predicts the severity of its mutant phenotype, suggesting an unexpectedly straightforward variable underlying the effects of genetic context.

Vu et al., 2015, Cell *162*, 391–402
July 16, 2015
http://dx.doi.org/10.1016/j.cell.2015.06.037

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Natural Variation in Gene Expression Modulates the Severity of Mutant Phenotypes

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http://dx.doi.org/10.1016/j.cell.2015.06.037

SUMMARY

Many mutations cause genetic disorders. However, two people inheriting the same mutation often have different severity of symptoms, and this is partly genetic. The effects of genetic background on mutant phenotypes are poorly understood, but predicting them is critical for personalized medicine. To study this phenomenon comprehensively and systematically, we used RNAi to compare loss-of-function phenotypes for ~1,400 genes in two isolates of *C. elegans* and find that ~20% of genes differ in the severity of phenotypes in these two genetic backgrounds. Crucially, this effect of genetic background on the severity of both RNAi and mutant phenotypes can be predicted from variation in the expression levels of the affected gene. This is also true in mammalian cells, suggesting it is a general property of genetic networks. We suggest that differences in the manifestation of mutant phenotypes between individuals are largely the result of natural variation in gene expression.

INTRODUCTION

Many mutations affect phenotype. Intriguingly, however, an identical genetic mutation can have very different effects on phenotype in different individuals of the same species. In part this is due to differences in genetic background, that is, to the specific combination of rare and common variants that comprise each individual genome. In model organisms, this impact of differences in genetic background on the severity of mutant phenotypes has been known for around a century. However, although there are many isolated examples in organisms ranging from mouse (Dietrich et al., 1993; Hamilton and Yu, 2012; Nadeau, 2001) to yeast (Dowell et al., 2010), we still understand relatively little about the general mechanisms underlying this effect of genetic background. This is a major gap in our basic understanding of how genotype determines phenotype.

Understanding the effect of genetic background on mutant phenotypes also has specific medical relevance. Mendelian disorders such as cystic fibrosis (CF) and sickle cell anemia are the best understood genetic diseases in humans—each disorder is caused by inheritance of a mutation in a single gene (reviewed in Antonarakis and Beckmann, 2006 and cataloged extensively in the Online Inheritance in Man [OMIM] database). However, even classic "monogenic" disorders such as CF show clear genetic background effects—around half of all CF patients of European ancestry are homozygous for the ΔF508 *CFTR* allele (Bobadilla et al., 2002), yet they present with a broad range of clinical symptoms due to variation in so-called "modifier genes," including *MBL2* and *TGF-β* (Wright et al., 2011). Similar effects of genetic background and modifier genes on disease severity are observed in many Mendelian disorders (Dorfman, 2012; Nadeau, 2001), and the picture emerging is that although disease risk may be largely monogenic and its heritability predictable, the severity of the disease phenotype is the outcome of interaction between multiple genes (reviewed extensively in Cooper et al., 2013). We cannot yet predict these effects of genetic background on disease severity with any accuracy, nor do we understand the general mechanisms underlying this. Here we endeavored to carry out a systematic study to examine the effects of genetic background on mutant phenotypes.

In *C. elegans*, many genome-scale RNAi screens have been carried out. These screens have identified the majority of genes whose knockdown causes an obviously detectable phenotype (Kamath et al., 2003). This set of genes is analogous to the set of human genes that cause Mendelian disorders, that is, inheritance of mutant alleles of any one of these genes will have a detectable effect on the phenotype of the individual. We selected 1,353 of these genes to cover a diverse set of RNAi phenotypes, ranging from sterility and lethality to more subtle phenotypes such as tissue development defects (Fraser et al., 2000; Kamath et al., 2003)—our test set thus allows us to perturb most aspects of *C. elegans* biology. By carrying out RNAi on each of these genes in different *C. elegans* wild-type isolates, we can systematically assess the effects of genetic background on the severity of loss-of-function phenotypes in an intact animal model.

Here we report a comparison of the RNAi phenotypes of these ~1,400 test genes in two isolates of *C. elegans*: the canonical N2 Bristol isolate and the CB4856 Hawaiian isolate. They differ genetically by ~1 SNP per 800 bp (Wicks et al., 2001) as well as having many copy-number variants (Maydan et al., 2010). They are the two best characterized *C. elegans* isolates—N2 is the standard laboratory strain (Brenner, 1974), and CB4856 has also been widely used. Furthermore, the efficacy of RNAi itself has been extremely well studied in this pair of isolates (Pollard and Rockman, 2013; Tijsterman et al., 2002). Our results

show that there is extensive variation in the severity of loss-of-function phenotypes between individuals and that this variation in mutant phenotypes is partly predictable from variation in gene expression. We thus suggest that variations in personal gene expression levels are one of the key causes of the variation in disease severity in Mendelian disorders.

RESULTS

A Quantitative RNAi Screen Identifies Many Genes with Different RNAi Phenotypes in Two *C. elegans* Isolates

To assess how differences in genetic background affect the severity of loss-of-function phenotypes, we compared the RNAi phenotypes of each of 1,353 test genes in two isolates of *C. elegans*: the canonical N2 Bristol isolate and the CB4856 Hawaiian isolate. To measure RNAi phenotypes, we used a quantitative phenotyping pipeline that we adapted from methods we had previously described (Lehner et al., 2006). In outline, in any single RNAi assay, ~15 L1-stage worms are mixed with double-stranded RNA (dsRNA)-expressing bacteria targeting a gene of interest and incubated for 4 days in 96-well plates. The contents of each well are then analyzed using a commercially available worm sorter—the body length and the optical density of each worm or embryo in the sample are measured (shown schematically in Figure 1A). In control cultures fed with non-targeting dsRNA-expressing bacteria, there are ~800 animals—~15 adults and their progeny (at L1, L2, or L3 larval stages). When any of our test genes is targeted, however, we can measure changes in brood size, embryonic lethality, and growth rate, as illustrated schematically in Figure 1B. Almost all (1,319/1,353; 97%) of the test genes have a detectable RNAi phenotype in at least one of these assays.

We compared RNAi phenotypes for each of the 1,353 test genes in N2 and CB4856 in three independent biological repeats, each with three technical replicates as shown in Figure 1A; details of data analysis are found in Experimental Procedures. In broadest outline, 9% (120/1,353) of genes screened had significantly more severe phenotypes in CB4856, and 42% (570/1,353) had more severe phenotypes in N2 (all data in Table S1). Before analyzing these gene sets, we carried out a series of validation steps described in the next section.

Data Validation and Confirmation of Differences in RNAi Phenotype with Mutant Alleles

To validate our data, we carried out a series of tests. First, to confirm the differences in RNAi phenotypes we observed with the quantitative phenotyping pipeline, we repeated the phenotyping manually. We had captured images of each well that was analyzed using the worm sorter; two observers scored all images and categorized phenotypes into five classes: N2 more severe (strongly or weakly), similar phenotype in N2 and CB4856, and CB4856 more severe (strongly or weakly) (all data in Table S1). Overall, the overlap between quantitative and manual phenotype was 55%; that is, 382/690 of the genes that had more severe phenotypes in N2 or CB4856 could be confirmed by manual screening. We note that almost all differences are due to the greater sensitivity of the quantitative sorter assay because the agreement is > 90% for the largest differences in phenotype (see Figures S1A and S1B). We also set up an additional three replicates of all RNAi experiments and phenotyped them manually, and the results were again similar (Figures S1A and S1B). We thus believe that the sorter data give accurate measures of the differences in RNAi phenotype between the isolates.

Second, we removed any confounding effects in which differences in RNAi phenotype between N2 and CB4856 were due to differences in RNAi efficacy—this can vary between isolates of *C. elegans.* Differences in RNAi efficacy between N2 and CB4856 are well-characterized—CB4856 has less effective RNAi than N2 (Pollard and Rockman, 2013; Tijsterman et al., 2002). Thus, whereas more severe RNAi phenotypes in the CB4856 isolate are indeed due to true differences in severity of loss-of-function phenotype, more severe RNAi phenotypes in N2 may be due to greater levels of knockdown in the N2 background. CB4856 has a naturally occurring null mutation in *ppw-1*, a member of the Argonaute family, and genetic studies have shown that this is the sole relevant variant affecting RNAi efficacy between N2 and CB4856 (Pollard and Rockman, 2013). We can thus control for the effect of variation in RNAi efficacy by directly assessing the contribution of *ppw-1* activity to differences in RNAi phenotypes between N2 and CB4856.

