

Cancer-driving mutations are enriched in genic regions intolerant to germline variation

Dimitrios Vitsios^{1,*}, Ryan S. Dhindsa^{2,3,4,*}, Jonathan Mitchell¹, Dorota Matelska¹, Zoe Zou¹, Joshua Armenia⁵, Quanli Wang², Ben Sidders⁵, Andrew R. Harper¹, Slavé Petrovski^{1,6,*}

¹Centre for Genomics Research, Discovery Sciences, BioPharmaceuticals R&D, AstraZeneca, Cambridge, UK

²Centre for Genomics Research, Discovery Sciences, BioPharmaceuticals R&D, AstraZeneca, Waltham, MA, USA

³Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

⁴Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, Houston, TX, USA

⁵Bioinformatics and Data Science, Research and Early Development, Oncology R&D, AstraZeneca, Cambridge, UK

⁶Department of Medicine, University of Melbourne, Austin Health, Melbourne, Victoria, Australia

*These authors contributed equally

+Correspondence: dimitrios.vitsios@astrazeneca.com (D.V.), ryan.dhindsa@astrazeneca.com (R.S.D.), slav.petrovski@astrazeneca.com (S.P.)

Abstract

1 Large reference datasets of protein-coding variation in human populations have allowed us to
2 determine which genes and genic sub-regions are intolerant to germline genetic variation. There
3 is also a growing number of genes implicated in severe Mendelian diseases that overlap with
4 genes implicated in cancer. Here, we hypothesized that mitotically mutable genic sub-regions
5 that are intolerant to germline variation are enriched for cancer-driving mutations. We introduce
6 a new metric, OncMTR, which uses 125,748 exomes in the gnomAD database to identify genic
7 sub-regions intolerant to germline variation but enriched for hematologic somatic variants. We
8 demonstrate that OncMTR can significantly predict driver mutations implicated in hematologic
9 malignancies. Divergent OncMTR regions were enriched for cancer-relevant protein domains,
10 and overlaying OncMTR scores on protein structures identified functionally important protein
11 residues. Finally, we performed a rare variant, gene-based collapsing analysis on an
12 independent set of 394,694 exomes from the UK Biobank and find that OncMTR dramatically
13 improves genetic signals for hematologic malignancies. Our web app enables easy visualization
14 of OncMTR scores for each protein-coding gene ([https://astrazeneca-cgr-](https://astrazeneca-cgr-publications.github.io/OncMTR-Viewer/)
15 [publications.github.io/OncMTR-Viewer/](https://astrazeneca-cgr-publications.github.io/OncMTR-Viewer/)).

16 **Introduction**

17 The availability of large-scale human genetic variation reference datasets has revolutionized our
18 ability to identify disease-causing mutations (Karczewski et al., 2020; Wang et al., 2021).
19 Through the effective process of natural selection, variants with severe clinical outcomes are
20 generally depleted in these datasets. We and others have leveraged this paradigm to develop
21 intolerance metrics that quantify the extent to which natural selection constrains germline
22 variation in genes and genic-sub regions (Dhindsa et al., 2020; Petrovski et al., 2013; Samocha
23 et al., 2014; Traynelis et al., 2017). These methods have proven invaluable in prioritizing which
24 of the roughly 20,000 protein-coding variants observed in any given individual are most likely to
25 contribute to disease. Interpreting variants in the context of cancer suffers from similar
26 challenges as interpreting germline variation: cancer cells often carry thousands of somatic
27 mutations, but only some of these drive the oncogenic process. Despite their success in
28 prioritizing germline variants, population genetics-based approaches have yet to be applied in
29 the context of distinguishing between somatic cancer driving mutations and neutral “passenger”
30 mutations.

31 Many developmental disorder-causing germline mutations occur in essential genic
32 subregions, leading to dysfunction of crucial cellular biology pathways. We postulated that if
33 these same mutations arise mitotically later in life, they will not cause the same developmental
34 disease due to more limited expression of the mutation but could have equally as profound
35 impacts on cellular biology. Consistent with this, there are several examples whereby identical
36 point mutations that cause severe developmental syndromes when mutated in the germline
37 cause cancer when mutated somatically (Hoischen et al., 2014; Petrovski et al., 2016), including
38 identical mutations in *PTEN*, *ASXL1* (Hoischen et al., 2011), *EZH2* (Gibson et al., 2012), and
39 others (Kaplanis et al., 2020). Many of these genes are involved in cell proliferation, chromatin
40 remodeling, genome maintenance, and signal transduction pathways. This convergence
41 highlights a subset of genes in the human genome that are crucial to cell biology, whereby
42 disruptive mutations can cause different clinical outcomes depending on their timing,
43 localization, and cellular context.

44 Here, we hypothesized that regions of genes that are under strong negative selection for
45 germline variation but are exceptionally mitotically mutable would be enriched for variants that
46 increase cancer risk. Identifying germline-constrained but mitotically mutable genic subregions
47 could help prioritize cancer-driving mutations. Here, we focus on missense variants as they are
48 the most observed protein-coding variant class, are becoming increasingly clinically actionable
49 (Hyman et al., 2017), but importantly are also more difficult to interpret than protein-truncating

50 annotated variants. We previously introduced the missense tolerance ratio (MTR), a sliding
51 window-based approach that detects genetic sub-regions depleted of missense variation
52 (Traynelis et al., 2017). In this study, we extended this method to produce a score (OncMTR) to
53 identify germline intolerant but mitotically mutable genic sub-regions by using exome data from
54 125,748 individuals in GnomAD (Karczewski et al., 2020). We demonstrate that OncMTR
55 effectively predicts driver mutations of hematologic malignancies. We also use 394,694 UK
56 Biobank exomes to illustrate the utility of OncMTR in prioritizing variants in genetic discovery for
57 cancer phenotypes. This work introduces a population genetics approach to identify genic
58 subregions enriched for cancer-related somatic missense mutations.

