Clinical Analysis of PGP Participants with *BRCA* frameshifts

We used the default CAVA impact factors for mutations [1]. A variant is given impact 1 if it is a stop-gain variant caused by a base substitution, a variant that alters essential splice-site base (+1, +2, -1, -2), or a frameshifting insertion and/or deletion. A variant is given impact 2 if it a variant that alters the +5 splice-site base and is not impact 1, a variant that alters the first or last 3 bases of an exon but not the frame of the coding sequence, a variant that alters the initiating methionine start coding, a stop-loss variant, an inframe insertion and/or deletion, or a nonsynonymous variant. Other types of mutations are given impact 3. Below, we report all variations observed that would cause a variant of impact 1 or 2, regardless of whether they are preceded by a stop-gain variant introduced by a frameshift. We assume that though they might be reported to be on the same phase, the phase groups are not necessarily well defined, meaning the frameshift and stop-gain variants could easily be out of phase with other mutations in the genome.

Overall, we would like to observe that *BRCA1* contains fewer variations overall and fewer frameshifts causing premature stop codons. The number of tile variants in *BRCA2* is partially due to poorly sequenced regions and partially due to a higher number of genomic variations. Given the importance of *BRCA2*, we suggest further research into improving quality of calls in *BRCA2*. We also observe that the only female reporting breast cancer, who has a frameshift variant in *BRCA2*, also has a large number of homozygous recessive variants in *BRCA1*. Further studies on the effect of *BRCA1/2* variations might focus on the effect of normally benign or unclassified mutations when inherited with a pathogenic frameshift variation.

All visualizations shown below may be viewed at `science.curoverse.com/tiling/brca/pgp-graph`.

`hu72C17A`

Participant `hu72C17A` is a male in his 50's with no self-reports concerning cancer who has a pathogenic frameshift/splice region variant at *BRCA2*, exon 24, K3084N that adds a stop-gain variant at 3086 by removing the exon-splice site. The tile library around 00.247.0bc8, the tile position containing the pathogenic frameshift variant is shown in Fig. 1. All tile variants occurring in `hu72C17A`

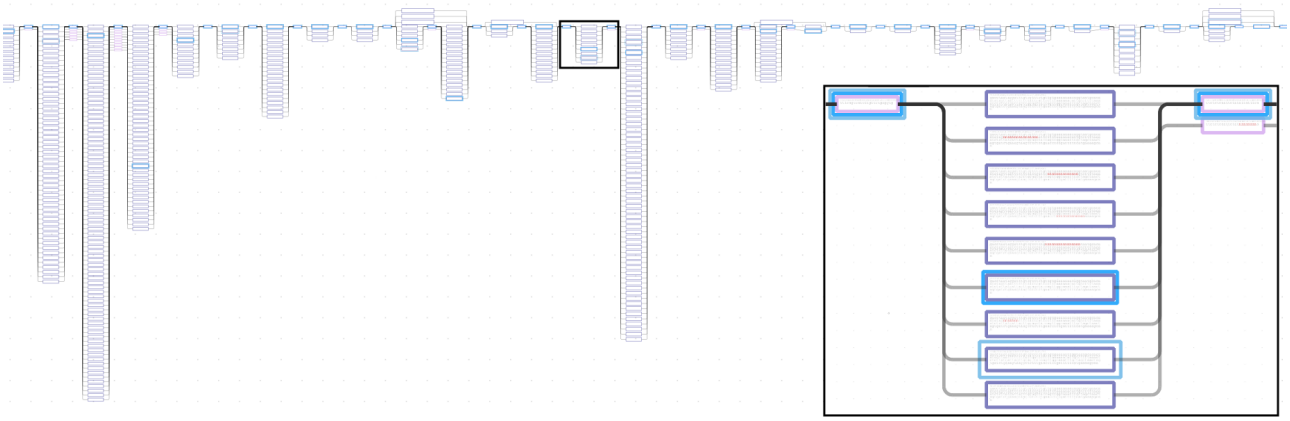

Figure 1. Visualization of the *BRCA2* tile library around the pathogenic tile variant found in `hu72C17A`. `hu72C17A` is highlighted in blue: phase 1 is highlighted with the inner blue overlay; phase 2 is highlighted with a lighter outer blue overlay. Position 00.247.0bc8 is boxed in black and shown zoomed in on the bottom right of the image. Tile variants are ordered into columns by tile position. Spanning tiles are shown above the center row. The center row is the GRCh37 reference sequence. Poorly sequenced regions can be seen as red marks in the zoomed in visualization.

with at least one genomic variant given impact 1 or 2 by CAVA, described in the order which they appear along the chromosome, are shown in Table 1. He has 2 potentially worrying variants in *BRCA2* and 1 potentially worrying *BRCA1* variant. Participant `hu72C17A` is homozygous for the variant *BRCA2*, exon 14, V2466A, classified by ClinVar to be benign. ExAC reports its allele frequency to be 99.4% ($n = 121398$). He is heterozygous for the variant *BRCA2*, Ex24, K3084N*fs*, a frameshift variant submitted to ClinVar by Breast Cancer Information Core and Sharing Clinical Reports Project, and classified as pathogenic. This variant is not found in ExAC. He is heterozygous for the *BRCA1*, Ex10, P871L variant, which is not in ClinVar. ExAC reports it allele frequency to be 41% ($n = 121412$).