To test whether the more severe RNAi phenotypes that we see in the N2 isolate are due to differences in *ppw-1* activity between N2 and CB4856, we compared the RNAi phenotypes of all 570 genes that had more severe RNAi phenotypes in N2 in three worm strains: N2, CB4856, and the NL2550 strain. The NL2550 strain is identical to N2 except that (like CB4856) it is homozygous for a null mutation in *ppw-1*. If the RNAi phenotype of any gene is severe in N2 but similarly weak in both CB4856 and NL2550, then the difference in phenotype between N2 and CB4856 is due to differences in RNAi efficacy. Conversely, if both N2 and NL2550 have similarly severe phenotypes and CB4856 is weaker, then the difference in phenotype between N2 and CB4856 is not due to variation in *ppw-1*; rather, it is a true effect of the difference in genetic background between N2 and CB4856 (see Figure S1C for schematic). We used a rapid manual screen to compare the RNAi phenotypes of all 570 genes that had more severe phenotypes in N2 in the N2, CB4856, and NL2550 strains. Each gene was placed into one of three classes: class A, wherein the phenotype in NL2550 is similar to that in N2 (difference in RNAi phenotype between N2 and CB4856 is not due to *ppw-1*); class C, wherein NL2550 is similar to CB4856 (difference is due to *ppw-1*); and class B, wherein NL2550 has an intermediate phenotype. We validated these data by testing a subset with our quantitative pipeline (Figure S1D): the median contribution of variation in *ppw-1* activity to the difference in RNAi phenotype between N2 and CB4856 in the three classes is 25%, 39%, and 74% for classes A, B, and C, respectively. We find that in over 75% of the cases where genes had more severe RNAi phenotypes in N2 than CB4856, this is largely the result of differences in RNAi efficacy (genes in class C). Removing these artifactual hits from our data, we thus find that 9% (127/1353) of the genes screened have more severe loss-of-function phenotypes in N2 due largely to differences in genetic background rather than to differences in RNAi efficacy.

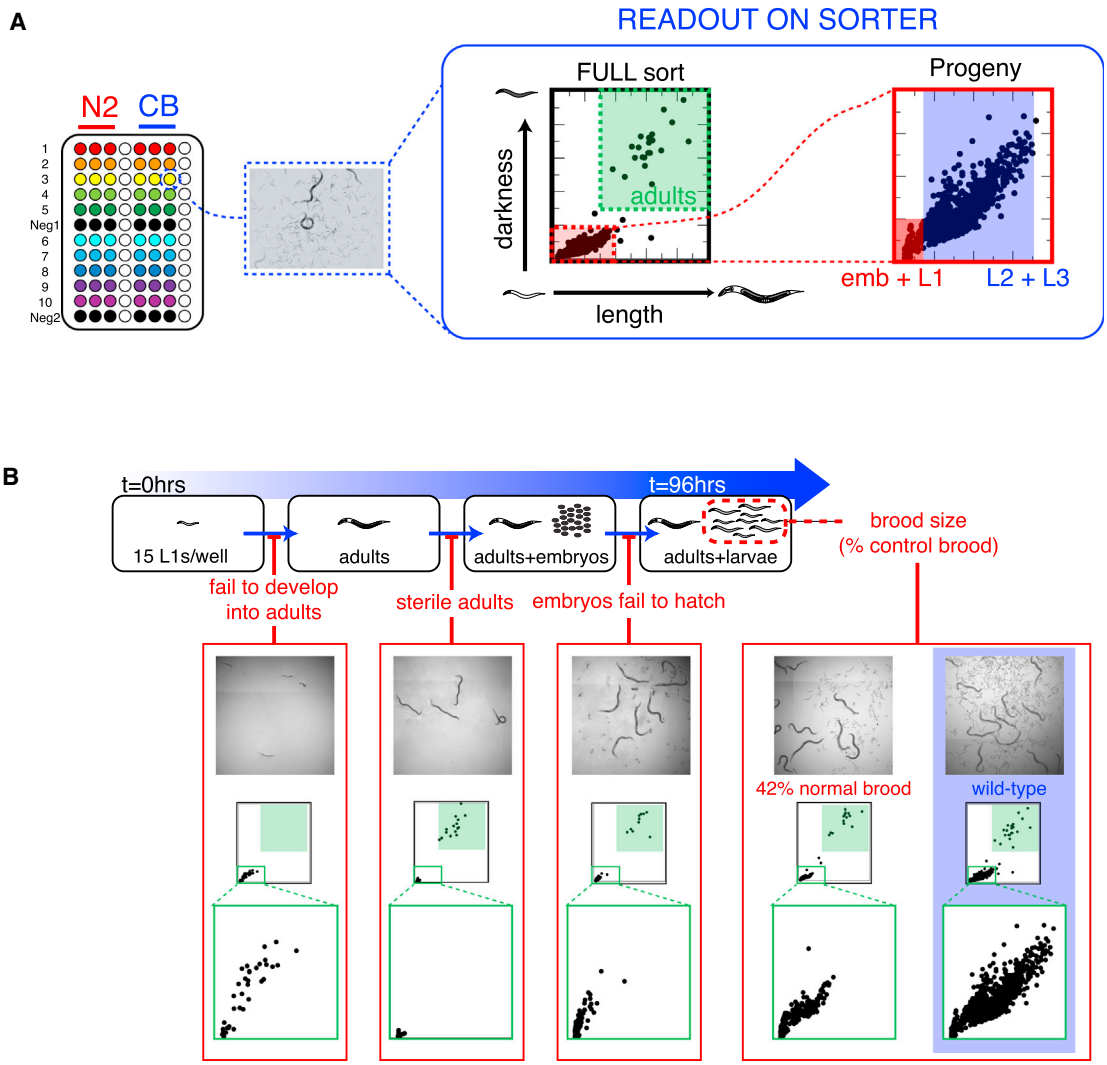

Figure 1. Outline of Quantitative Phenotyping Pipeline

(A) Experimental set up and sorter output. dsRNA-expressing bacteria are fed to worms in 96-well plates. Each gene is targeted in three independent cultures in each isolate, and every plate includes six negative control cultures ("Neg1" and "Neg2"). The set up shown has 10 genes being targeted in N2 and CB4856 (CB). After 96 hr, cultures are aspirated into a worm sorter that analyzes the length and darkness of each animal—we show here the full sorter data for a control culture ("Full sort") as well as an expanded view of the progeny ("Progeny"). Adults lie in the region marked in green, embryos and L1 animals lie in the red region, and L2 and L3 larvae in the purple region. All data appear in Table S1.

(B) Biological interpretation of sorter data. Here we show how a range of worm phenotypes appear both in sorter data and under light microscopy. Each photograph is a composite of several high-magnification images of any single well that are computationally stitched together.

Finally, and most importantly, to validate that any difference in RNAi phenotype between N2 and CB4856 truly reflects a difference in the severity of mutant phenotypes, we tested a subset of our hits using genetic mutants. For example, CB4856 has a more severe RNAi phenotype when we target the gene *mua-3*. We constructed a CB4856 strain that is homozygous for the characterized loss-of-function *mua-3(rh195)* allele (see Experimental Procedures) and compared its phenotype to that of N2 worms that are homozygous for the same *mua-3(rh195)* mutant allele. The result is shown in Figure 2A: whereas N2 *mua-3(rh195)* worms are mostly viable, over 80% of CB4856 *mua-3(rh195)* animals die as larvae, thus confirming our RNAi data. We tested eight genes like this, restricting our choice to genes with viable, well-characterized mutant alleles. These eight genes included five with more severe RNAi phenotypes in CB4856 and three that are more severe in N2 and cover diverse molecular pathways—*mua-3, vab-10,* and *unc-52* encode components of the fibrous organelle (FO), *nuo-6* encodes a subunit of the electron transport chain (ETC), *cgh-1* encodes an RNA helicase, *gld-1* encodes an RNA-binding protein, *unc-32* encodes a subunit of the vacuolar ATPase, and *unc-45* affects thick filaments in muscle cells.