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61 **Results**

62 **Putative somatic variants in gnomAD**

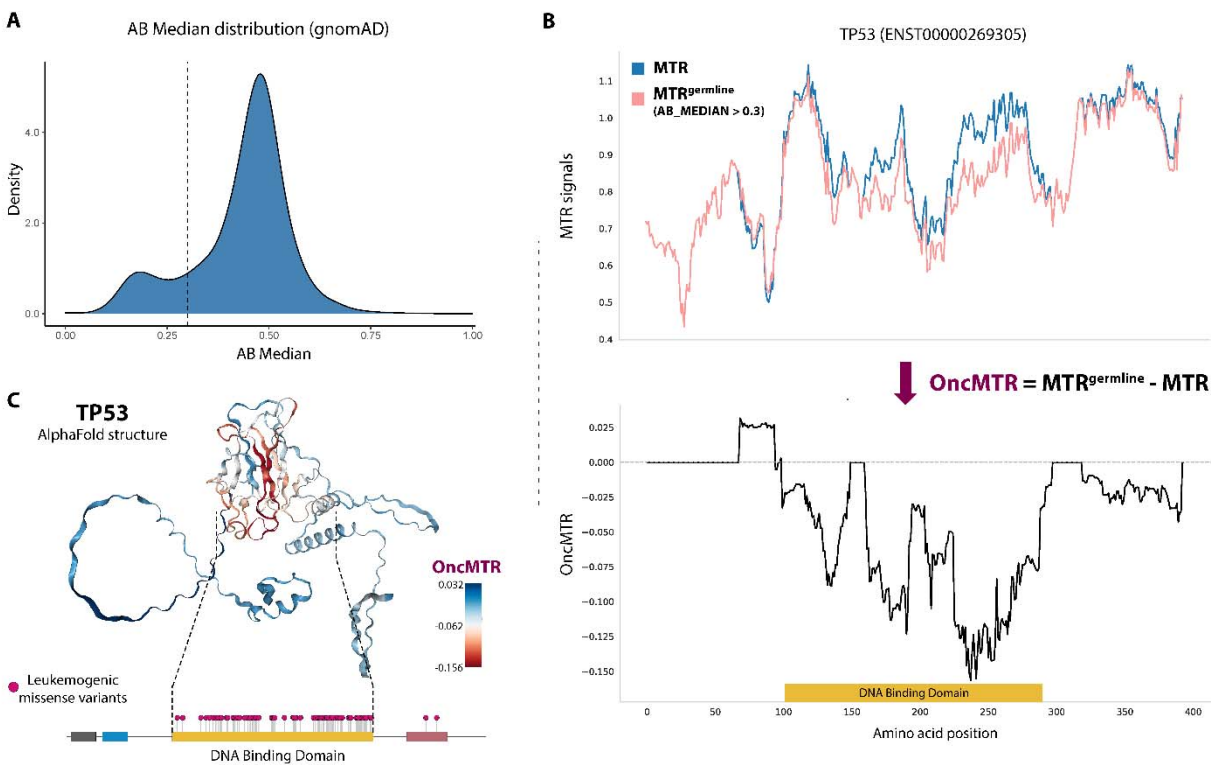
63 Population-level catalogues of human genetic variation allow for the investigation of selective
64 constraint and mutational patterns in the exome. We used the gnomAD database of 125,748
65 human exomes to survey both germline and somatic variants (Karczewski et al., 2020).
66 Although the gnomAD variant calling pipeline was tuned to detect germline variation, we
67 reasoned that we may also be able to identify somatic variants that reach a sufficiently high
68 variant allele frequency to be detected through their germline variant caller. Inherited
69 heterozygous germline variants are expected to have an allelic ratio close to 50%. We observed
70 that the distribution of median allelic balance (AB_median) values for gnomAD variants followed
71 a bimodal distribution, with one distribution centered around 50% and another, smaller
72 distribution centered around 20% (**Fig. 1A**).

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74 **Defining OncMTR**

75 We previously introduced a sliding window-based metric, the missense tolerance ratio (MTR),
76 that measures purifying selection on missense variation in genic sub-regions (Traynelis et al.,
77 2017). This score demonstrably detects crucial functional domains of proteins that can cause
78 Mendelian disease when mutated in the germline. Motivated by the overlap between mutations
79 associated with Mendelian disease and cancer, we set out to create a cancer-relevant version
80 of MTR (methods) that captures regions that are depleted of germline variation but also
81 enriched for somatic variation. In this study, we defined another variation of the MTR score,
82 namely MTR^{germline} . In its construction, MTR^{germline} is restricted to only those variants achieving
83 an $AB_median > 0.3$. Taking the well-known cancer gene *TP53* as an example, we can observe

84 those genic subregions where the two MTR formulations diverged (**Fig. 1B**). We then define
 85 OncMTR as the difference between these two MTR formulations for each codon and using a 31-
 86 codon sliding window (**Fig. 1B**). Negative scores correspond to regions with the greatest
 87 divergence between germline intolerance and somatic variant enrichment. Overlaying OncMTR
 88 scores on the AlphaFold-predicted structure of TP53 (Jumper et al., 2021) illustrated that the
 89 strongest negative scores correspond to the DNA-binding domain, which is the domain enriched
 90 for mutations known to drive hematologic malignancies (**Fig. 1C**).



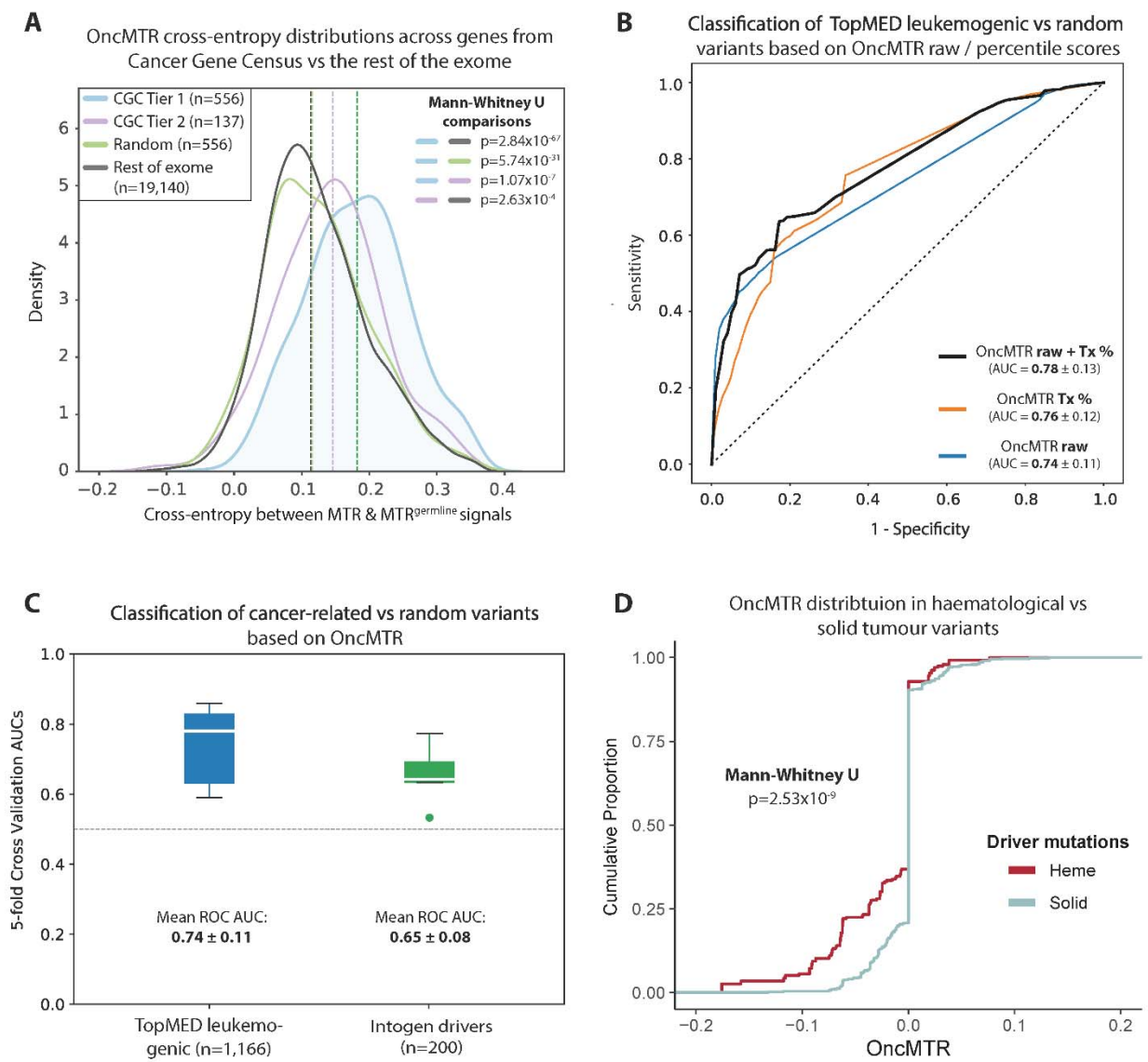
91
 92 **Figure 1. Defining the OncMTR score. (A)** Bi-modal distribution of median allelic
 93 balance values for heterozygous variants in the gnomAD database. We defined putative
 94 somatic variants as those with an AB median ≤ 0.3 (dashed line). **(B)** The top figure
 95 demonstrates the missense tolerance ratio (MTR) distribution of *TP53* when considering
 96 all missense variants (blue) and when restricted to only germline variants (i.e., AB
 97 Median > 0.3, depicted in pink). We defined OncMTR as the difference between these
 98 two distributions (bottom panel). **(C)** OncMTR scores overlaid on the AlphaFold structure
 99 for TP53. The most intolerant region maps to the DNA-binding domain of the protein,
 100 which is strongly enriched for mutations known to drive hematologic malignancies.