`hu82436A`

Participant `hu82436A` is a 29-year-old male with no self-reports relating to cancer, who has a pathogenic frameshift variation in *BRCA1*, exon 10, D1162V that introduces a stop-gain variant at codon 1209. The tile library around position 00.2c5.04c0, the position containing the pathogenic tile variant, is shown in Fig. 2. Tile variants in `hu82436A` with at least one genomic variant given impact 1 or 2 by CAVA, described in the order which they appear along the chromosome, are shown in Table 2. He has 3 potentially worrying variants in *BRCA2* and 5 potentially worrying *BRCA1* variants. He is heterozygous for a SNV causing

Table 1. *BRCA1* and *BRCA2* tile variants `hu72C17A` tile variants which have a possibly damaging genomic variant. ExAC frequency is not shown if the variant is not found in ExAC. Phases are assigned by Complete Genomics. All loci are 0-based, in GRCh37 coordinates. Protein changes were predicted by CAVA.

Tile position, Variant name, Phase	Covers loci	Freq. of Tile	Freq. of Variant in ExAC	Genomic Variants in Tile	Predicted Protein Change
00.247.0b6b, 088b78ec892d503c6aaab1f3d053b7a9, phase A	chr13: 32,929,173 - 32,929,423	65.6% (1360)	99.4% (121,398)	32,929,386 SNP T→C	*BRCA2*, Ex14, V2466A
00.247.0b6b, 877d2ebddc414a8f759fb7d9f3d92045, phase B	chr13: 32,929,173 - 32,929,423	19.7% (1360)	22.4% (121,408)	32,929,231 SNP A→G	-
			99.4% (121,398)	32,929,386 SNP T→C	*BRCA2*, Ex14, V2466A
00.247.0bc8, d16d51ee6d027ae066ea827dc181d038, phase B	chr13: 32,954,218 - 32,954,467	0.073% (1360)	-	32,954,277 INDEL AACA→TT	*BRCA2*, Ex24, K3084N*fs*
00.2c5.04c4, d288f8636dfe8818f72dfbda76138c46, phase B	chr17: 41,244,787 - 41,245,036	45.2% (1360)	41% (121,412)	41,244,935 SNP G→A	*BRCA1*, Ex10, P871L

the *BRCA2* N372H variant, which has conflicting interpretations in ClinVar. 4 submissions classify the variant as benign, 1 classifies the variant as pathogenic, and 1 submission did not classify the variant. This variant is known to ExAC, and has a reported allele frequency of 27.7% ($n = 121208$). He is heterozygous for a SNP causing the *BRCA2* R2108H not found in ClinVar, though it is in ExAC, and has a reported allele frequency of 0.1269% ($n = 121398$). `hu82436A` is homozygous for a SNP resulting in the *BRCA2* V2466A missense mutation, which is classified by ClinVar to be benign. ExAC reports this allele frequency to be 99.4% ($n = 121398$). He is heterozygous for a SNV resulting in *BRCA1* S1634G, which is not present in ClinVar or ExAC. He is heterozygous for a SNV, classified by ClinVar as benign, which results in *BRCA1* K1183R. ExAC reports this SNV to have an allele frequency of 34.8% ($n = 121406$). He is heterozygous for a frameshift variant resulting in *BRCA1* D1162V, which results in a stop-gain variant at codon 1209 variant is a frameshift in exon 10 classified by ClinVar as pathogenic. This variant is not in ExAC. `hu82436A` is heterozygous for a SNV resulting in the mutation *BRCA1* E1038G, which is not in ClinVar. ExAC reports the allele frequency of this variant to be 34.3% ($n = 121404$). Finally, he is also heterozygous for a SNV not in ClinVar that results in the mutation *BRCA1* P871L. This SNV has an ExAC allele frequency of 41% ($n = 121412$).

Table 2. *BRCA1* and *BRCA2* tile variants `hu82436A` tile variants which have a possibly damaging genomic variant. ExAC frequency is not shown if the variant is not found in ExAC. Phases are assigned by Complete Genomics. All loci are 0-based, in GRCh37 coordinates. Protein changes were predicted by CAVA.