Our results are shown in Figures 2 and 3 and Table S2—as predicted from our RNAi data, *mua-3, unc-52*, *vab-10,* and *unc-32* mutants all have more severe mutant phenotypes in the

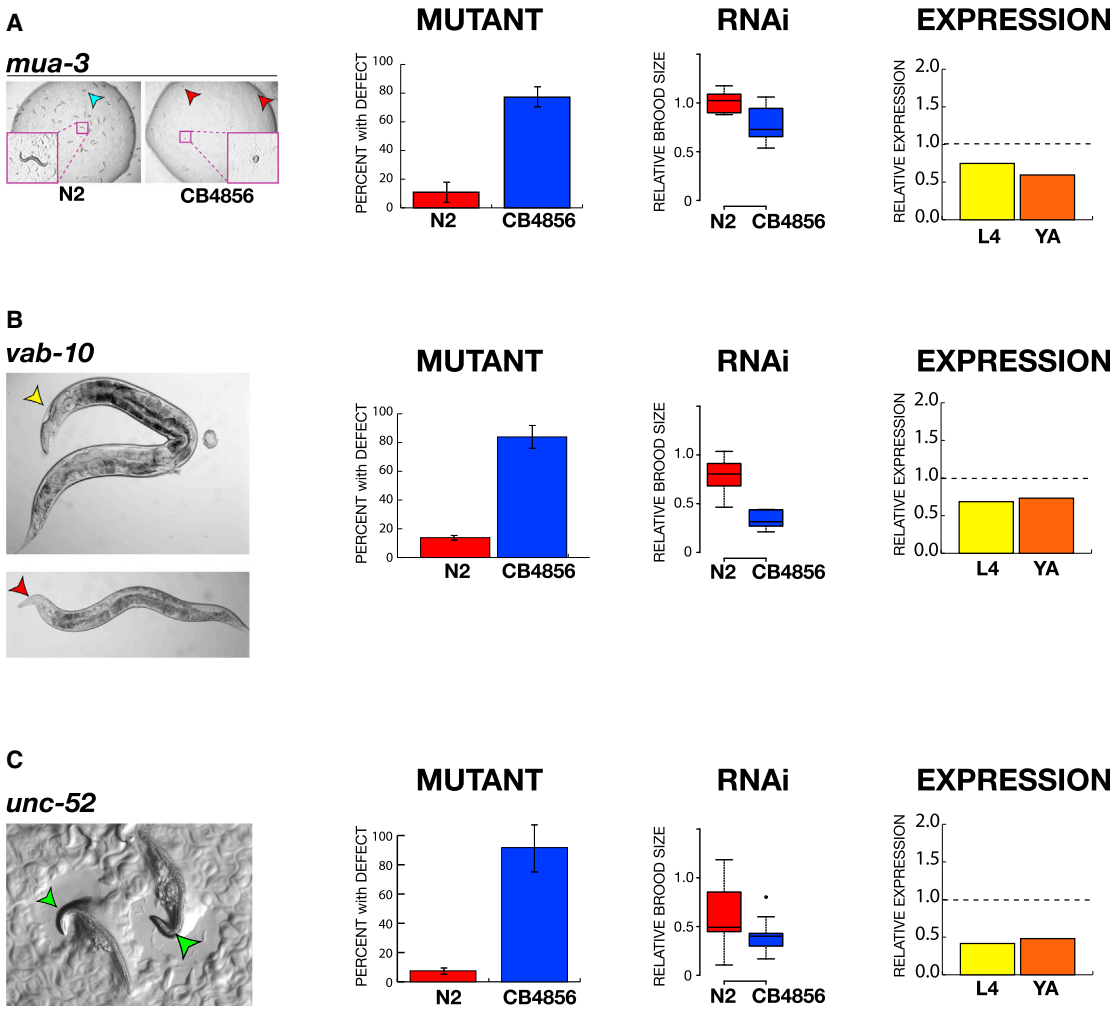

Figure 2. Mutations in Components of the FO Have More Severe Phenotypes in the CB4856 Background

We generated CB4856 strains homozygous for loss-of-function mutations in *mua-3*, *vab-10*, or *unc-52* and compared their phenotypes to N2 strains carrying the same mutant alleles.

(A) Most N2 worms homozygous for *mua-3(rh195)* develop into adults (cyan arrow and inset), but most CB4856 *mua-3(rh195)* homozygotes die as early larvae (red arrow and inset). This difference in severity is quantified in the lefthand (LH) column graph ("MUTANT," $p < 0.001$, chi-square, error bars denote SD from five independent measurements) and is concordant with differences in severity of RNAi phenotypes in our primary screen (boxplot labeled "RNAi" shows distribution of nine measurements of brood sizes after targeting *mua-3* in either N2 or CB4856, $p < 0.01$). Finally, the column graph at the righthand (RH) side ("EXPRESSION") shows the relative expression of *mua-3* in either L4 animals (yellow) or young adults (YA, orange) expressed as the ratio of CB4856:N2 expression.

(B) Adult worms homozygous for the hypomorphic *vab-10(e698)* allele have a detachment of the hypoderm from the cuticle (yellow arrow) and head defects (red arrow). This is more severe in the CB4856 background (column graph "MUTANT," $p < 0.001$, chi-square, error bars denote SD from five independent measurements), and this recapitulates the differences in severity of RNAi phenotypes (boxplot labeled "RNAi," $p < 0.001$). The RH column graph ("EXPRESSION") shows the expression of *vab-10* in L4s (yellow) or young adults (YA, orange) expressed as the ratio of CB4856:N2 expression.

(C) Adult worms homozygous for the *unc-52(e669su250)* allele show paralysis at 20°C (green arrow). This is more severe in the CB4856 background (column graph "MUTANT," $p < 0.001$, chi-square, error bars denote SD from five independent measurements), and this recapitulates the differences in severity of RNAi phenotypes seen in our primary screen (boxplot labeled "RNAi," $p < 0.01$). The RH column graph ("EXPRESSION") shows the expression of *unc-52* in either L4s (yellow) or young adults (YA, orange) expressed as the ratio of CB4856:N2 expression.

All data appear in Table S2.

CB4856 background, whereas *cgh-1* and *nuo-6* mutants have more severe phenotypes in the N2 background. Although we detected no difference in phenotype for mutants in either *unc-45* or *gld-1* between the N2 and CB4856 backgrounds, we note that for these two genes, the mutant alleles have weaker loss-of-function phenotypes than we generated by RNAi, and this might explain the difference in result. The alternative is that these might be false positives from our RNAi screen. Our data thus show that in most cases where we found a difference in RNAi phenotype between N2 and CB4856, this difference in phenotype can be confirmed using genetic mutants. This validates our approach of using RNAi-based screens to investigate the effect of

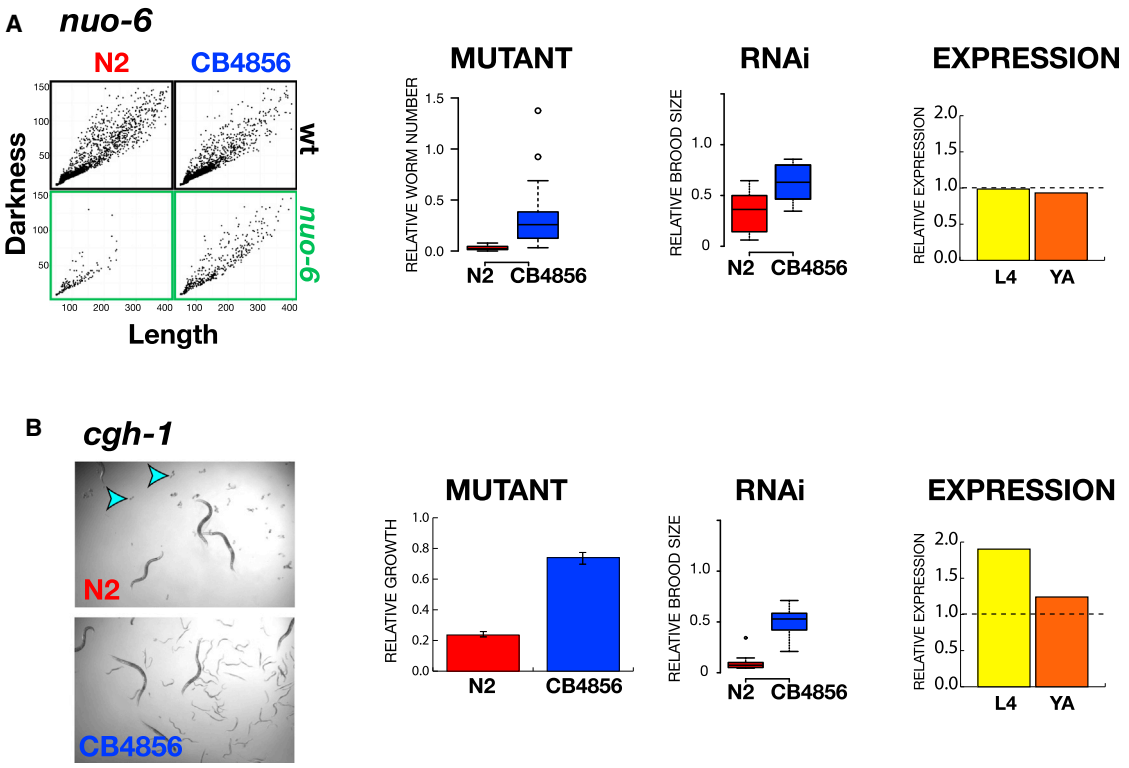

Figure 3. Mutations in Components of the ETC and in P Granule Regulators Cause More Severe Phenotypes in the N2 Background

We generated CB4856 strains that were homozygous for the hypomorphic *nuo-6(qm200)* allele or the temperature-sensitive *cgh-1(tn691)* allele and compared their phenotypes to N2 strains homozygous for the same alleles.