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103 Using OncMTR to prioritize driver mutations in hematologic malignancies

104 Motivated by the positive proof-of-concept demonstrated for *TP53*, we next tested whether the
 105 MTR and MTR^{germline} distributions differed across other oncogenes included in the Catalogue of
 106 Somatic Mutations in Cancer (COSMIC) Cancer Gene Census (CGC). The CGC is divided into

107 two tiers, with Tier 1 containing *bona fide* cancer genes (n=556) and Tier 2 containing genes
 108 that have strong indications of playing a role in cancer but with less expansive evidence than
 109 Tier 1 (n=137). The difference between MTR and MTR^{germline} distributions per gene, calculated
 110 via cross entropy, was significantly higher for Tier 1 genes than a random selection of 556 non-
 111 CGC genes ($p = 5.7 \times 10^{-31}$), the remainder of the exome ($p = 2.8 \times 10^{-67}$), and Tier 2 genes ($p =$
 112 1.1×10^{-7}) (**Fig. 2A**). The cross entropy was also significantly larger for Tier 2 genes than the
 113 remaining genes in the exome ($p = 2.6 \times 10^{-4}$) (**Fig. 2A**). Together, these results support the
 114 hypothesis that mitotically mutable genic sub-regions that are intolerant to germline variation are
 115 broadly relevant to cancer.
 116



117

118 **Figure 2. OncMTR regions are enriched for somatic variants associated with**
119 **hematologic malignancies. (A) Cross entropy between the** distribution MTR and
120 **MTR^{AB} distributions for Catalogue of Somatic Mutations in Cancer (COSMIC) Cancer**
121 **Gene Census (CGC) genes, a random selection of genes, and the rest of the exome. (B)**
122 **Receiver operator curve (ROC) depicting the ability of random forest models based on**
123 **either the raw OncMTR score, the OncMTR transcript-level percentile scores (“Tx%”),**
124 **and a joint model in discriminating between 1,166 leukemogenic variants and a random**
125 **size-matched set of variants. AUC = area under the curve. (C) Mean ROC AUCs (with**
126 **fivefold cross-validation) of random forest models based on raw OncMTR in predicting**
127 **variants involved in leukemia (same variant set as figure B) and hematologic driver**
128 **mutations annotated in IntoGen (Tamborero et al., 2018). The putatively neutral variant**
129 **sets comprise of random, size-matched selection of variants. (D) The OncMTR**
130 **distributions of driver mutations for hematologic malignancies versus solid tumors is**
131 **derived from the Cancer Genome Interpreter.**
132
133

134 Distinguishing between cancer-causing driver mutations and neutral passenger mutations
135 remains a central challenge in cancer genomics. We thus tested whether OncMTR could help
136 prioritize somatic mutations that cause hematologic malignancies. We found that the OncMTR
137 scores of a previously defined list of 1,166 leukemogenic driver mutations (Bick et al., 2020)
138 (**Supplementary Table 1**) were significantly lower than a size-matched set of random variants
139 (Mann Whitney U $p=2.97 \times 10^{-86}$; **Supplementary Fig. 1A**). A random forest model using
140 OncMTR achieved an area under the receiving operator curve (AUC) of 0.74 in discriminating
141 between these leukemogenic variants and the random set (**Fig. 2B**). We also calculated
142 transcript-level percentiles for the MTR scores, in which lower percentiles corresponded to lower
143 OncMTR scores. The AUC or the OncMTR transcript percentiles was 0.76, and a combined
144 model that incorporated both the raw OncMTR scores and transcript percentiles achieved an
145 even higher AUC of 0.78 (**Fig. 2B**).

146 To further assess the capacity of OncMTR to prioritize driver mutations, we trained
147 random forest models with raw OncMTR scores using 5-fold cross-validation. The mean AUC
148 for predicting leukemogenic variants was 0.74 (**Fig. 2C**). We next compared the performance of
149 OncMTR in distinguishing between a set of random variants and 200 established driver
150 mutations implicated in acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML),
151 chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), or multiple
152 myeloma (MM), achieving an AUC of 0.65 (**Fig. 2C**) and having significantly disparate OncMTR
153 distributions from each other (Mann Whitney U $p=4.89 \times 10^{-5}$; **Supplementary Fig. 1B** and
154 **Supplementary Table 2**). Logistic regression-based classifiers achieved similar, albeit
155 marginally lower, AUCs than the random forest models (with AUCs of 0.73 and 0.62 for the two
156 variant sets, respectively), likely due to a small degree of non-linear distribution of OncMTR
157 scores (**Supplementary Fig. 2**). Altogether, these results demonstrate the utility of our

158 population genetics-based approach in identifying genic sub-regions relevant to hematologic
159 malignancies.

160 Because the somatic mutations used to calculate OncMTR arose in the blood, we
161 expected that OncMTR would more reliably prioritize driver mutations in hematologic
162 malignancies than in solid tumors. As expected, the OncMTR scores of driver mutations
163 implicated in heme malignancies were significantly lower (Mann Whitney U $p=2.53 \times 10^{-9}$; **Fig.**
164 **2D**). To determine whether OncMTR performs better for certain subtypes of heme malignancies,
165 we compared OncMTR distributions of putative driver and passenger mutations identified in a
166 recent comprehensive *in silico* saturation mutagenesis experiment (Muiños et al., 2021). This
167 dataset includes simulated variants across 3 genes for CLL, 9 genes for AML, 2 genes for non-
168 Hodgkin lymphoma, 5 genes for lymphoma, 6 genes for multiple myeloma, and 2 genes for ALL
169 (**Supplementary Table 10**). The OncMTR scores of predicted driver mutations were
170 significantly lower than those of passenger mutations for each cancer subtype, though we
171 observed the strongest separation in CLL (Wilcoxon $p < 2 \times 10^{-308}$) and AML (Wilcoxon $p = 1.4 \times 10^{-$
172 ¹⁵⁵) (**Supplementary Fig. 3**).