Tile position, Variant name, Phase	Covers loci	Freq. of Tile	Freq. of Variant in ExAC	Genomic Variants in Tile	Predicted Protein Change
0.8 00.247.0b11, 66b2b2ff2b6a27a4f094fc4b17231a1a, phase B	chr13: 32,906,623 - 32,906,872	22.5% (1360)	27.7% (121,208)	32,906,728 SNP A→C	*BRCA2*, Ex10, N372H
00.247.0b31, d1076b181410eab60e91ea07a9f48e2a, phase B	chr13: 32,914,593 - 329,14,842	0.15% (1360)	0.127% (121,398)	32,914,814 SNP G→A	*BRCA2*, Ex11, R2108H
00.247.0b6b, 088b78ec892d503c6aaab1f3d053b7a9, phase A and B	chr13: 32,929,173 - 32,929,423	65.6% (1360)	99.4% (121,398)	32,929,386 SNP T→C	*BRCA2*, Ex14, V2466A
00.2c5.0473, 386bf7e30ef14a0b0b52676e68728700, phase B	chr17: 41,222,921 - 41,223,170	26.6% (1360)	-	41,223,093 SNP T→C	*BRCA1*, Ex15, S1634G
00.2c5.04c0, 84fa1b2665461533586d77aa8c21393e, phase B	chr17: 41,243,883 - 41,244,132	0.073% (1360)	34.8% (121,406)	41,243,999 SNP T→C	*BRCA1*, Ex10, K1183R
			-	41,244,062 INDEL T→-	*BRCA1*, Ex10, D1162Vfs
00.2c5.04c2, ae7ce1e177b20af63a5d28c5e7620012, phase B	chr17: 41,244,333 - 41,244,586	30.1% (1360)	34.3% (121,404)	41,244,434 SNP T→C	*BRCA1*, Ex10, E1038G
00.2c5.04c4, d288f8636dfe8818f72dfbda76138c46, phase B	chr17: 41,244,787 - 41,245,036	45.2% (1360)	41% (121,412)	41,244,935 SNP G→A	*BRCA1*, Ex10, P871L

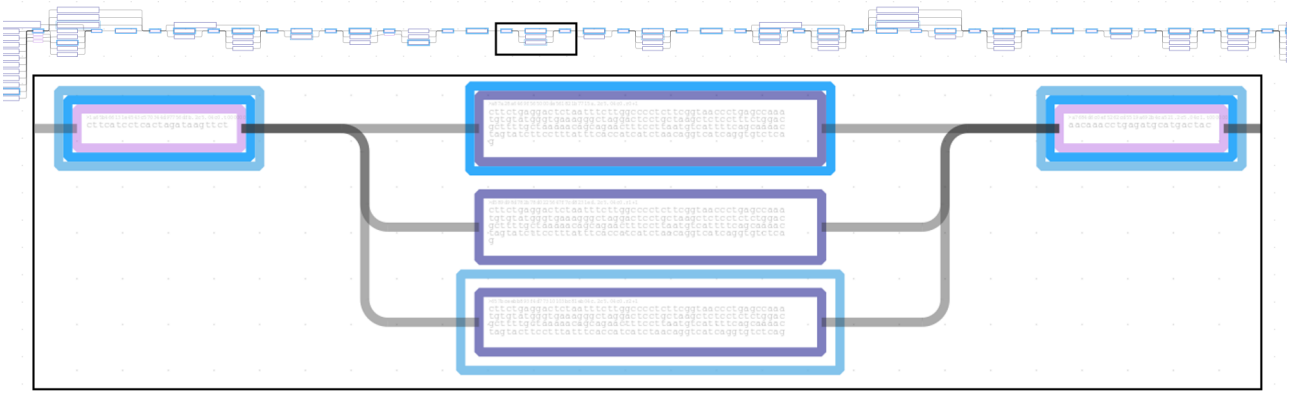

Figure 2. Visualization of the *BRCA1* tile library around the pathogenic tile variant found in `hu82436A`. `hu82436A` is highlighted in blue: phase 1 is highlighted with the inner blue overlay; phase 2 is highlighted with a lighter outer blue overlay. Position 00.2c5.04c0 is boxed in black and shown zoomed in on the bottom of the image. Tile variants are ordered into columns by tile position. Spanning tiles are shown above the center row. The center row is the GRCh37 reference sequence. This library section is one of low variance, partially resulting from well sequenced regions.

hu92C40A

Participant `hu92C40A` is a 71-year-old female who reports having breast cancer at age 66 and who is heterozygous for a frameshift variant in *BRCA2*, exon 11, F1866L, which introduces a stop-gain variant at 1873. The tile library around position 00.247.0b2e, the position containing the tile variant with this mutation, with participant `hu92C40A`'s sequence highlighted is shown in Fig. 3. Tile variants in `hu92C40A` with at least one genomic variant given impact 1 or 2 by CAVA, described in the order which they appear along the chromosome, are shown in Table 3. She has 3 potentially pathogenic variants in *BRCA2* and 5 potentially pathogenic variants in *BRCA1*. The first variant is a frameshift variant, resulting in *BRCA2* F1866L and a stop-gain variant at 1873. We classified this variant as pathogenic, using the definitions outlined by Rebbeck *et al*, 2015 [2], because it introduces a stop-gain variant in *BRCA2*, exon 11. This variant is not in ClinVar or ExAC. She is heterozygous for a SNV resulting in *BRCA2* S2186T, which also not in ClinVar or ExAC. She is homozygous for a SNP, resulting in *BRCA2* V2466A, classified by ClinVar to be benign. ExAC reports this allele frequency to be 99.4% ($n = 121398$). She is homozygous for a SNV, resulting in the *BRCA1* S1634G, which is not found in ClinVar or ExAC. She is also homozygous for a SNV that results in the *BRCA1* K1183R and that is classified by ClinVar as benign. ExAC reports this variant to have an allele frequency of 34.8% ($n = 121406$). She is homozygous for a SNV resulting in *BRCA1* E1038G, which is not in ClinVar. ExAC reports this variant to have an

