

THE FUNCTIONAL SPECTRUM OF HUMAN VARIATION NOTES

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ABSTRACT. We examine in great detail the conclusion from Figure 4 in the 1000 Human Genomes paper.

1. UNDERSTANDING PURIFYING SELECTION

The purpose of figure 4 from *An integrated map of genetic variation from 1,092 human genomes* [1] is to illustrate the role of purifying selection within and between populations. Critically, this first requires a discussion of purifying selection. **Purifying selection** is a type of background selection resulting in lower genetic diversity [2]. When mutations occur in the genome which are highly deleterious, offspring do not survive long enough to pass on these mutations to subsequent generations, at least on longer timescales [2]. When this background selection occurs, the observed genetic diversity is lower than what would be expected under neutral substitution. However, these dynamics exist within the broader population level (longer) timescales and periodic deleterious mutations do appear to exist on shorter term time scales.

2. QUANTIFYING HUMAN VARIATION

2.1. Genomic Evolutionary Rate Profiling Score.

The Genomic Evolutionary Rate Profiling Score (GERP(2)) scores provide a way of measuring which sites likely lead to deleterious mutations. This statistical framework provides a way of estimating the difference between the expected number of mutations under a neutral substitution model (which assumes no impact on fitness) and the observed variation. Positive GERP(2) scores thus represent fewer mutations than would be expected, likely indicating a more conserved site, while negative scores would indicate more substitutions than expected. Put another way, we would expect that high GERP(2) scores will occur in regions which are important for survival to reproductive age and are largely conserved in the population (i.e., at higher levels in the population with fewer mutations in these regions).

2.2. Derived Allele(1) Frequency.

We can examine the derived allele(1) frequency (DAP) to assess the overall distribution of alleles within a population. As a reminder, a derived allele(1) are variants which have arisen since the last common ancestor. The derived allele(1) frequency is then a summarization of the pattern and frequency that these variant alleles appear. Taking the population sampled as a whole we can calculate the frequency with which each allele(1) variant appears.

3. EXAMINATION OF FIGURE 3

3.1. Panel A.

In Figure 1 we see the following:

- X-axis: the GERP(2) score representing the evolutionary conservation where higher scores are more conserved.
- Y-axis: the proportion of variants with a DAF(3) < 0.5% where higher values indicate lower frequencies in the studied population
- Colored lines: the different functional elements
- cross on the x and y axes representing the average values for GERP(2) score and proportion of variants with a DAF(3) < 0.5%, respectively.

From this figure we can conclude that:

- More generally, there are fewer mutation observed in more highly conserved sites (i.e., higher GERP(2) scores and higher proportions).
- Specifically, we see that additional Stop(4) codons, Splice(5) mutations, and non-synonymous (Nonsyn(6)) mutations appear very rare and are likely associated with deleterious effects. Later in the paper these are identified as “loss of function” mutations.
- The addition of Stop(4) codons continues to be relatively rare at most sites across GERP(2) scores. This implies that the addition of the codons likely results in a severe loss of function. As stop(4) codons stop(4) transcription prematurely, these additions will result in macromolecules not being transcribed more generally. This pattern is similar amongst the splice(5) mutations which impact the assembly of said macromolecules.
- We see an interesting phenomena with splice(5) variants having higher mutation in less conserved locations.
- The authors note that rare variant loads are similar for synonymous and nonsynonymous locations suggesting weak selective constraints

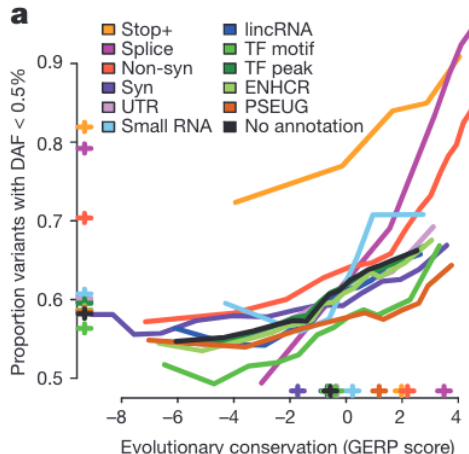


FIGURE 1. The relationship between evolutionary conservation (measured by GERP(2) score) and rare variant proportion (fraction of all variants with derived allele frequency (DAF(3)) < 5% for variants occurring in different functional elements and with different coding con-

sequences. Crosses indicate the average GERP(2) score at variant sites (x axis) and the proportion of rare variants (y axis) in each category.

3.2. Panel B.

In Figure 2 examines the CTCF-binding motif(15) within the CTCF-binding peaks. The transcription repressor CTCF has been characterized as playing a vital role in transcription regulation including the recombination of the antibody loci and the regulation of chromatin architecture [3, 4]. Intuitively, we would expect relatively low diversity in this gene as chromatin structural formation is vital for transcription (and cellular generation more generally).

The binding motif(15) is shown in the picto-graphic (called the “logo plot”) for the actual nucleotide. In all cases, red represents the “out peak” and blue represents the “in peak” from the Chip-seq (ChipSeq(16)) analysis which is used to map binding sites. Those sites that are located within the peak are likely related to binding and associated with function.

3.2.1. Upper panel.

The y axis again represents the GERP(2) score in the different regions. Higher values represent more likely to be a conserved region as it changes less than expected under a natural substitution model.

3.2.2. Lower panel.

The y axis represents the average diversity as defined as the per-nucleotide pair-wise distance with higher values representing more differences (i.e., more distance and differences)

3.2.3. Figure conclusions.

As suspected, in an important gene we see that those sites associated with function (“in peak”) vary less than expected under natural substitution as shown by the GERP(2) scores and lower pair-wise nucleotide distances in the lower panel. This is not to say that there isn’t a more complex story as there is a hint of degeneracy in position 8.