173 We next assessed whether OncMTR can successfully distinguish between ClinVar
174 pathogenic and benign somatic variants. Logistic regression classification between pathogenic
175 and benign or random variants across all protein-coding genes reached an AUC of 0.60 and
176 0.58, respectively (**Supplementary Fig. 4**; $P=815$ unique pathogenic vs $B=58$ unique benign
177 variants; a set R [random] of equal size to P was sampled to compile the random variants - see
178 also Methods). We next restricted the set of pathogenic somatic variants to those occurring in
179 genes associated with hematologic malignancies and compared to benign or random variants.
180 The AUC was 0.62 in distinguishing between pathogenic and benign variants in hematologic
181 malignancy genes ($P=64$ vs $B=20$) and 0.67 when comparing to benign variants across the
182 entire exome ($P=64$ vs $B=58$). The AUCs for pathogenic hematologic malignancy variants
183 versus random variants were 0.61 for random variants restricted to heme genes ($P=64$ vs $R=64$)
184 and 0.64 for random variants pulled from all protein-coding genes ($P=815$ vs $R=815$)
185 (**Supplementary Fig. 4**). These results provide support to this blood-based sequencing version
186 of OncMTR being more powerful in identifying pathogenic mutations implicated with heme
187 malignancies.

188 Finally, to further explore OncMTR's power to agnostically detect putative oncogenic
189 regions, we scanned all protein-coding genes in ClinVar in search of transcripts that are
190 preferentially enriched for ClinVar pathogenic somatic variants in regions with OncMTR scores
191 at the bottom 20-percentile of the full OncMTR distribution (see Methods). We identified 101

192 such transcripts from 24 unique genes (Fisher's exact test $p < 0.05$; **Supplementary Table 11**),
193 with several known cancer driver genes captured, such as *TP53*, *IDH1*, *ALK* and *HNRNPA1*
194 (Martínez-Jiménez et al., 2020). Many of the top ranked genes are implicated in hematologic
195 malignancies, including *MYC*, *MSH2*, and *FBXW7* (**Supplementary Fig. 5**) (Bhatia et al., 1993;
196 King et al., 2013; Whiteside et al., 2002).

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198 **Genes carrying mutations implicated in both human Mendelian disease and cancer**

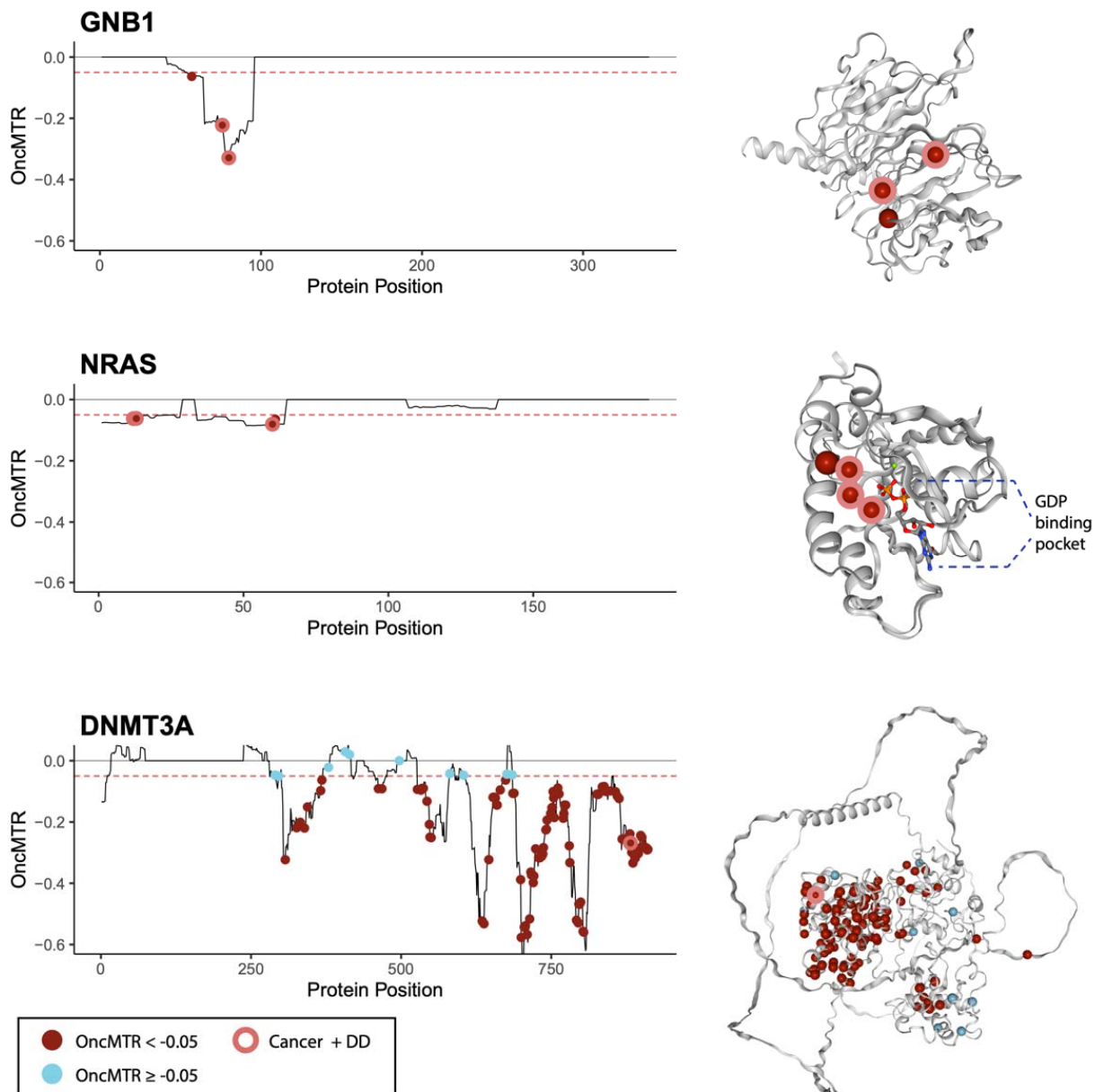
199 The underlying hypothesis in deriving OncMTR is that certain genic regions are critically
200 important to human biology, and thus germline mutations in these regions cause severe
201 Mendelian phenotypes, whereas identical somatic mutations—occurring later in life and localized
202 to specific tissue(s)—in these regions may have an oncogenic effect. To evaluate this, we plotted
203 OncMTR distributions for three genes implicated in both neurodevelopmental disease and
204 leukemia: *GNB1*, *NRAS*, and *DNMT3A* (**Fig. 3 A-C** and **Supplementary Table 4**).

205 Germline *de novo* mutations in *GNB1* cause a severe developmental syndrome
206 characterized by intellectual disability (ID) and other variable features, including hypotonia,
207 seizures, and poor growth (Petrovski et al., 2016). Somatic mutations in this gene have been
208 associated with ALL, CLL, and myelodysplastic syndrome (Yoda et al., 2015). Three of the four
209 somatic driver mutations in this gene overlap with *de novo* mutations implicated in
210 developmental delay (p.Asp76Gly, p.Ile80Thr, and p.Ile80Asn) (**Fig. 3A**) (Petrovski et al., 2016).
211 All four mutated residues reside in a low OncMTR region (OncMTR < -0.05) of the gene, which
212 corresponds to the G β -protein surface that interacts with G α subunits and downstream effectors
213 (**Fig. 3A**).

214 *NRAS* encodes a RAS protein with intrinsic GTPase activity that has been implicated in
215 multiple hematologic and solid malignancies (Oliveira et al., 2007). There are 28 somatic
216 missense variants in this gene at four distinct amino acid positions associated with juvenile
217 myelomonocytic leukemia and AML, and all residing in low OncMTR regions (**Fig. 3B**) (Bick et
218 al., 2020). Two of these mutations have also been reported as causal germline *de novo*
219 mutations for Noonan syndrome, a developmental delay syndrome that includes congenital
220 heart defects, short stature, and other features (p.Gly13Asp, p.Gly60Glu) (**Fig. 3B**) (Cirstea et
221 al., 2010; Matsuda et al., 2007).