(A) The hypomorphic *nuo-6(qm200)* allele causes more severe growth defects in the N2 background. Parallel liquid cultures of N2 or CB4856 parental isolates and of strains homozygous for the *nuo-6(qm200)* allele in either the N2 or CB4856 background were set up and grown at 20°C. Samples were taken after 8 days and analyzed using the worm sorter. The LH panel shows the raw sorter output—N2 and CB4856 populations show similar growth, but N2 *nuo-6(qm200)* grows more slowly than CB4856 *nuo-6(qm200)*. The LH boxplot ("MUTANT") shows the number of L4 and adult worms in N2 *nuo-6(qm200)* or CB4856 *nuo-6(qm200)* strains relative to the number in the respective wild-type backgrounds. The RH boxplot ("RNAi") shows the differences in severity of RNAi phenotypes in our primary screen ($p < 0.01$). Finally, the column graph ("EXPRESSION") shows the relative expression of *nuo-6* in either L4 animals (yellow) or young adults (YA, orange) expressed as the ratio of CB4856:N2 expression.

(B) The temperature-sensitive *cgh-1(tn691)* allele causes highly penetrant embryonic lethality in the N2 background. Embryos homozygous for the temperature-sensitive *cgh-1(tn691)* allele fail to hatch at 22°C in the N2 background, whereas most CB4856 *cgh-1(tn691)* embryos hatch normally (LH panel; cyan arrowheads show dead embryos). This can be quantified as a difference in the ratio of L2 and L3 larvae to embryos (LH column graph, "MUTANT," $p < 0.01$, t test, error bars denote SD from five independent measurements). The boxplot ("RNAi") shows the differences in severity of RNAi phenotypes in our primary screen (relative brood size, $p < 0.001$). Finally, the RH column graph ("EXPRESSION") shows relative expression of *cgh-1* in L4s (yellow) or young adults (YA, orange) expressed as the ratio of CB4856:N2 expression.

All data appear in Table S2.

differences in genetic background on the severity of phenotypes that arise from inheritance of a mutation.

Many Genes with Different Severity of Loss-of-Function Phenotype Fall into Distinct Functional Classes

We identified 120 genes that have more severe phenotypes in CB4856 and 127 that have more severe phenotypes in the N2 background; this is due mainly to differences in genetic background. To gain some insight into the underlying causes of these differences in phenotypes, we first examined whether these genes tend to encode components of similar molecular machineries. We manually annotated all 1,353 genes into one of 19 major functional classes that comprise over 40 more specific classes (annotations in Table S1). We identified three significant enrichments: N2 has more severe phenotypes for genes involved in the ETC (8/28 genes more severe in N2) and for genes encoding RNA-binding proteins (8/22 genes more severe in N2); this latter enrichment is due to genes that affect P granules (7/11 screened). CB4856 has more severe phenotypes for genes involved in protein synthesis (31/200 screened). All enrichments are shown in Figure 4A and listed in Table S3. We note that because some of the functional classes are broad, they hide more fine-grained enrichments. For example, we screened five genes that encode subunits of the FO, the structure that links muscle cells to the cuticle. Three of these genes (*vab-10, unc-52,* and *mua-3*) have significantly more severe phenotypes in CB4856, and the other two (*mup-4* and *let-805*) also have more severe phenotypes in CB4856 but narrowly miss the statistical cut-off (Z scores of 1.67 and 1.63, respectively).

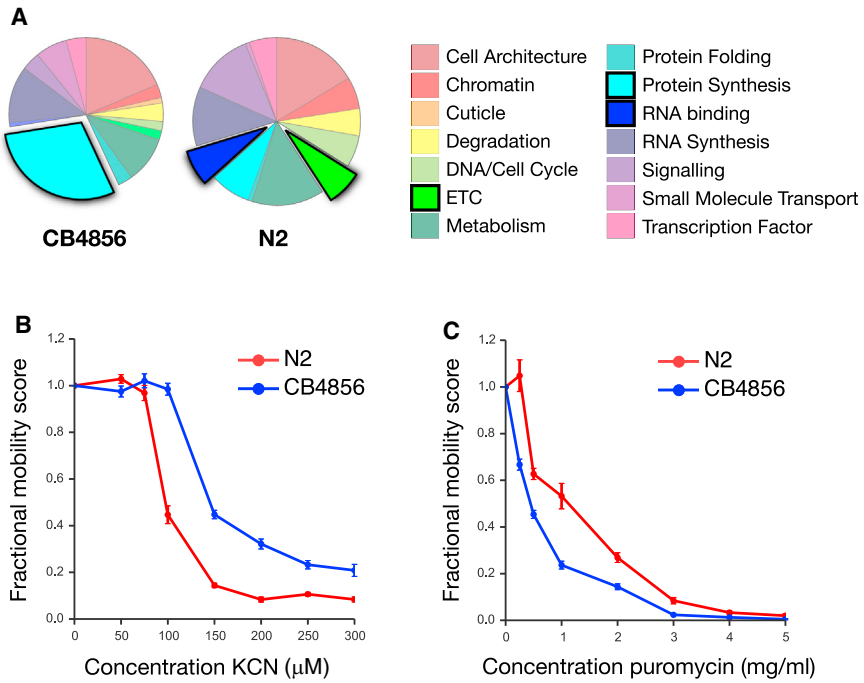

Figure 4. Distribution of Functional Classes of Genes with More Severe Phenotypes in Either N2 or CB4856 Isolates

(A) All genes were placed into one of 40 functional classes. The pie charts shows the distribution of the genes that had more severe RNAi phenotypes in either N2 or CB4856 in each class. Only classes with > 20 members are shown; classes that are significantly enriched ($p < 0.05$; Bonferroni corrected hypergeometric test) are shown as separated segments of the pie chart. Enrichments are all found in Table S3.

(B and C) The N2 isolate is more sensitive to treatment with KCN, and the CB4856 isolate is more sensitive to puromycin. Approximately 120 L1 worms were treated with a range of concentrations of KCN or puromycin, and their movement quantified after 90 min—the graph shows the fraction of worms that are moving (fractional mobility score; mean of six replicates; error bars represent SEM).

The enrichments that we found involve many of the genes that we retested using mutants (Figures 2 and 3)—mutations in *mua-3, unc-52,* or *vab-10* (all encode FO components) have more severe phenotypes in CB4856, whereas mutations in *nuo-6* (encodes a component of complex I in the ETC) or *cgh-1* (an RNA helicase that affects P granules) cause more severe phenotypes in N2. Furthermore, we can confirm these data using drugs—N2 is more sensitive to targeting ETC components by RNAi and is more severely affected by treatment with cyanide, an ETC inhibitor, whereas CB4856 is more sensitive to targeting genes encoding protein translational machinery and is more sensitive to treatment with puromycin, an inhibitor of protein synthesis (Figures 4B and 4C). We thus suggest that the same disease-causing mutation may have different effects in different individuals because of variation in the level of the requirement for the entire module in which the mutated gene acts.

Natural Variation in Gene Expression Causes Differences in Sensitivity to Perturbation in Each Individual

Almost 20% of the genes we examined have different severity of loss-of-function phenotypes in two isolates. Why is this? The great majority of the genes that have different loss-of-function phenotypes have identical coding sequences in the two isolates, and we thus focused on whether variation in gene expression could contribute to the differences in severity of RNAi and mutant phenotypes in N2 and CB4856. There is extensive variation in gene expression between N2 and CB4856, but the functional consequences of this are still largely unknown. We used RNA-seq to measure mRNA expression levels in two stages of the life cycle (the L4 larval stage and the young adult stage) in both N2 and CB4856 (see Experimental Procedures) and used this dataset to investigate whether variation in gene expression might affect the severity of loss-of-function phenotypes.