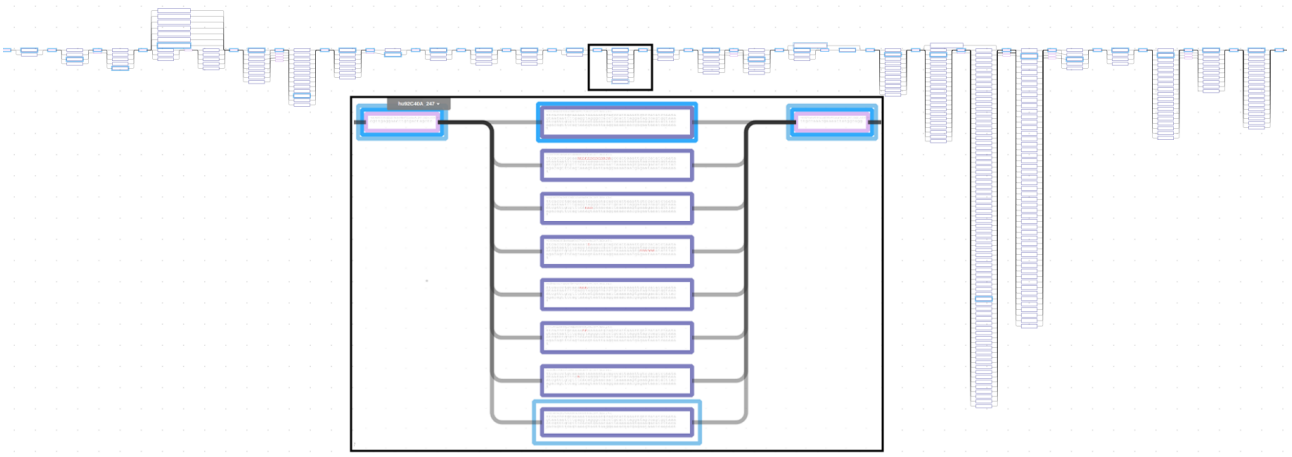

Figure 3. Visualization of the *BRCA2* tile library around the pathogenic tile variant found in `hu92C40A`. `hu92C40A` is highlighted in blue: phase 1 is highlighted with the inner blue overlay; phase 2 is highlighted with a lighter outer blue overlay. Position 00.247.0b2e is boxed in black and shown zoomed in at the bottom center of the image. Tile variants are ordered into columns by tile position. Spanning tiles are shown above the center row. The center row is the GRCh37 reference sequence. Poorly-sequenced regions can be seen as red marks in the zoomed in visualization.

allele frequency of 34.3% ($n = 121404$). She is homozygous for a SNV, which is not in ClinVar, that results in the *BRCA1* P871L mutation. ExAC reports this variant to have an allele frequency of 41% ($n = 121412$). She is heterozygous for a SNV not in ClinVar, which results in the *BRCA1* D693N variant. ExAC reports this variant to have an allele frequency of 5.7% ($n = 121408$).

`huCD380F`

Participant `huCD380F` is a 33-year-old female who does not report any phenotypes relating to cancer and who is heterozygous for a frameshift variant: *BRCA1*, exon 10, V240G, which introduces a stop-gain variant at 345. The tile library at tile position 00.2c5.04cb, the position that contains this variant, with `huCD380F` highlighted, is shown in Fig. 4. Tile variants in `huCD380F` with at least one genomic variant given impact 1 or 2 by CAVA, described in the order which they appear along the chromosome, are shown in Table 4. She has 2 potentially worrying variants in *BRCA2* and 2 potentially worrying *BRCA1* variants. She is heterozygous for a SNV resulting in the mutation *BRCA2* N372H, which has conflicting interpretations in ClinVar. 4 submissions classify the variant as benign, 1 classifies the variant as pathogenic, and 1 submission did not classify the variant. This variant is known to ExAC, and has a reported allele frequency of 27.7% ($n = 121208$). She is homozygous for the *BRCA2* V2466A

Table 3. *BRCA1* and *BRCA2* tile variants `hu92C40A` tile variants which have a possibly damaging genomic variant. ExAC frequency is not shown if the variant is not found in ExAC. Phases are assigned by Complete Genomics. All loci are 0-based, in GRCh37 coordinates. Protein change was predicted by CAVA.