222 *DNMT3A* encodes a DNA methyltransferase essential for DNA methylation during
223 human embryogenesis and, when mutated somatically, increases risk of acute myeloid
224 leukemia (Kosaki et al., 2017). In a large study on clonal hematopoiesis of indeterminate
225 potential (CHIP), *DNMT3A* was found to harbor the largest proportion of CHIP variants of all

226 CHIP-associated genes (Jaiswal et al., 2017), suggesting it is highly mitotically mutable. In line
227 with this, the OncMTR distribution of this gene is highly enriched for negative values, even
228 compared to *GNB1* and *NRAS* (**Fig. 3C**). The R882 amino acid residue of *DNMT3A*
229 corresponds to a DNA-binding residue that is a major somatic mutation hotspot in CHIP and
230 AML (Kosaki et al., 2017). *De novo* germline mutations at this residue are associated with an
231 overgrowth syndrome called Tatton-Brown-Rahman syndrome characterized by tall stature and
232 impaired intellectual development (Tatton-Brown et al., 2014). Mutations at the R882 residue
233 are thought to interfere with DNA binding, resulting in functional impairment of the protein and
234 aberrant DNA methylation patterns (Zhang et al., 2018). As expected, we identify that the
235 leukemogenic variants in this gene are enriched in low OncMTR regions (**Fig. 3C**). Altogether,
236 these results support the notion that some critically important genic sub-regions are
237 exceptionally mitotically mutable, and mutations in these regions result in different phenotypic
238 outcomes depending on timing and cellular context (Hoischen et al., 2011).



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Enrichment of low OncMTR scores in protein domains

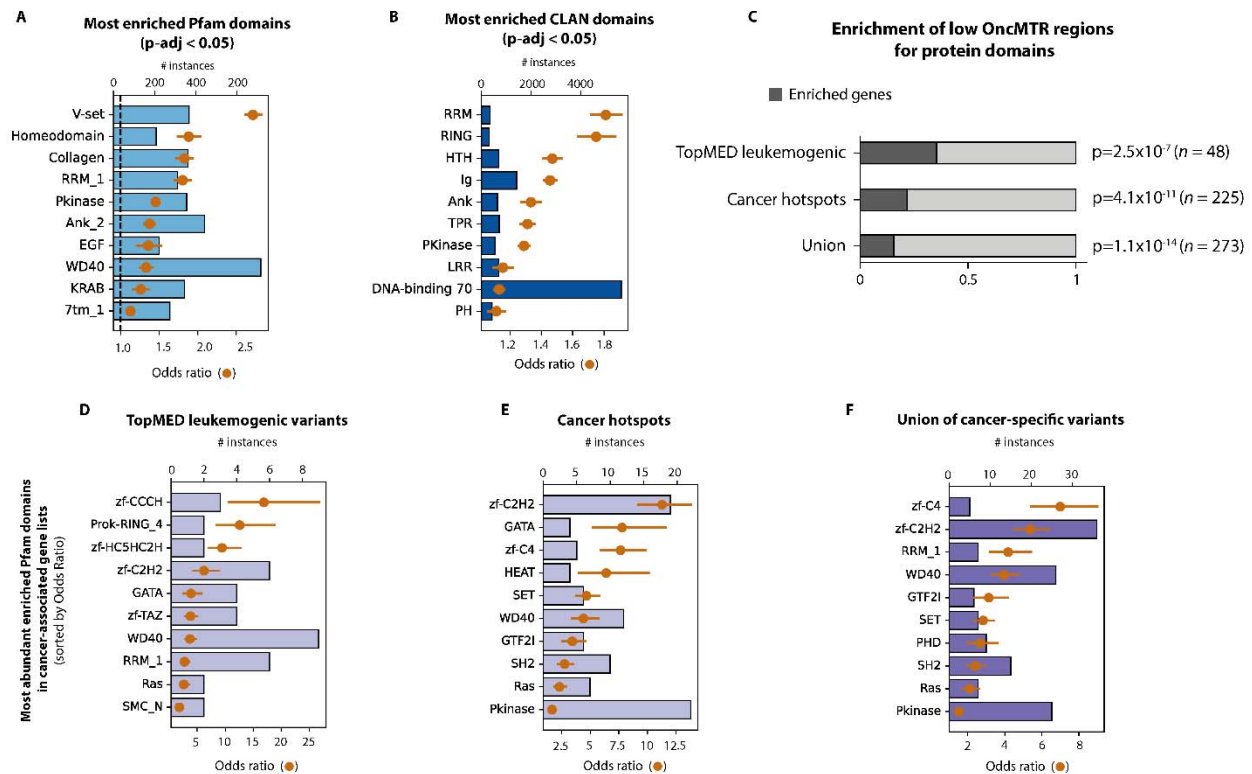
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One strength of the sliding window approach implemented in OncMTR is that its estimates are

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independent of biological boundaries, such as annotated protein domains, which are not always

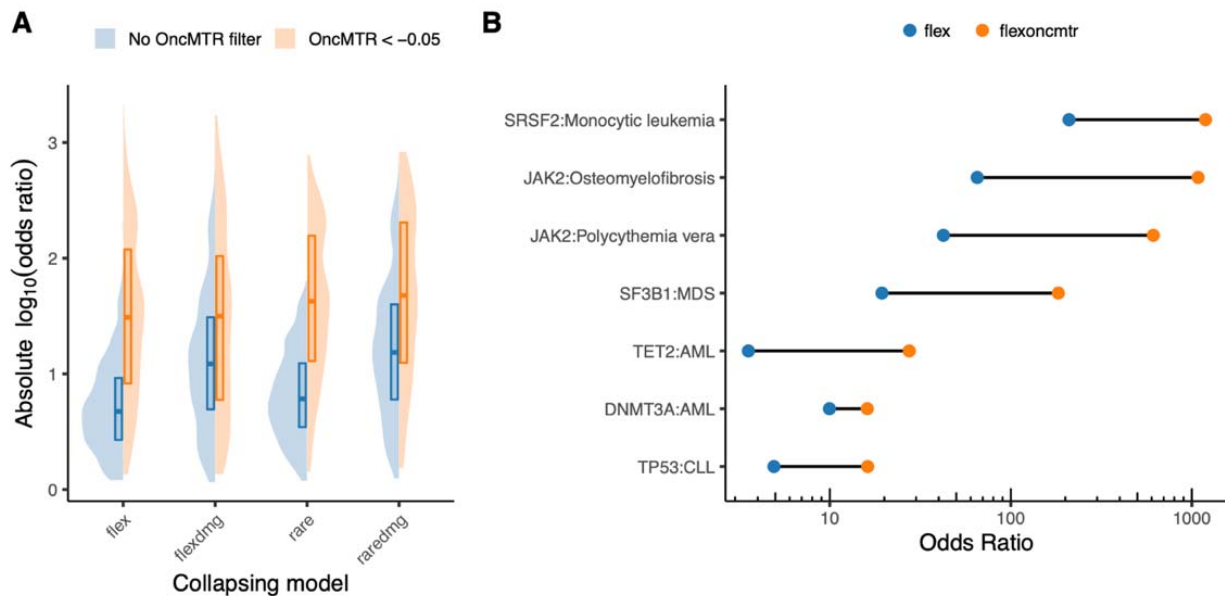
254 well-annotated. However, it is known that cancer-causing missense mutations tend to cluster in
 255 certain functional domains. We thus tested whether Pfam domains and domain superfamilies
 256 were enriched for low OncMTR regions (defined as OncMTR < -0.05). Across human protein-
 257 coding genes, low OncMTR regions were significantly enriched for several protein domains
 258 previously implicated in cancer, such as homeodomains (Fisher's exact adjusted p-
 259 value= 4.9×10^{-46}), protein kinase domains (Fisher's exact adjusted p-value= 5.25×10^{-110}), RING
 260 domains (Fisher's exact adjusted p-value= 3.22×10^{-48}), and others (**Figure 4 A,B** and
 261 **Supplementary Tables 5,6**). Furthermore, we found that proteins that had functional domains
 262 enriched for low OncMTR scores are significantly enriched in genes with TOPMed
 263 leukemogenic variants and known cancer hotspots (Chang et al., 2018) (**Figure 4C** and
 264 **Supplementary Tables 1-3;7-9**). Among these two lists of genes, zinc finger motifs were found
 265 to be the most strongly enriched for low OncMTR scores (**Figures 4D-F**; most significant
 266 adjusted p-value= 2.3×10^{-52} from the union list, based on Fisher's exact test), in line with their
 267 well-established role in cancer development (Cassandri et al., 2017). Remarkably, although the
 268 calculation of OncMTR is agnostic to domain annotations, it independently identifies cancer-
 269 relevant functional genic sub-regions.
 270