We first focused on the functional modules that showed systematic differences in loss-of-function phenotypes between the two isolates. As described above, N2 is more sensitive to perturbations affecting either the ETC or P granule regulators, whereas CB4856 is more sensitive to changes in activity of the protein synthesis machinery or the FO. We find that the ETC and P granule regulators are lower expressed in N2 than in CB4856, whereas components of the FO and the protein synthesis machinery (specifically, genes affecting ribosome biogenesis; see Figure S2A) are lower expressed in CB4856 (Figure 5A). This suggests that variation in gene expression may affect the severity of mutant phenotypes—the lower the expression in any individual, the more severe the phenotype. This is not specific to RNAi-induced phenotypes because we see the same sensitization to drugs (e.g., N2 has lower expression of the ETC and is more sensitive to cyanide; CB4856 has lower expression of the protein synthesis machinery and is more sensitive to puromycin; Figures 4B and 4C). Most importantly, we see that differences in expression levels also correlate with the severity of the mutant phenotypes of genes—*mua-3, unc-52, vab-10*, and *unc-32* are lower expressed in CB4856, and CB4856 has more severe mutant phenotypes for these genes (Figure 2). Conversely, *cgh-1* is lower expressed in N2, and *nuo-6* encodes a subunit of the ETC that is lower expressed in N2; both have more severe mutant phenotypes in N2 (Figure 3). The differences in expression are generally subtle (see Table S1), but at least for *cgh-1*, the expression differences are larger (~2-fold lower in N2). We find that qPCR confirms the lower expression of *cgh-1* in N2 (Figure S2D) and also that *cgh-1* expression is lower in the N2 *cgh-1(tn691)* strain than in the CB4856 *cgh-1(tn691)* strain. This confirms that lower expression of *cgh-1* correlates with increased severity of mutant phenotype (see Figure 3B).

Our data suggest that natural variation in gene expression affects the severity of mutant phenotypes—individuals with lower expression tend to have more severe phenotypes. To examine this further, we tested whether experimentally altering expression levels could modulate the severity of mutant phenotypes. We examined two functional modules—the FO and the P granule regulators. CB4856 has lower FO expression and more severe mutant phenotypes of FO components—if we reduce the expression of FO components in the higher-expressing N2 isolate, does this increase the severity of FO mutant phenotypes? Conversely, most P granule regulators have lower expression and more severe phenotypes in the N2 background—if we reduce expression of P granule regulators, does this increase the severity of their mutant phenotypes in the higher-expressing CB4856 background?

We used RNAi to reduce expression of *vab-10* or *unc-52* in N2 wild-type worms or in N2 strains homozygous for hypomorphic alleles of either *vab-10* or *unc-52*. Reducing *vab-10* expression greatly increases the severity of the *vab-10* mutant phenotype (Figure 5B), and reducing *unc-52* expression increases the severity of the *unc-52* mutant phenotype (Figure 5C). Importantly, reducing *vab-10* expression also increases the severity of the *unc-52* mutant phenotype and vice versa (Figure 5C). This indicates that the severity of the mutant phenotype of any specific gene in any individual can be affected by the level of either its own expression or the expression of other genes that act in the same functional module. We find very similar results when we alter expression of P granule regulators (higher expressed in CB4856)—reducing expression of either *mex-3* or *pos-1* significantly enhances the severity of the *cgh-1* mutant phenotype in the CB4856 background (see Figure 5D). In all cases, the combined phenotype is more severe than the simple additive effect of each perturbation alone.

Together, these data suggest that natural variation in gene expression can significantly affect the severity of mutant phenotypes. This can either be variation in the expression level of the mutant gene itself or variation in the expression of other genes that act in the same functional module. In either case, the trend is the same—individuals with lower expression tend to have a more severe mutant phenotype.

Natural Variation in Gene Expression Can Predict the Severity of Loss-of-Function Phenotypes

A central goal of this study was to improve our ability to predict the severity of mutant phenotypes in each affected individual. As described above, we found that variation in gene expression correlates with the severity of mutant phenotypes—lower expression of a mutant gene or of the functional module in which it acts results in a more severe loss-of-function phenotype. This trend is most obvious in the functional modules that are enriched for phenotypic differences but is still predictive across all genes. For example, genes that are at least 50% lower expressed in CB4856 are around twice as likely (1.8 times) to have a more severe phenotype in CB4856 than expected and around half as likely (0.4 times) to have a more severe phenotype in N2 (see Figure S2B). However, predicting the relative severity of a mutant phenotype in two individuals is not our goal—we want to predict the severity of a mutant phenotype for any *single* individual relative to some "average phenotype" for the species, that is, to answer "how severe will my phenotype be?" To examine this for the *C. elegans* species, rather than simply between N2 and CB4856, we selected two other isolates (AB1 from Australia and ED3040 from South Africa) that represent additional major branches of the phylogenetic tree (Andersen et al., 2012).

We used RNA-seq to measure gene expression in these isolates at the L4 larval stage and examined whether the expression level of any gene in a given isolate predicts the severity of its loss-of-function phenotype in that isolate. To address this, rather than screen all ~1,400 genes again in all 4 isolates, we focused on the ETC and used RNAi to compare loss-of-function phenotypes for 35 ETC genes in all 4 isolates. We found that ETC expression varies between isolates (Figure 6A). This does not reflect any general differences in expression of mitochondrial genes, e.g., the 75 mitochondrial ribosome genes are similarly expressed in 3 isolates, and the only exception (ED3040) shows lower expression of the mitochondrial ribosome and higher levels of ETC (see Figure S2C). We find that the isolates with lower ETC expression (N2 and AB1) tend to have more severe phenotypes for ETC genes (Figure 6B). Crucially, this trend is predictive at the single-gene level: the lower the expression of any ETC gene in an isolate, the more severe the phenotype of that gene in that isolate (Figure 6C). For example, in individuals with at least a 20% difference in expression level compared to the species average, we predict with over 85% accuracy their relative severity of phenotype (23/27 correct predictions, $p < 0.001$, binomial test). We note that these results are unlikely to be an effect of differences in RNAi efficacy between the isolates—not only did we confirm the difference in severity of the loss-of-function phenotype of a component of the ETC between N2 and CB4856 using a genetic mutant (Figure 3A), but we see the predicted differential sensitivity to cyanide between the isolates, e.g., ED3040 and CB4856 have higher ETC expression (Figure 6A), less severe RNAi phenotypes for ETC genes (Figure 6B), and weaker sensitivity to cyanide (Figure 6D), whereas AB1 and N2 have lower ETC expression (Figure 6A) and more severe RNAi phenotypes for ETC genes and are more sensitive to cyanide (Figures 6B and 6D).

Finally, to test whether differences in expression level result in differences in severity of loss-of-function phenotype in another biological system, we examined data from a large number of genome-scale RNAi screens in mammalian cells. We identified 507 genes that are required for cell growth or viability and tested whether lower expression of each of these genes in a number of different cell lines correlates with increased severity of their RNAi phenotype (see Experimental Procedures for methods and Table S5 for list of genes). We find that the severity of phenotype is indeed greater in cell lines that have lower expression levels of the target gene (see Figure 6E and an example for the gene *PAF1* in Figure 6F). Thus, although we have not tested our model in another whole animal model, or indeed in human populations, this suggests that the finding that differences in gene expression lead to differences in severity of loss-of-function phenotypes is a general feature of biological networks.

Taken together, our results suggest that one of the major ways in which variation in genetic background affects the severity of Mendelian disorders in humans is via variation in the expression