Tile position, Variant name, Phase	Covers loci	Freq. of Tile	Freq. of Variant in ExAC	Genomic Variants in Tile	Predicted Protein Change
00.247.0b2e, 3552b9fc68c47f5e7783addc4c2877a2, phase B	chr13: 32,913,918 - 32,914,167	0.073% (1360)	-	32,914,086 INDEL AT→C	*BRCA2*, Ex11, F1866L*fs*
00.247.0b32 (spans 2 positions), 9b139321a46717d287369ee083b7ed40, phase B	chr13: 32,914,818 - 32,915,067	0.073% (1360)	99.3% (121,384)	32,915,004 SNP G→C	-
			-	32,915,047 SNP T→A	*BRCA2*, Ex11, R2108H
00.247.0b6b, 877d2ebddc414a8f759fb7d9f3d92045, phase B	chr13: 32,929,173 - 32,929,423	19.7% (1360)	22.4% (121,408)	32,929,231 SNP A→G	-
			99.4% (121,398)	32,929,386 SNP T→C	*BRCA2*, Ex14, V2466A
00.2c5.0473, 386bf7e30ef14a0b0b52676e68728700, phase A and B	chr17: 41,222,921 - 41,223,170	26.6% (1360)	-	41,223,093 SNP T→C	*BRCA1*, Ex15, S1634G
00.2c5.04c0, bcbbe9c21fa647da52e97c9c0b59fbdc, phase A and B	chr17: 41,243,883 - 41,244,132	31.8% (1360)	34.8% (121,406)	41,243,999 SNP T→C	*BRCA1*, Ex10, K1183R
00.2c5.04c2, ae7ce1e177b20af63a5d28c5e7620012, phase A and B	chr17: 41,244,333 - 41,244,586	30.1% (1360)	34.3% (121,404)	41,244,434 SNP T→C	*BRCA1*, Ex10, E1038G
00.2c5.04c4, d288f8636dfe8818f72dfbda76138c46, phase A and B	chr17: 41,244,787 - 41,245,036	45.2% (1360)	41% (121,412)	41,244,935 SNP G→A	*BRCA1*, Ex10, P871L
00.2c5.04c6 (spans 2 positions), 1aeba432fc45eb70741e4c5de472c19d, phase B	chr17: 41,245,237 - 41,245,486	4.85% (1360)	34.8% (121,406)	41,245,465 SNP G→A	-
			5.7% (121,408)	41,245,470 SNP C→T	*BRCA1*, Ex10, D693N

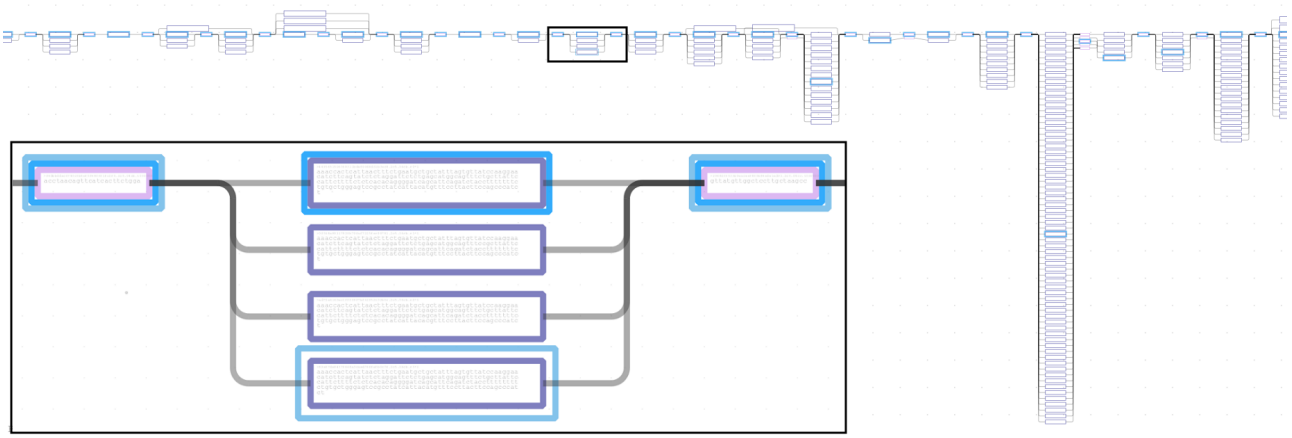

Figure 4. Visualization of the *BRCA1* tile library around the pathogenic tile variant found in `huCD380F`. `huCD380F` is highlighted in blue: phase 1 is highlighted with the inner blue overlay; phase 2 is highlighted with a lighter outer blue overlay. Position 00.2c5.04cb is boxed in black and shown zoomed in at the bottom center of the image. Tile variants are ordered into columns by tile position. Spanning tiles are shown above the center row. The center row is the GRCh37 reference sequence.

mutation, caused by a SNV and classified by ClinVar as benign. ExAC reports its allele frequency to be 99.4% ($n = 121398$). She is heterozygous for all *BRCA1* variations. She has a SNV resulting in *BRCA1* S1634G, which is not known to ClinVar or ExAC. Additionally, she has a frameshift variant not found in ExAC, and classified by ClinVar as pathogenic.