272 **Figure 4. Overlap between OncMTR regions and protein domains. (A)** Pfam protein
273 domains most strongly enriched with low OncMTR regions (OncMTR < -0.05). **(B)** Pfam
274 domain clans most strongly enriched with low OncMTR regions. The DNA-binding
275 superfamily set was defined in a prior publication (Bahrami et al., 2015). **(C)** Proportions
276 of genes enriched with low OncMTR scores in annotated protein domains in various
277 cancer-related gene sets: genes carrying TopMED leukemogenic variants, annotated
278 cancer hotspots, as well as the union of these three lists. **(D-F)** The most abundant Pfam
279 domains enriched with low OncMTR regions in proteins encoded by the labeled sets of
280 cancer genes. Error bars in each panel represent 95% confidence intervals. P-values
281 were calculated with Fisher's exact test and adjusted via Bonferroni correction.
282

283 **Informing rare-variant collapsing analysis with OncMTR**

284 With increasing adoption of next generation sequencing to generate case-control cohorts, rare
285 variant collapsing analysis has emerged as a powerful approach to detect disease-associated
286 genes for both rare and complex disorders. In this approach, the proportion of cases with a
287 qualifying variant is compared to the proportion of controls with a qualifying variant in the same
288 gene. We have previously shown that incorporating an MTR filter in defining QVs dramatically
289 improves rare variant collapsing analyses (Wang et al., 2021). In that phenome-wide
290 association study (PheWAS) on approximately 300,000 exomes in the UK Biobank, the
291 collapsing analyses detected seven genes associated with hematologic malignancies (Wang et
292 al., 2021). Here, we sought to test whether OncMTR would further improve collapsing analysis
293 signals for hematologic malignancy associations by performing a collapsing analysis on 394,694
294 European exomes contained in the UK Biobank focused on 1,394 chapter IX (neoplasm)
295 phenotypes. We defined a total of eight collapsing models with and without OncMTR filters
296 (**Supplementary Table 12**). Imposing an OncMTR filter of -0.05 (i.e., only considering missense
297 QVs that fall below this threshold) significantly increased the effect sizes of gene-phenotype
298 associations ($p < 0.0001$) for each model (**Fig. 5A**). We observed genome-wide significant
299 ($p < 1 \times 10^{-8}$) associations between several heme malignancies and *DNMT3A*, *FBXW7*, *IDH2*,
300 *IGLL5*, *JAK2*, *SF3B1*, *SRSF2*, *TET2*, and *TP53*, in certain cases the effect sizes were 10-fold
301 greater than without adopting the OncMTR filter (**Fig. 5B**). We also found that the association
302 between *TP53* and CLL only reached significance in models including our OncMTR filter; for
303 example, in the 'raremg' model, this association had a p-value of 1.2×10^{-7} (odds ratio [OR] =
304 8.8; 95% confidence interval [CI]: 4.8-16.0), whereas in the 'raremgoncmtr' model, the same
305 association reached a p-value of 3.4×10^{-10} (OR = 33.2; 95%CI: 16.1-68.7). Thus, applying the
306 OncMTR filter effectively reduces background variation in the setting of gene-level collapsing
307 analysis for haematological malignancy phenotypes and we advise future large-scale
308 haematological malignancy discovery studies to consider adopting OncMTR filter for improved
309 signal detection.



310

311 **Figure 5. Collapsing analyses using OncMTR. (A)** Effect sizes of gene-phenotype
312 associations derived from a gene-level collapsing analysis performed on neoplasm
313 phenotypes in 394,694 UK Biobank exomes. Collapsing models are defined in
314 Supplemental Table 12. **(B)** Changes in odds ratios observed for selected gene-
315 phenotype associations. MDS = myelodysplastic syndrome; AML = acute myeloid
316 leukemia; CLL = chronic lymphocytic leukemia.

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319 Discussion

320 Determining the clinical relevance of missense variants in oncogenes remains a central
321 challenge in cancer genetics (Chang et al., 2018; Hyman et al., 2017). Motivated by the
322 observation that missense variants in certain genic sub-regions can cause severe Mendelian
323 disease when mutated in the germline and cancer when mutated somatically, we introduced a
324 population genetics-based framework called OncMTR to quantitate the divergence between
325 germline constraint and somatic mutability across the human exome.

326 First, we demonstrated that oncogenes are enriched for these critically important regions
327 that do not tolerate germline missense variants but harbor somatic mutations. We then
328 illustrated that OncMTR can effectively distinguish between leukemogenic driver mutations and
329 passenger mutations. Although OncMTR is calculated using a sliding window without any input
330 of domain annotations, we found that genic sub-regions that have low OncMTR scores are
331 significantly enriched for protein domains known to be relevant to cancer. Illustrative of our
332 hypothesis was the observation that identical point mutations implicated in both severe
333 Mendelian disease and leukemia in the genes *GNB1*, *NRAS*, and *DNMT3A* occur in low
334 OncMTR regions. Finally, we found that incorporating OncMTR in a gene-level collapsing

335 analysis on hematologic malignancy phenotypes using 394,694 UKB exomes improved the
336 signal-to-noise ratio for detecting hematologic malignancy associations. We have also
337 developed web server for visualization of OncMTR scores for each human protein-coding gene:
338 <https://astrazeneca-cgr-publications.github.io/OncMTR-Viewer/>.

339 Our findings have important implications for the disease biology of both severe
340 Mendelian disorders and cancer. The convergence of genes and genic sub-regions between
341 these two disease areas suggest that similar biological processes play a fundamental role in
342 these two groups of phenotypes. Indeed, cellular proliferation, chromatin remodeling, cell
343 migration, and other cancer-relevant processes have been implicated in neurodevelopmental
344 diseases (De Rubeis et al., 2014; Dhindsa et al., 2021; Feng et al., 2019; Kaplanis et al., 2020).
345 Furthermore, our work supports the notion that mutations in these genes have different
346 phenotypic manifestations based on timing (i.e., zygote versus adulthood), localization
347 (systemic versus hematological), and cellular context.