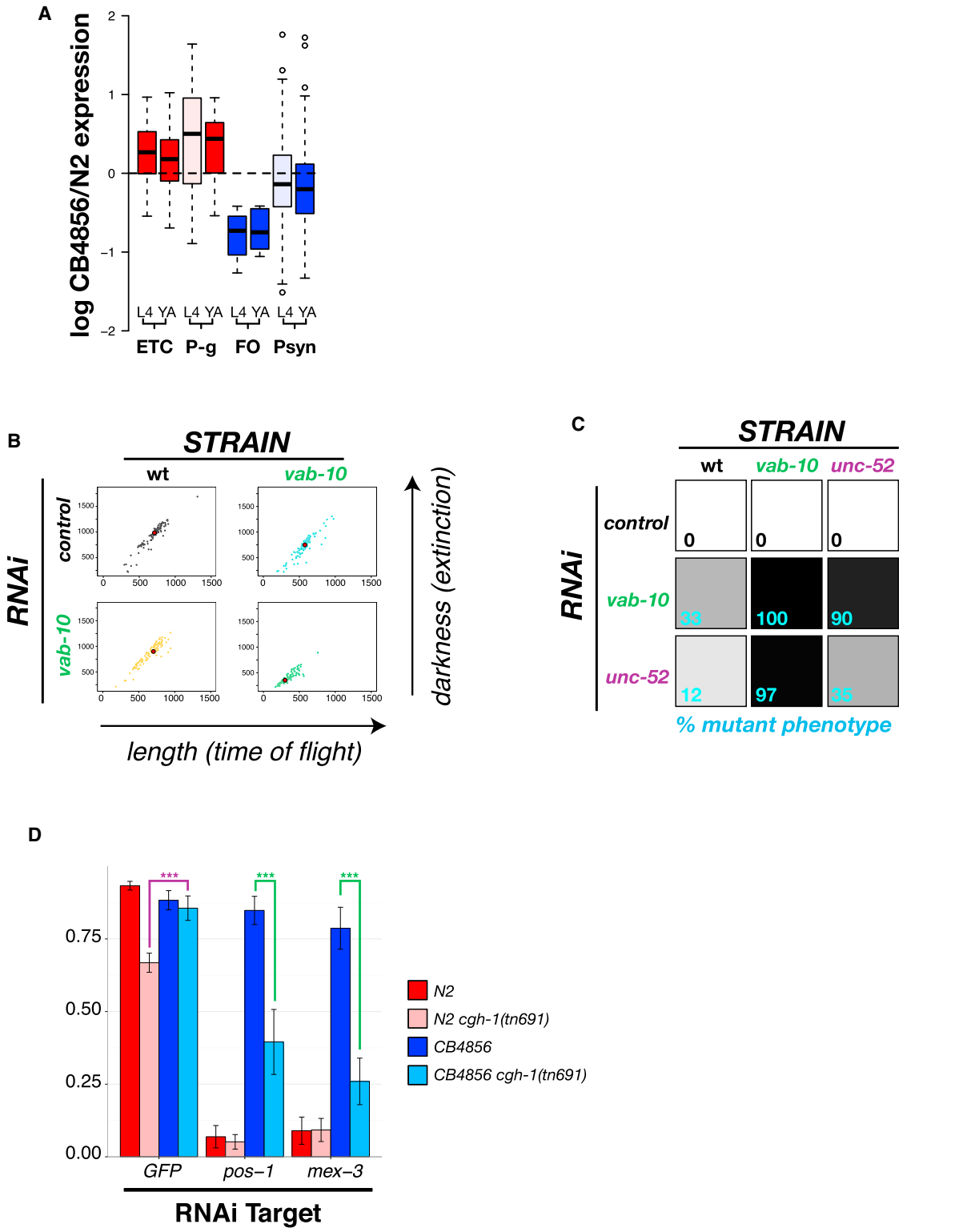

Figure 5. Natural Variation in Gene Expression Affects Loss-of-Function Phenotypes

(A) Differences in expression of specific functional modules in N2 and CB4856 mRNA expression levels were measured in either L4 or young adult (YA) animals using RNA-seq. The boxplot shows $\log_2$-transformed relative expression levels of genes encoding components of the ETC, P granule regulators (P-g), components of the FO, and protein synthesis machinery (Psyn) in N2 and CB4856. Boxes are bold if expression differences are significant ($p < 0.05$, paired t test).

(legend continued on next page)

levels of the mutated genes—the variants that affect gene expression can ultimately affect the severity of the inherited disorder.

DISCUSSION

Two individuals inheriting the same mutation may have very different severity of mutant phenotypes. Many factors can affect this, but in this study we focus specifically on the effect of genetic background. We used RNAi to knock down the expression of each of 1,353 genes in two different *C. elegans* natural isolates and measured the effect of each knockdown on phenotype. This set of ~1,400 genes comprises the majority of genes that have a readily detectable mutant phenotype in *C. elegans*, that is, the genes whose mutation causes a "genetic disorder" in worms. The set of genes we are studying is thus broadly analogous to the set of genes whose mutation causes genetic disorders in humans (for example, those described in the OMIM database). Our conclusions should therefore generalize to the impact of genetic background on disease severity following the inheritance of a disease-related variant. We believe our data support two main conclusions.

First, genetic background frequently has a marked impact on the phenotypic outcome of a genetic perturbation. Approximately 20% of genes have different severity of RNAi phenotypes due to differences in genetic background even when we compare only two individuals. As more individuals are examined (e.g., when we compared the loss-of-function phenotypes of components of the ETC in 4 isolates), this impact of genetic background on variation in loss-of-function phenotypes becomes more obvious. Over two-thirds of the genes encoding components of the ETC showed major differences (at least 50% different to the species average) in phenotype severity in at least one isolate—these isolates are representatives of major branches of the phylogenetic tree for *C. elegans* (Andersen et al., 2012). These data suggest that the majority of disease-causing mutations will have phenotypes that are affected by differences in genetic background.

Second, we find that natural variation in gene expression has a significant impact on the severity of mutant phenotypes. The lower the expression of a gene in any individual, the more likely it is that the effect of an inherited mutation in that gene will be severe. We also find that the mutant phenotype of any gene is affected by the expression of other genes that act in the same functional module—again, reduced expression correlates with increased severity. Importantly, we find that personal expression levels are good predictors of personal severity of mutant phenotypes. If an individual isolate has at least a 20% lower expression of a given gene than the species average, we can predict with over 85% accuracy that the mutant phenotype of that gene will be more severe in that isolate; conversely, if the isolate has at least 20% higher expression, this predicts with over 90% accuracy that the mutant phenotype will be less severe in that isolate. Thus, our data suggest that our personal levels of gene expression provide a strong predictor of the likely effects of the severity of the phenotype arising from rare variants that drive Mendelian disorders. This model is illustrated schematically in Figure 7.

We note that there are already indications that variations in expression levels can modulate severity of Mendelian disorders in humans. For example, Marfan syndrome is a dominantly inherited connective tissue disorder that has a complex array of symptoms. Marfan syndrome is primarily caused by mutations in *FBN1* (Dietz et al., 1991; Lee et al., 1991)—the *C. elegans* ortholog is *mua-3*, which we studied here. Just as variations in *mua-3* expression in different isolates of *C. elegans* affects the severity of the *mua-3* mutant phenotype, so variations in *FBN1* levels have been found to correlate with severity of Marfan syndrome in humans (Hutchinson et al., 2003). This relationship between the level of expression and the severity of phenotypes appears to hold in several other dominantly inherited syndromes (e.g., retinitis pigmentosa 11; Vithana et al., 2003). It will be intriguing to see first whether this correlation generally holds in other syndromes, including those that are inherited in a recessive manner, and second whether variations in expression levels of other hemidesomosome components such as perlecan (*HSPG2*) or dystonin (*DST*) can also modulate the effect of *FBN1* mutations on Marfan severity as we find for variations in *unc-52* and *vab-10* in *C. elegans*.

In summary, our data suggest that most rare variants that cause Mendelian disorders will have phenotypic outcomes of different severity in different individuals. We believe that one way to view the genetics of Mendelian disorders is that there is a difference between the genetics underlying the risk of having a Mendelian disorder and the genetics underlying the severity of the symptoms. The risk is binary, monogenic, and predictable from genome sequence—one either inherits or does not inherit a disease-causing mutation like the ΔF508

(B) Reducing expression of *vab-10* increases the severity of the *vab-10(e698)* mutant phenotype. Wild-type N2 animals or N2 animals homozygous for the *vab-10(e698)* allele were fed with bacteria targeting either *vab-10* or GFP (control) for 4 days and populations analyzed on a worm sorter. Almost all of the initial added L1 worms developed to adulthood (TOF > 400) in the N2 wild-type animals either with RNAi targeting GFP (control; black) or *vab-10* (cyan). The same is true for *vab-10(e698)* animal control cultures (yellow); however, *vab-10(e698)* animals fed with dsRNAs targeting *vab-10* (green) grew more slowly, and few reached adulthood. Red circles mark median TOF and extinctions in each plot.

(C) Altering expression of FO components affects severity of FO mutant phenotypes. Wild-type N2 animals or N2 strains homozygous for either *vab-10(e698)* or *unc-52(e669su250)* mutant alleles were exposed to bacteria expressing dsRNAs targeting either GFP (control), *vab-10,* or *unc-52*. The percentage of L1 animals that failed to develop to adulthood or that had severe movement defects was scored after 4 days.