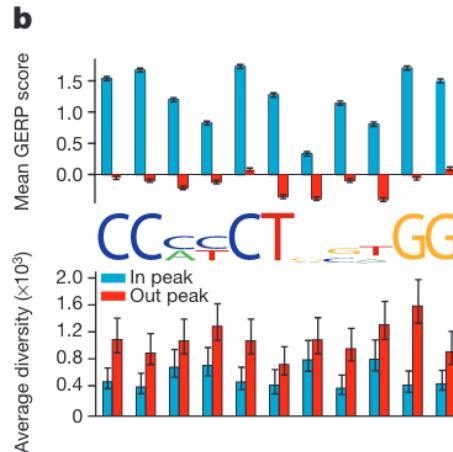


FIGURE 2. Levels of evolutionary conservation (mean GERP(2) score, top) and genetic diversity (per-nucleotide pairwise differences, bottom) for the sequences matching the CTCF-binding motif within CTCF-binding peaks, as identified experimentally by ChIP-seq in the ENCODE project (blue) and in a matched set of motifs outside peaks (red). The logo plot shows the distribution of identified motifs within peaks.

3.3. Key conclusions.

There are some highly conserved regions and likely purifying selection is driven by the addition of stop(4) codons, splice(5) mutations, and non-synonymous mutations at these conserved sites due to their lower observed frequencies of occurring.

GLOSSARY

allele(1) A variation in the same sequence of nucleotides at the same place on a long DNA molecule.

→ [https://en.wikipedia.org/wiki/Allele\(1\)](https://en.wikipedia.org/wiki/Allele(1))

GERP(2) The Genomic Evolutionary Rate Profiling is a statistical approach to quantify the deficit of substitutions in base pairs based on neutral the neutral rate of substitution. The neutral rate of substitution is an estimate of the number of new mutations per each generation multiplied by the probability that the substitution reaches fixations. In cases where neutral mutations exist, the rate of substitution will be equal to the rate of mutations. The GERP(2)

Score thus indicates the deviation from the neutral rate of substitution indicating that positive scores will indicate that fewer substitutions have taken place than would be expected under neutral substitution while negative scores indicate that more substitutions have taken place than would be expected under the neutral model.

→ <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1172034/>

DAF(3) Derived allele(1) frequency which represents the distribution of alleles at a given loci.

→ https://en.wikipedia.org/wiki/Allele_frequency_spectrum

Stop(4) A nucleotide substitution leading to the addition of a stop(4) codon (UAA, UAG, UGA) and leads to premature truncation of a protein during transcription. These are associated with a loss of function.

→ [https://illustrated-glossary.nejm.org/term/stop\(4\)-gain_variant](https://illustrated-glossary.nejm.org/term/stop(4)-gain_variant)

Splice(5) Often associated with post-translational changes in either the N-terminal region or a change in the availability of glycosylation sites through a change in the signal peptide. This can lead to secondary changes due to changes in the structure and is associated with a loss of function.

→ <https://onlinelibrary.wiley.com/doi/10.1002/prot.10568>

Nonsyn(6) A non-synonymous mutation is a change in the DNA sequence which results in a change in the encoded amino acid.

→ <https://bmccancer.biomedcentral.com/articles/10.1186/s12885-019-5572-x>

Syn(7) A synonymous mutation is a change in the DNA sequence which does not result in a change in the encoded amino acid.

→ <https://bmccancer.biomedcentral.com/articles/10.1186/s12885-019-5572-x>

UTR(8) The untranslated region. These appear on either side of the coding region with the 5' untranslated region called the leader sequence and the 3' untranslated region called the trailer sequence. The role of the untranslated region is not well described and may have a role in transcription regulation and mutations in this region may be associated with increased risk of certain cancers.

→ https://en.wikipedia.org/wiki/Untranslated_region

SmallRNA(9) Small RNA sequences are short, normally non-coding regions with a nucleotide length less than 200 bases. RNA silencing has been associated with these sequences.

→ https://en.wikipedia.org/wiki/Small_RNA

lincRNA(10) Large intergenic non-coding RNA are associated with remodeling of chromatin, genome architecture, RNA stabilisation, and transcription regulation.

→ <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5889127/>

TFmotif(11) Transcription factor motif(15) is the sequence associated with the proteins which are involved in the transcription of DNA to mRNA.

→ https://en.wikipedia.org/wiki/Transcription_factor

TFpeak(12) Transcription factor motif(15)

→ https://en.wikipedia.org/wiki/Transcription_factor

ENHCR(13) Enhancers regulate the transcription of genes by increasing the levels than without them.

→ <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4445073/#:~:text=Enhancers%20are%20DNA%2Dregulatory%20>

PSEUG(14) Pseudogenes are nonfunctional segments of DNA which resemble genes. These could be artefacts from mutations of prior functioning genes.

→ <https://en.wikipedia.org/wiki/Pseudogene>

motif(15) A motif(15) is a nucleotide or amino acid sequence associated with a specific structure and are associated with functionally important sites.

→ https://www.ncbi.nlm.nih.gov/CBBresearch/Przytycka/download/lectures/PCB_Lect08_Bind_Motifs.pdf

ChipSeq(16) Chromatin immunoprecipitation followed by sequencing (ChIP-seq) is extensively used to determine transcription factor binding sites (TFBSs)

→ <https://academic.oup.com/bioinformatics/article/29/21/2705/195767>

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