348 There exist many other approaches that aim to predict which genic sub-regions are
349 relevant to cancer. These methods tend to look for nonrandom clustering patterns of somatic
350 mutations in either the linear protein sequence or three-dimensional space (Porta-Pardo et al.,
351 2017). To the best of our knowledge, none of these approaches integrate population-level
352 inferences of genic constraint. OncMTR could improve the predictive performance of other,
353 orthogonal driver mutation prediction approaches, as a recent *in silico* saturation mutagenesis
354 experiment demonstrated the strength of incorporating multiple lines of evidence in prioritizing
355 driver mutations (Muiños et al., 2021).

356 One limitation of OncMTR in its current formulation is that it does not reflect the broader
357 range of solid tumor malignancies since it is based on somatic mutations observed in blood-
358 based sequencing. In future work, the general framework introduced in this study could be
359 applied to sufficiently large tumor-normal sequence datasets as those numbers increase.
360 Furthermore, we used gnomAD because it represents the largest collection of publicly available
361 aggregated allele frequency data. However, gnomAD variants were all called using a germline
362 variant caller. While we demonstrated that we could detect somatic variants in this database, we
363 were likely limited to those that reached a sufficiently high variant allele frequency to be
364 detected. Use of somatic variant callers adopted on these large-scale datasets could further
365 improve the sensitivity of OncMTR.

366

367

368 **Methods**

369 **Reconstructing the Missense Tolerance Ratio with 125K samples from gnomAD**

370 We first reconstruct the Missense Tolerance Ratio (MTR) using a cohort of 125,748 exomes
371 from the gnomAD consortium (v2, GRCh38 liftover). The formula for deriving the window-based
372 MTR scores has been introduced in the original paper (Traynelis et al., 2017):

373

$$MTR = \frac{\frac{missense (obs)}{missense (obs) + synonymous (obs)}}{\frac{missense (exp)}{missense (exp) + synonymous (exp)}}$$

374

375 where the numerator represents the observed proportion of missense variants among the total
376 observed protein-coding variation. The numerator is then scaled by the same proportion
377 computed from the collection of all possible protein-coding variants in the corresponding protein-
378 coding window. A window size of 31 codons has been employed for calculating MTR based on
379 the gnomAD cohort, in agreement with the previously published score (Traynelis et al., 2017).

380

381 The expected proportion of missense variants in a given protein-coding window was calculated
382 by annotating all possible variants of a protein-coding transcript with SnpEff 4.3t using
383 GRCh38.92 as the reference annotation and assuming all events were equally likely to occur.
384 Annotation with SnpEff focused on single nucleotide variants (SNVs) that were flagged as PASS
385 variants in the original gnomAD release (v2). Variants annotated as 'missense_variant' or
386 'missense_variant&splice_region_variant' by SnpEff represent the set of 'missense' variants in
387 the MTR formula. Variants annotated as 'synonymous_variant', 'stop_retained_variant',
388 'splice_region_variant&stop_retained_variant' or 'splice_region_variant&synonymous_variant'
389 by SnpEff were considered as the 'synonymous' variants in the same formula.

390

391 **OncMTR score construction**

392 Using MTR as our basis, we construct the OncMTR score (i.e. Oncology MTR score) to capture
393 protein-coding subregions that are depleted of germline missense variants but observe somatic
394 mutations. We observe that the total distribution of AB_MEDIAN values across all gnomAD
395 variants (**Fig. 1A**) is bimodal, with the main peak centered close to 0.5 and a second one
396 emerging for values approximately around 0.2. The AB_MEDIAN metric represents the allelic
397 ratio between the alleles for each variant, with values close to 0.5 reflecting an equal number of

398 copies being inherited from each parent in heterozygous settings, while truly biological variants
399 that approach zero increasingly reflect variants that more likely arose somatically.

400

401 We leverage this observation to construct an alternative version of the original MTR score:
402 excluding any putative somatic variants and employing only germline variants from the gnomAD
403 dataset. We achieve that by selecting only variants with AB_MEDIAN > 0.3, constructing the
404 MTR^{germline} version of the score. OncMTR is then defined as the difference of the original MTR
405 score from the MTR^{germline} version:

$$406 \quad \text{OncMTR} = MTR^{\text{germline}} - MTR$$

407 Negative OncMTR values (i.e. $MTR^{\text{germline}} < MTR$) represent regions that are depleted of
408 germline variants and are instead enriched for somatic variation, thus allowing to highlight
409 putative oncogenic subregions in protein coding genes.

410

411 **Compilation of variant sets**

412 We used a pre-compiled set of variants known to be drivers of haematologic malignancies in a
413 total of 160 genes (Jaiswal et al., 2014). This list was generated from recurrent haematologic
414 somatic mutations in the literature and COSMIC, excluding genes with a relatively high
415 proportion of loss-of-function germline mutations. A second, smaller pre-compiled list, focused
416 on genes which were recurrent drivers specifically for myeloid malignancies (Bick et al., 2020).
417 A third validation set included a list of annotated driver mutations provided through the IntoGen
418 database (Tamborero et al., 2018). We restricted this set to “Tier 1” (highest confidence) driver
419 mutations observed in hematologic malignancies, which included ALL, AML, CLL, DLBC, and
420 MM.

421

422 **Classification of oncogenic variant sets with OncMTR**

423 We have performed classification of different oncogenic variant sets (TOPMed leukemogenic
424 and Intogen drivers) against random variant sets of equal size. We employ two supervised
425 models for the binary classification task: Logistic Regression with ‘max_iter’=1000 and a
426 Random Forest classifier with ‘max_depth’=2, to avoid overfitting on the training set. Each
427 classification was performed as a 5-fold cross-validation task and the mean Area Under Curve
428 (AUC) from all folds is reported to reflect the total average performance of each learning task.
429 The implementations of Logistic Regression and Random Forest were derived from the sklearn
430 Python package (v0.22.1).

431

432 We also estimated the optimal OncMTR cut point for each classification by calculating the
433 Youden's index from each learning task. The average Youden index from all classification tasks
434 performed with Logistic Regression was $Y_{LR} = -0.0409$ (standard deviation: 0.00126) while for
435 Random Forest it was $Y_{RF} = -0.0614$ (standard deviation: 0.00057). The mean of the two
436 averages of Youden indexes is -0.05115 or -0.05, after rounding it up to one decimal point for
437 simplicity. We thus consider OncMTR values below -0.05 to have the most distinctive power.

438

439 **Identifying OncMTR regions significantly enriched for ClinVar somatic variants**

440 For this analysis, we use all ClinVar somatic variants (ORIGIN=2) from the GRCh38 release
441 (last accessed on 9 June 2019), focusing on those annotated as missense or synonymous. We
442 consider as pathogenic variants those annotated as "Pathogenic" or "Likely_pathogenic" and as
443 benign those annotated as "Benign" or "Likely_benign" (based on ClinVar). Classification
444 between pathogenic and benign (or random) variant sets was performed with a logistic
445 regression classifier with 5-fold cross validation (sklearn, Python package v0.22.1). When
446 restricting the classification to heme-implicated genes, we derived those gene sets based on the
447 Intogen annotation (**Supplementary Table 10**).