(D) Altering expression of P granule regulators affects severity of *cgh-1* mutant phenotypes. We assessed the effect of targeting different P granule regulators using RNAi in four different worm strains: N2 and CB4856 wild-type isolates and N2 and CB4856 strains each homozygous for the temperature-sensitive *cgh-1(tn691)* allele. In each case, ~15 L1 worms were fed with dsRNAs targeting either GFP (control), *pos-1*, or *mex-3* for 4 days at 20°C, and brood sizes were measured using the worm sorter and expressed relative to brood sizes in control cultures. The effect of the *cgh-1(tn691)* allele on brood size is greater in the N2 background than in the CB4856 background (purple lines). Importantly, whereas targeting *pos-1* or *mex-3* has only a weak effect on brood size in the CB4856 wild-type isolate, it has a strong defect on brood size in the CB4856 strain with the *cgh-1(tn691)* allele (green lines). All marked differences are significant at $p < 0.01$. Error bars denote SD from three independent measurements

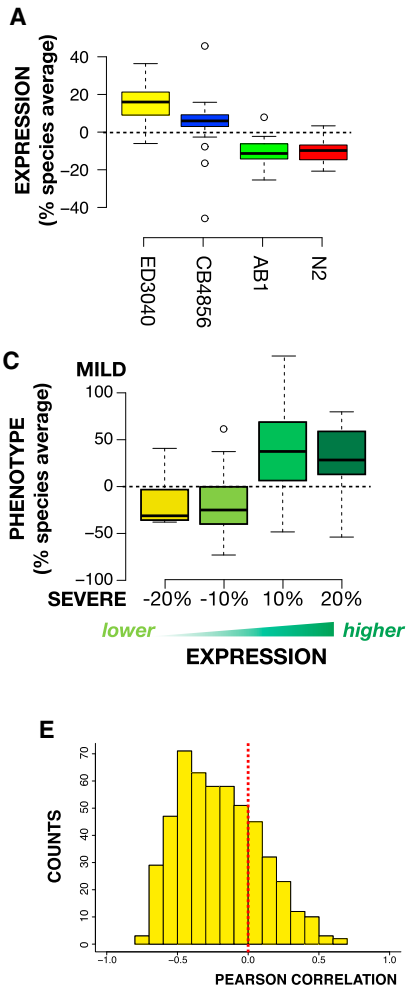

Figure 6. Differences in Gene Expression Predict the Relative Severity of Loss-of-Function Phenotypes

(A) Expression levels of the ETC in four isolates. mRNA levels in L4 larvae were measured in all four isolates using RNA-seq. The expression level of any gene is expressed relative to the average expression of that gene across all four isolates. The plot shows expression levels of genes encoding ETC components; all isolates differ at $p < 0.05$ (paired t test) except AB1 and N2. See also Figure S2 for more expression analyses.

(B) Severity of RNAi phenotypes of ETC components in four isolates. We used RNAi to target each of 35 components of the ETC in all isolates and measured the effect on brood size. The RNAi phenotype of each gene in any isolate is expressed relative to the average RNAi phenotype of that gene across all four isolates and plotted in the box-and-whisker plot. All isolates have different severity of phenotypes ($p < 0.05$, paired t test) except AB1 and N2.

(C) Differences in gene expression predict differences in severity of mutant phenotypes. Thirty-five genes encoding components of the ETC were placed into bins based on their relative expression in any isolate: 20% or more lower than the average expression (−20%); 10%–20% lower (−10%); 10%–20% higher (10%); and 20% or more higher than average expression (20%). In each bin, the box-and-whisker plot shows the distribution of severity of RNAi phenotypes; the RNAi phenotype of each gene in any single isolate is expressed relative to the average phenotype of that gene across all four isolates.

(D) Sensitivity of different *C. elegans* isolates to KCN treatment. Approximately 120 ED3040, CB4856, N2, and AB1 L1 worms were treated with different concentrations of KCN, and their movement quantified after 90 min—the graph shows the fraction of worms that were moving (fractional mobility score; data are mean of eight assays; error bars represent SEM). AB1 and N2 are more sensitive to KCN than CB4856 and ED3040 ($p < 0.01$, t test).

(E) Correlation between expression level and severity of RNAi phenotypes in mammalian cell lines. We identified 507 human genes that are required for normal cell growth (see Experimental Procedures) and tested whether their relative expression in a range of 18 pancreatic and ovarian cell lines predicted their relative severity of RNAi phenotype in each cell line. Here we show the distribution of correlations between the severity of RNAi phenotype and the expression level for the 507 genes across a panel of 18 pancreatic and ovarian cell lines (see Experimental Procedures). The mean correlation is −0.20, which is significantly lower than zero ($p < 2.2 \times 10^{-16}$, t test).

(F) As an example of the data that are contained in Figure 6E, we show a plot of the correlation between essentiality and expression level for an individual gene, *PAF1*.

CFTR mutation in CF. However, the severity of the outcome is genetically complex and is at least in part due to variants that affect gene expression. These findings may ultimately allow us to predict the phenotypic differences between individuals who inherit the same Mendelian mutation and, more broadly, to understand how the interplay between rare variants and modifiers affects the phenotype of any individual.

EXPERIMENTAL PROCEDURES

Strain Maintenance and Worm Handling

C. elegans strains were maintained at 20°C on NGM agar plates seeded with OP50 *E.coli* according to standard protocols (Stiernagle, 2006). All strains were supplied by the *Caenorhabditis* Genetics Centre (CGC), University of Minnesota, USA.

RNAi Screening and Data Processing

RNAi by feeding was carried out as described in Lehner et al. (2006). Genes were screened in triplicates in each RNAi screen, and three biological repeats were carried out. Strains were also grown with non-targeting control bacteria (bacteria expressing dsRNA-targeting GFP); over 100 replicates of these were screened in each experiment. Each well was analyzed using the Reflex module of a Union Biometria worm sorter to acquire the time of flight (*TOF*) and Extinction (*EXT*) for each object—bubble counts were removed using custom Perl scripts. We measured three parameters for each well—the number of adults, the brood size, and a measure of the rate of growth of the progeny population (the ratio between the number of objects with TOF below 75 [embryos and L1 larvae] and objects with TOF > 75 and < 350 [L2 and L3 worms]). To compute Z scores for differences in any parameter between the isolates, we used the distribution of the differences in the controls.

Genes were classed as having more severe phenotypes in either isolate as follows. If the Z score for differences in brood size was either < −2 or > 2, genes

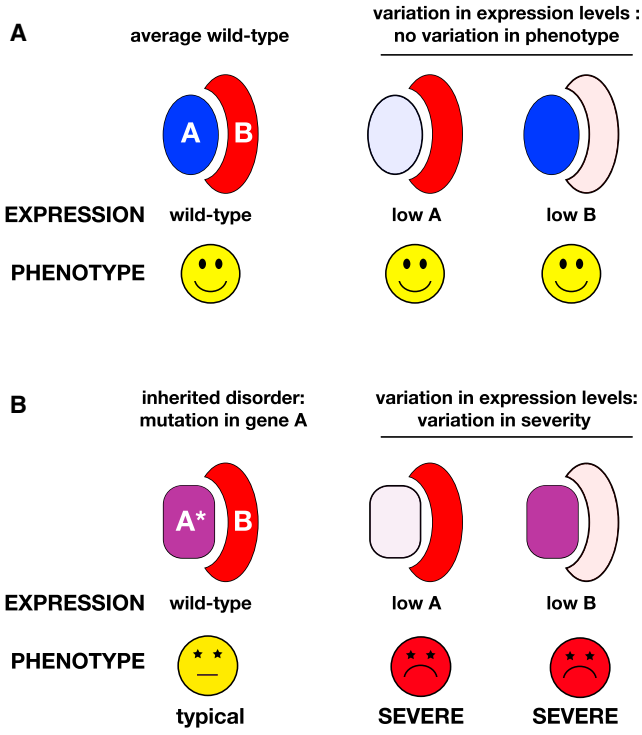

Figure 7. Natural Variation in Gene Expression Results in Variation in Severity of Mutant Phenotypes

In this schematic, we illustrate how natural variation in gene expression can lead to differences in the severity of mutant phenotypes. Lower expression either of a mutated gene ('gene A*') or of other components of the functional module in which that gene acts ('gene B') can equally result in a more severe mutant phenotype. We also suggest that this variation in expression levels only has a significant effect on phenotype in the context of a mutant allele. In (A), natural variation in gene expression has little or no effect on phenotype in the context of wild-type alleles; in (B), the same variation in gene expression modulates the severity of the mutant phenotype.

were annotated as being more severe in either N2 or CB4856, respectively. However, if brood sizes were < 10% of control levels in both isolates, we required a greater significance of $z < -2.5$ or $z > 2.5$. If there was no significant difference in brood size, we next examined the Z scores for differences in growth—if $z < -2$ or $z > 2$, we annotated genes as being more severe in either N2 or CB4856, respectively. To reduce false positives, we also required that brood-size differences did not contradict the growth severity, i.e., we only scored differences in severity if z (growth) > 2 AND z (brood) > 0 or z (growth) < −2 AND z (brood) < 0.

Enrichment Analysis

Genes were manually annotated into each of 19 major functional classes (that comprise 40 more specific functional classes) using all available gene information in WormBase. An enrichment analysis was performed to identify which of the 19 major functional classes were under- or over-represented for genes with more severe RNAi phenotypes in N2 or CB4856, and p values were calculated based on a hypergeometric test and Bonferroni-corrected for multiple hypothesis testing.

Correlation between Expression and Severity of RNAi Phenotype

We used standard RNA-seq protocols to measure gene-expression levels in L4-staged animals of four isolates and also young adults of N2 or CB4856 isolates. In cases where genes had multiple isoforms, we combined these to give a single mean rpkm value for each gene.