`huD3A569`

Participant `huD3A569` is a male in his 40's who reports 2 non-melanoma skin cancer occurrences in his 30's. He also reports that his mother has 20+ occurrences of skin cancer. He has a frameshift variant: *BRCA2*, exon 11, S1982R, which introduces a stop-gain variant at codon 2003. This variant is a founder mutation in the Ashkenazi Jewish population. The tile library around tile position 00.247.0b30, which contains the tile variant with this pathogenic frameshift variant, with participant `huD3A569`'s sequence highlighted is shown in Fig. 5. Tile variants appearing in participant `huD3A569` with at least one genomic variant given impact 1 or 2 by CAVA, described in the order which they appear along the chromosome, are shown in Table 5. He has 3 potentially worrying variants in *BRCA2* and 5 potentially worrying *BRCA1* variants. He is heterozygous for a SNV which has conflicting interpretations in ClinVar, which results in the *BRCA1* N372H mutation. 4 submissions classify the variant as benign, 1 classifies the variant as pathogenic, and 1 submission did not classify the variant.

Table 4. *BRCA1* and *BRCA2* tile variants `huCD380F` tile variants which have a possibly damaging genomic variant. ExAC frequency is not shown if the variant is not found in ExAC. Phases are assigned by Complete Genomics. All loci are 0-based, in GRCh37 coordinates. Protein change prediction was produced by CAVA.

Tile position, Variant name, Phase	Covers loci	Freq. of Tile	Freq. of Variant in ExAC	Genomic Variants in Tile	Predicted Protein Change
00.247.0b11, 66b2b2ff2b6a27a4f094fc4b17231a1a, phase B	chr13: 32,906,623 - 32,906,872	22.5% (1360)	27.7% (121,208)	32,906,728 SNP A→C	*BRCA2*, Ex10, N372H
00.247.0b6b, 088b78ec892d503c6aaab1f3d053b7a9, phase A and B	chr13: 32,929,173 - 32,929,423	65.6% (1360)	99.4% (121,398)	32,929,386 SNP T→C	*BRCA2*, Ex14, V2466A
00.2c5.0473, 386bf7e30ef14a0b0b52676e68728700, phase B	chr17: 41,222,921 - 41,223,170	26.6% (1360)	-	41,223,093 SNP T→C	*BRCA1*, Ex15, S1634G
00.2c5.04cb, e318e2ddb54bb3d6cc3a5ca6749482c5, phase B	chr17: 41,246,365 - 41,246,614	0.073% (1360)	-	41,246,531 INDEL -→T	*BRCA1*, Ex10, V340G*fs*

This variant is known to ExAC, and has a reported allele frequency of 27.7% ($n = 121208$). He is heterozygous for a frameshift variant, resulting in *BRCA2* S1982Rfs, which is not in ExAC, and which is classified by ClinVar as pathogenic, or at least a risk factor, in familial breast cancer, familial breast-ovarian cancer, pancreatic cancer, and fanconi anemia. He is homozygous for a SNV classified by ClinVar to be benign, which results in the mutation *BRCA2* V2466A. ExAC reports its allele frequency to be 99.4% ($n = 121398$). `huD3A569` is heterozygous for all *BRCA1* variations. His first variant is a SNV not in ClinVar or ExAC that causes the mutation *BRCA1* S1634G. His second variant is a SNV, resulting in *BRCA1* K1183R, classified by 9 submissions in ClinVar to be benign. ExAC reports its allele frequency to be 34.8% ($n = 121406$). His third variant in *BRCA1* is a SNV, which results in the mutation *BRCA1* E1038G. It is not known to ClinVar, but has an ExAC allele frequency of 34.3% ($n = 121404$). His fourth variant in *BRCA1* is a SNV not found in ClinVar that results in the mutation *BRCA1* P871L. ExAC reports its allele frequency to be 41% ($n = 121412$). His last variant is a SNV in *BRCA1* that is also not in ClinVar, and which ExAC reports an allele frequency of 5.7% ($n = 121408$). This SNV results in the *BRCA1* D693N mutation.

`huFFB09D`

Participant `huFFB09D` is a female in her 40's who does not report any phenotypes relating to cancer, who is heterozygous for the mutation *CASP10*-V410I, which has a possibly protective effect on breast cancer, and who is heterozygous for a

Table 5. *BRCA1* and *BRCA2* tile variants `huD3A569` tile variants which have a possibly damaging genomic variant. ExAC frequency is not shown if the variant is not found in ExAC. Phases are assigned by Complete Genomics. All loci are 0-based, in GRCh37 coordinates. Protein change prediction was produced by CAVA.