448

449 In order to identify genes/transcripts across the exome that are preferentially enriched for
450 ClinVar somatic pathogenic variants in regions with low OncMTR scores we employ Fisher's
451 exact test. Specifically, we scan across each transcript and identify what percentage of the
452 codons in each transcript achieve an OncMTR score at the bottom 20-percentile of the full
453 OncMTR distribution (across the entire transcript). Then, we check whether known pathogenic
454 or likely pathogenic ClinVar missense variants preferentially land in these codons (i.e.
455 corresponding to low OncMTR scores) compared to the rest of the transcript. We apply a
456 Fisher's exact test (FET) to evaluate the enrichment of each set of regions, i.e., those with low
457 OncMTR scores vs the rest of the transcript. Eventually, we rank each transcript based on the
458 odds ratio and significance of the FET enrichments (**Supplementary Table 11**).

459

460 **Enrichment of low OncMTR scores in protein domains**

461 To describe the functional context of OncMTR, we calculated enrichment of constrained regions
462 in protein domain families. Residues within each canonical transcript (as defined by UniProtKB)
463 were divided into two classes based on their corresponding OncMTR scores: below -0.05
464 (constrained; as defined by Youden's index) and greater or equal -0.05 (relaxed). Domain and
465 clan annotations for the human proteome were taken from the Pfam 34.0 database. DNA-

466 binding domains were pulled from a previous compendium (Bahrami et al., 2015). The final set
467 of the canonical human proteome consisted of 18,313 annotated proteins. Enrichments of the
468 constrained regions in protein domains were tested with the Fisher's exact test followed by
469 Bonferroni correction, and significance level of adjusted p -value of 0.05.

470

471 **UK Biobank Collapsing analysis**

472 Collapsing analyses were performed using the 394,694 exomes available in the UK Biobank
473 (UKB) cohort (Bycroft et al., 2018). The UKB is a prospective study of approximately 500,000
474 participants aged 40–69 years at time of recruitment. Participants were recruited in the UK
475 between 2006 and 2010 and are continuously followed. The average age at recruitment for
476 sequenced individuals was 56.5 years and 54% of the sequenced cohort is of female genetic
477 sex. Participant data include health records that are periodically updated by the UKB, self-
478 reported survey information, linkage to death and cancer registries, collection of urine and blood
479 biomarkers, imaging data, accelerometer data and various other phenotypic end points. All
480 study participants provided informed consent and the UK Biobank has approval from the North-
481 West Multi-centre Research Ethics Committee (MREC; 11/NW/0382).

482 We performed a gene-based collapsing analysis on 1,394 chapter IX (neoplasm)
483 phenotypes adopting our previously described approach (Wang et al., 2021). We implemented a
484 total of eight dominant collapsing models with and without OncMTR filters (**Supplementary**
485 **Table 12**). Using SnpEff (Cingolani et al., 2012), we defined PTVs as variants annotated as
486 exon_loss_variant, frameshift_variant, start_lost, stop_gained, stop_lost,
487 splice_acceptor_variant, splice_donor_variant, gene_fusion, bidirectional_gene_fusion,
488 rare_amino_acid_variant, and transcript_ablation. We defined missense as:
489 missense_variant_splice_region_variant, and missense_variant. Non-Synonymous variants
490 included: exon_loss_variant, frameshift_variant, start_lost, stop_gained, stop_lost,
491 splice_acceptor_variant, splice_donor_variant, gene_fusion, bidirectional_gene_fusion,
492 rare_amino_acid_variant, transcript_ablation, conservative_inframe_deletion,
493 conservative_inframe_insertion, disruptive_inframe_insertion, disruptive_inframe_deletion,
494 missense_variant_splice_region_variant, missense_variant, and protein_altering_variant. We
495 derived allele frequencies from gnomAD (Karczewski et al., 2020). The raredmg,
496 raredmg_OncMTR, flexdmg, and flexdmg_oncMTR models incorporated a REVEL cutoff of
497 REVEL ≥ 0.5 to restrict to putatively damaging missense variants (Ioannidis et al., 2016).

498 To compute p -values, the carriers of at least one qualifying variant (QV) in a gene were
499 compared to the non-carriers. The difference in the proportion of cases and controls carrying

500 QVs in a gene was tested using a Fisher's exact two-sided test. we applied the following quality
501 control filters: minimum coverage 10X; annotation in CCDS transcripts (release 22;
502 approximately 34 Mb); at most 80% alternate reads in homozygous genotypes; percent of
503 alternate reads in heterozygous variants ≤ 0.25 and ≥ 0.8 ; binomial test of alternate allele
504 proportion departure from 50% in heterozygous state $P < 1 \times 10^{-6}$; GQ ≤ 20 ; FS ≥ 200 (indels) \geq
505 60 (SNVs); MQ ≤ 40 ; QUAL ≤ 30 ; read position rank sum score ≤ -2 ; MQRS ≤ -8 ; DRAGEN
506 variant status = PASS; the variant site achieved ten-fold coverage in $\leq 25\%$ of gnomAD
507 exomes, and if the variant was observed in gnomAD exomes, the variant achieved exome z-
508 score ≤ -2.0 and exome MQ ≤ 30 . We excluded 46 genes that we previously found associated
509 with batch effects (Wang et al., 2021).

510 For all models, we applied the following quality control filters: minimum coverage 10X;
511 annotation in CCDS transcripts (release 22; approximately 34 Mb); at most 80% alternate reads
512 in homozygous genotypes; percent of alternate reads in heterozygous variants ≤ 0.25 and ≥ 0.8 ;
513 binomial test of alternate allele proportion departure from 50% in heterozygous state $P < 1 \times 10^{-6}$;
514 GQ ≤ 20 ; FS ≥ 200 (indels) ≥ 60 (SNVs); MQ ≤ 40 ; QUAL ≤ 30 ; read position rank sum score
515 ≤ -2 ; MQRS ≤ -8 ; DRAGEN variant status = PASS; the variant site achieved ten-fold coverage
516 in $\leq 25\%$ of gnomAD exomes, and if the variant was observed in gnomAD exomes, the variant
517 achieved exome z-score ≤ -2.0 and exome MQ ≤ 30 . We excluded 46 genes that we previously
518 found associated with batch effects¹⁰.

519 We defined the study-wide significance threshold as $p < 1 \times 10^{-8}$. We have previously
520 shown using an n-of-1 permutation approach and the empirical null synonymous model that this
521 threshold corresponds to a false positive rate of 9 and 2, respectively, out of ~ 346.5 million tests
522 for binary traits in the setting of collapsing analysis PheWAS (Wang et al., 2021).

523

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527

528

529 **Author Contributions**

530 D.V., R.S.D., and S.P. designed the study. D.V., R.S.D., J.M., D.M., J.A., Q.W., B.S., A.R.H.,
531 and S.P. performed analyses and statistical interpretation. Q.W. performed bioinformatic
532 processing. D.V., R.S.D., and S.P. wrote the manuscript. D.V. and D.M. developed the web
533 portal. D.V., R.S.D., J.M., D.M., Z.Z., J.A., Q.W., B.S., A.R.H, and S.P. reviewed the manuscript.

534

535

536 **Competing Interests**

537 D.V., R.S.D., J.M., D.M., Z.Z., J.A., Q.W., B.S., A.R.H, and S.P. are current employees and/or
538 stockholders of AstraZeneca.

539

540

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