Construction of CB4856 Strains Carrying Mutant Alleles

All mutant alleles used were initially isolated in an N2 background. To generate CB4856 background strains carrying these same alleles, we crossed CB4856 and the N2 strain carrying the mutant of interest. We then carried out multiple rounds of backcrossing with CB4856 (in all cases, at least five rounds of backcrossing) following by at least five generations of selfing before isolating CB4856 lines that were homozygous for the mutation of interest. Multiple CB4856 lines were generated for each gene tested; results are reported for a single representative line.

Using Expression to Predict Relative Fitness in Human Cell Lines

Gene-expression and siRNA knockdown data for human cell lines were obtained from the COLT database (http://colt.ccbr.utoronto.ca/cancer/) and analyzed based on Z scores (Marcotte et al., 2012) and on Bayes Factors (Hart et al., 2014). We identified genes that are required for normal cell growth by fitting a logistic regression model from the COLT RNAi Bayes Factors in different cell lines to a set of genes whose orthologs are known to be required for viability in *C. elegans* and *S. cerevisiae*. When tested against the training set, we observed a bimodal distribution of response scores. We considered the upper peak to be genes that affect cell growth or viability and identified genes likely to be drawn from that distribution using a Gaussian mixture model with a cutoff probability of 0.95, giving us 507 genes. We calculated the Pearson correlation between expression and severity of RNAi phenotype across different cell lines for each one of the 507 genes we identified and examined the entire distribution for evidence of global correlation.

Acute Effect of Potassium Cyanide (KCN) or Puromycin Treatment

L1 larvae in M9 buffer were exposed to a range of concentrations of KCN or puromycin. For each concentration, six replicate wells were analyzed each containing ~120 worms. After 90 min at 20°C, each well was photographed twice at a 500 ms interval under bright-field illumination. A fractional paralysis score was determined by counting the number of worms that did not move between the consecutive images.

ACCESSION NUMBERS

The SRA accession numbers for the RNA-seq data reported in this paper are SAMN03839005, SAMN03839006, SAMN03839007, and SAMN03839008.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, two figures, and five tables and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2015.06.037.

ACKNOWLEDGMENTS

We thank Brent Derry for the generous gift of a worm sorter and Ben Lehner, Jovana Drinjakovic, and Leonid Kruglyak for helpful discussions. This research was primarily supported by a CIHR grant (488367) and also by an NSERC (487143) grant to A.G.F. V.V. was supported by OGS and NSERC PGS-D; A.J.V. was supported by NSERC CGS-M and CGS-D. All strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

Received: February 28, 2014
Revised: November 4, 2014
Accepted: June 10, 2015
Published: July 16, 2015

REFERENCES

Andersen, E.C., Gerke, J.P., Shapiro, J.A., Crissman, J.R., Ghosh, R., Bloom, J.S., Félix, M.A., and Kruglyak, L. (2012). Chromosome-scale selective sweeps shape Caenorhabditis elegans genomic diversity. Nat. Genet. *44*, 285–290.

Antonarakis, S.E., and Beckmann, J.S. (2006). Mendelian disorders deserve more attention. Nat. Rev. Genet. *7*, 277–282.

Bobadilla, J.L., Macek, M., Jr., Fine, J.P., and Farrell, P.M. (2002). Cystic fibrosis: a worldwide analysis of CFTR mutations—correlation with incidence data and application to screening. Hum. Mutat. *19*, 575–606.

Brenner, S. (1974). The genetics of *C. elegans*. Genetics *77*, 71–94.

Cooper, D.N., Krawczak, M., Polychronakos, C., Tyler-Smith, C., and Kehrer-Sawatzki, H. (2013). Where genotype is not predictive of phenotype: towards an understanding of the molecular basis of reduced penetrance in human inherited disease. Hum. Genet. *132*, 1077–1130.

Dietrich, W.F., Lander, E.S., Smith, J.S., Moser, A.R., Gould, K.A., Luongo, C., Borenstein, N., and Dove, W. (1993). Genetic identification of Mom-1, a major modifier locus affecting Min-induced intestinal neoplasia in the mouse. Cell *75*, 631–639.

Dietz, H.C., Cutting, G.R., Pyeritz, R.E., Maslen, C.L., Sakai, L.Y., Corson, G.M., Puffenberger, E.G., Hamosh, A., Nanthakumar, E.J., Curristin, S.M., et al. (1991). Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature *352*, 337–339.

Dorfman, R. (2012). Modifier gene studies to identify new therapeutic targets in cystic fibrosis. Curr. Pharm. Des. *18*, 674–682.

Dowell, R.D., Ryan, O., Jansen, A., Cheung, D., Agarwala, S., Danford, T., Bernstein, D.A., Rolfe, P.A., Heisler, L.E., Chin, B., et al. (2010). Genotype to phenotype: a complex problem. Science *328*, 469.

Fraser, A.G., Kamath, R.S., Zipperlen, P., Martinez-Campos, M., Sohrmann, M., and Ahringer, J. (2000). Functional genomic analysis of C. elegans chromosome I by systematic RNA interference. Nature *408*, 325–330.

Hamilton, B.A., and Yu, B.D. (2012). Modifier genes and the plasticity of genetic networks in mice. PLoS Genet. *8*, e1002644.

Hart, T., Brown, K.R., Sircoulomb, F., Rottapel, R., and Moffat, J. (2014). Measuring error rates in genomic perturbation screens: gold standards for human functional genomics. Mol. Syst. Biol. *10*, 733.

Hutchinson, S., Furger, A., Halliday, D., Judge, D.P., Jefferson, A., Dietz, H.C., Firth, H., and Handford, P.A. (2003). Allelic variation in normal human FBN1 expression in a family with Marfan syndrome: a potential modifier of phenotype? Hum. Mol. Genet. *12*, 2269–2276.

Kamath, R.S., Fraser, A.G., Dong, Y., Poulin, G., Durbin, R., Gotta, M., Kanapin, A., Le Bot, N., Moreno, S., Sohrmann, M., et al. (2003). Systematic functional analysis of the Caenorhabditis elegans genome using RNAi. Nature *421*, 231–237.

Lee, B., Godfrey, M., Vitale, E., Hori, H., Mattei, M.G., Sarfarazi, M., Tsipouras, P., Ramirez, F., and Hollister, D.W. (1991). Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. Nature *352*, 330–334.

Lehner, B., Crombie, C., Tischler, J., Fortunato, A., and Fraser, A.G. (2006). Systematic mapping of genetic interactions in Caenorhabditis elegans identifies common modifiers of diverse signaling pathways. Nat. Genet. *38*, 896–903.

Marcotte, R., Brown, K.R., Suarez, F., Sayad, A., Karamboulas, K., Krzyzanowski, P.M., Sircoulomb, F., Medrano, M., Fedyshyn, Y., Koh, J.L., et al. (2012). Essential gene profiles in breast, pancreatic, and ovarian cancer cells. Cancer Discov *2*, 172–189.

Maydan, J.S., Lorch, A., Edgley, M.L., Flibotte, S., and Moerman, D.G. (2010). Copy number variation in the genomes of twelve natural isolates of Caenorhabditis elegans. BMC Genomics *11*, 62.

Nadeau, J.H. (2001). Modifier genes in mice and humans. Nat. Rev. Genet. *2*, 165–174.

Pollard, D.A., and Rockman, M.V. (2013). Resistance to germline RNA interference in a Caenorhabditis elegans wild isolate exhibits complexity and nonadditivity. G3 (Bethesda) *3*, 941–947.

Stiernagle, T. (2006). Maintenance of C. elegans. WormBook, 1–11.

Tijsterman, M., Okihara, K.L., Thijssen, K., and Plasterk, R.H. (2002). PPW-1, a PAZ/PIWI protein required for efficient germline RNAi, is defective in a natural isolate of C. elegans. Curr. Biol. *12*, 1535–1540.

Vithana, E.N., Abu-Safieh, L., Pelosini, L., Winchester, E., Hornan, D., Bird, A.C., Hunt, D.M., Bustin, S.A., and Bhattacharya, S.S. (2003). Expression of PRPF31 mRNA in patients with autosomal dominant retinitis pigmentosa: a molecular clue for incomplete penetrance? Invest. Ophthalmol. Vis. Sci. *44*, 4204–4209.

Wicks, S.R., Yeh, R.T., Gish, W.R., Waterston, R.H., and Plasterk, R.H. (2001). Rapid gene mapping in Caenorhabditis elegans using a high density polymorphism map. Nat. Genet. *28*, 160–164.

Wright, F.A., Strug, L.J., Doshi, V.K., Commander, C.W., Blackman, S.M., Sun, L., Berthiaume, Y., Cutler, D., Cojocaru, A., Collaco, J.M., et al. (2011). Genome-wide association and linkage identify modifier loci of lung disease severity in cystic fibrosis at 11p13 and 20q13.2. Nat. Genet. *43*, 539–546.