Tile position, Variant name, Phase	Covers loci	Freq. of Tile	Freq. of Variant in ExAC	Genomic Variants in Tile	Predicted Protein Change
00.247.0b11, 66b2b2ff2b6a27a4f094fc4b17231a1a, phase B	chr13: 32,906,623 - 32,906,872	22.5% (1360)	27.7% (121,208)	32,906,728 SNP A→C	*BRCA2*, Ex10, N372H
00.247.0b30, b0ee262cb5737ac19d609912fa2f0d40, phase B	chr13: 32,914,368 - 32,914,617	0.073% (1360)	-	32,914,437 INDEL T→-	*BRCA2*, Ex11, S1982R*fs*
00.247.0b6b, 088b78ec892d503c6aaab1f3d053b7a9, phase A and B	chr13: 32,929,173 - 32,929,423	65.6% (1360)	99.4% (121,398)	32,929,386 SNP T→C	*BRCA2*, Ex14, V2466A
00.2c5.0473, 386bf7e30ef14a0b0b52676e68728700, phase B	chr17: 41,222,921 - 41,223,170	26.6% (1360)	-	41,223,093 SNP T→C	*BRCA1*, Ex15, S1634G
00.2c5.04c0, bcbbe9c21fa647da52e97c9c0b59fbdc, phase B	chr17: 41,243,883 - 41,244,132	31.8% (1360)	34.8% (121,406)	41,243,999 SNP T→C	*BRCA1*, Ex10, K1183R
00.2c5.04c2, ae7ce1e177b20af63a5d28c5e7620012, phaseB	chr17: 41,244,333 - 41,244,586	30.1% (1360)	34.3% (121,404)	41,244,434 SNP T→C	*BRCA1*, Ex10, E1038G
00.2c5.04c4, d288f8636dfe8818f72dfbda76138c46, phase B	chr17: 41,244,787 - 41,245,036	45.2% (1360)	41% (121,412)	41,244,935 SNP G→A	*BRCA1*, Ex10, P871L
00.2c5.04c6 (spans 2 positions), 1aeba432fc45eb70741e4c5de472c19d, phase B	chr17: 41,245,237 - 41,245,486	4.85% (1360)	34.8% (121,406)	41,245,465 SNP G→A	-
			5.7% (121,408)	41,245,470 SNP C→T	*BRCA1*, Ex10, D693N

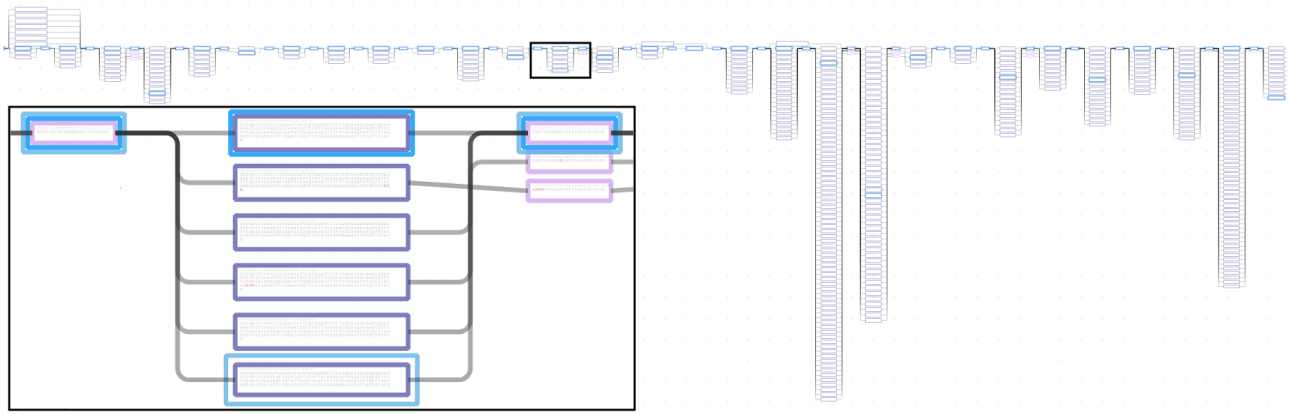

Figure 5. Visualization of the *BRCA2* tile library around the pathogenic tile variant found in `huD3A569`. `huD3A569` is highlighted in blue: phase 1 is highlighted with the inner blue overlay; phase 2 is highlighted with a lighter outer blue overlay. Position 00.247.0b30 is boxed in black and shown zoomed in at the bottom of the image. Tile variants are ordered into columns by tile position. Spanning tiles are shown above the center row. The center row is the GRCh37 reference sequence. Poorly-sequenced regions can be seen as red marks in the zoomed in visualization.

pathogenic frameshift variant in *BRCA2*, exon 11, N1198K, which introduces a stop-gain variant at codon 1199. The tile library around position 00.247.0b24, which contains the tile variant with the frameshift, is shown in Fig. 6, with the sequence of `huFFB09D` highlighted. Tile variants in `huFFB09D` which introduce a variation that CAVA classifies as impact 1 or 2 are shown in Table 6. Participant `huFFB09D` has 3 possibly pathogenic *BRCA2* variations and no possibly pathogenic *BRCA1* variations. She is heterozygous for a frameshift variant in *BRCA2*, which introduces the missense N1198K and a stop-gain variant at codon 1199. This frameshift variation is not known to ExAC or ClinVar. We classified the frameshift as pathogenic since it introduces a stop-gain variant in *BRCA2* exon 11, which, by the definitions used Rebbeck *et al*, 2015 [2], is pathogenic. She is heterozygous for a SNV, which is classified by 8 ClinVar submitters to be benign, by 1 ClinVar submitter to be likely benign, and 1 ClinVar submitter to be of uncertain significance. This SNV results in the *BRCA2* T1915M mutation and is reported by ExAC to have an allele frequency of 1.8% ($n = 121408$). Participant `huFFB09D` is homozygous for the *BRCA2* V2466A variant, which ClinVar classifies as benign. ExAC reports this variant to have an allele frequency of 99.4% ($n = 121398$).

Table 6. *BRCA1* and *BRCA2* tile variants huFFB09D tile variants which have a possibly damaging genomic variant. ExAC frequency is not shown if the variant is not found in ExAC. Phases are assigned by Complete Genomics. All loci are 0-based, in GRCh37 coordinates. Protein change prediction was produced by CAVA.

Tile position, Variant name, Phase	Covers loci	Freq. of Tile	Freq. of Variant in ExAC	Genomic Variants in Tile	Predicted Protein Change
00.247.0b24 (spans 2 positions), 91e94cf32f709225f17f37c159930451, phase B	chr13: 32,911,657 - 32,911,906	0.073% (1360)	29.3% (121,412)	32,911,887 SNP A→G	-
			-	32,912,080 INDEL -→A	*BRCA2*, Ex11, N1198K*fs*
00.247.0b2f, 824da8cbd6c5bd8de4e26f83b07100fd, phase B	chr13: 32,914,143 - 32,914,392	0.735% (1360)	1.8% (121,408)	32,914,235 SNP C→T	*BRCA2*, Ex11, T1915M
00.247.0b6b, 088b78ec892d503c6aaab1f3d053b7a9, phase A	chr13: 32,929,173 - 32,929,423	65.6% (1360)	99.4% (121,398)	32,929,386 SNP T→C	*BRCA2*, Ex14, V2466A
00.247.0b6b, 877d2ebddc414a8f759fb7d9f3d92045, phase B	chr13: 32,929,173 - 32,929,423	19.7% (1360)	22.4% (121,408)	32,929,231 SNP A→G	-
			99.4% (121,398)	32,929,386 SNP T→C	*BRCA2*, Ex14, V2466A

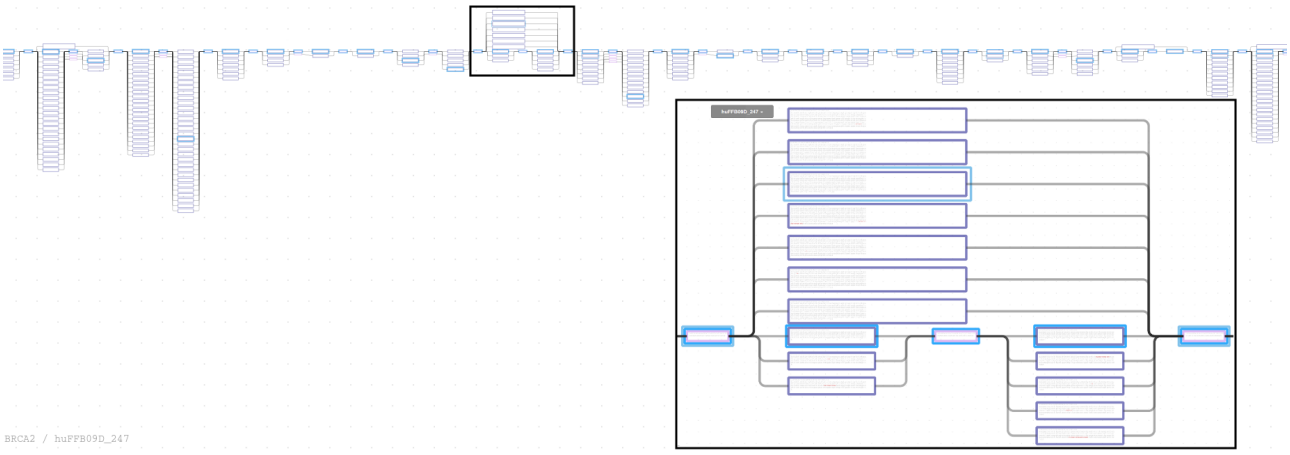

Figure 6. Visualization of the *BRCA2* tile library around the pathogenic tile variant found in huFFB09D. huFFB09D is highlighted in blue: phase 1 is highlighted with the inner blue overlay; phase 2 is highlighted with a lighter outer blue overlay. Position 00.247.0b24 is boxed in black and shown zoomed in at the bottom of the image. Tile variants are ordered into columns by tile position. Spanning tiles are shown above the center row. The center row is the GRCh37 reference sequence. Poorly-sequenced regions can be seen as red marks in the zoomed in visualization.

References

1. Muenz M, Ruark E, Renwick A, Ramsay E, Clarke M, Mahamdallie S, et al. CSN and CAVA: variant annotation tools for rapid, robust next-generation sequencing analysis in the clinic; 2015. Preprint. Available: doi: http://dx.doi.org/10.1101/016808. Accessed 30 March 2015.

2. Rebbeck TR, Mitra N, Wan F, Sinilnikova OM, Healey S, McGuffog L, et al. Association of Type and Location of *BRCA1* and *BRCA2* Mutations With Risk of Breast and Ovarian Cancer. JAMA. 2015;313(13): 1347-1361.