Supplemental Digital Content, containing 1 table and 2 figures. The Supplemental Digital Content was not copyedited by *Archives of Pathology & Laboratory Medicine*.

Supplemental Methods

Cell Lines

Three different cell lines from the Coriell Institute for Medical Research (Camden, NJ) were used between 2016 and 2020 including GM21781 (CAP#1), GM21846 (CAP#2), and GM24695 (CAP#3). The sex of each cell line was provided to the participants. CAP#1 was used for 2016 A/B and 2020 B mailings, CAP#2 was used for 2017, and 2019 A/B mailings, and CAP#3 was used for 2018 A/B, and 2020 A mailings. Each cell line was sequenced using whole exome and whole genome library preparation methods.

CAP#1 was subjected to four different sequencing methods including Ion Torrent exome sequencing (Life Technologies, now a part of Thermo Fisher Scientific, Waltham, MA), Illumina exome and Illumina genome sequencing (Illumina, San Diego, CA), Complete Genomics whole genome sequencing (Complete Genomics, San Jose, CA). Eventually by 2019, CAP#1 cell line was exhausted and regrown by Coriell. This regrown cell line was subjected to whole exome sequencing only. CAP#2 and CAP#3 were subjected to two sequencing methods including Illumina exome sequencing, and whole genome sequencing.

Bioinformatic analysis for data set

CAP#1 Ion torrent

CAP#1 deoxyribonucleic acid (DNA) was sent to Life Technologies (now part of Thermo Fisher) for sequencing. TargetSeq exome libraries were prepared according to the manufacturer's recommendation. Exome libraries were templated onto Ion Sphere Particles using an Ion OT2 200 Kit

and an Ion OneTouch 2 Instrument and template particles were enriched with an Ion OneTouch ES module. Ion Torrent 318 chips were loaded with enriched particles and sequenced on an Ion PGM system with the Ion PGM 200 Sequencing Kit. Raw data were transferred to the Torrent Server for analysis pipeline processing using TorrentSuite 3.6 with the default setting for standard deliverables at Life Technologies. All data were aligned to the human reference National Center for Biotechnology Information (NCBI) Build 37/hg19 human reference genome (National Library of Medicine, Bethesda, MD). The sequencing data quality control, alignment and variant calling were performed by Life Technologies and the variant output included single nucleotide variants (SNVs) as well as small insertions and deletions. Mean sequencing read coverage was 64-fold.

CAP#1 Illumina Exome Sequencing

DNA was sent to the ARUP Laboratories in Salt Lake City, Utah. Exome capture was performed using Roche Nimblegen SeqCap EZ Human Exome Library capture probes v3.0 (Roche, Basel, Switzerland) according to the manufacturer's instructions and was followed by sequencing on a single lane of the Illumina HiSeq 2000 (Illumina, San Diego, CA) with paired-end v3.0 sequencing chemistry to generate 2 X 100 (2 X 101) paired end reads. Read data in FASTQ file format were aligned to the NCBI37/hg19 human reference genome assembly using the Burrows-Wheeler Aligner (BWA) aligner (version 0.6.1, paired end, default settings; Illumina, San Diego, CA). Next, the Genomic Analysis Tool Kit (GATK) (version 1.5) IndelRealigner (Broad Institute, Cambridge, MA) was used for local realignment around indels. The dbSNP version 132 (National Library of Medicine, Bethesda, MD) was used with the realignment tool to make preliminary variant calls and to identify potential realignment positions. The Samtools (version 0.1.19) rmdup tool (Genome Research Ltd. Hinxton, Cambridgeshire, UK) was next used for duplicate read removal and GATK BaseRecalibrator (Broad Institute, Cambridge, MA) was used to adjust base quality scores. The modified alignment file (BAM format) was then passed to the GATK UnifiedGenotyper (Broad Institute, Cambridge, MA) for variant calling with the following non-default settings: Stand_call_conf 30, std_emit_conf 10 and base

alignment quality (-baq) CALCULATE_AS_NECESSARY. The resulting variants were then filtered using the GATK VariantFiltration tool (Broad Institute, Cambridge, MA), with values for SNVs set at QualByDepth (QD) < 2.0, RMSMappingQuality (MQ) < 40.0, Fisher Strand (FS) > 60.0, HaplotypeScore > 13.0, MQRankSum < -12.5, and ReadPosRankSum < -8.0. Similarly, Indel filter values were set for QD < 2.0, ReadPosRankSum < -20.0, and FS > 200.0. Mean sequencing coverage was 144-fold.

CAP#1 Illumina Whole Genome Sequencing

DNA was sent to the Illumina Clinical Services Laboratory in San Diego, California. A whole genome library was prepared by randomly shearing genomic DNA and sequencing was performed with a fragment library size of 300 nucleotides. To enrich the purified size-selected fragment library, the library was subjected to using whole genome amplification and re-purification prior to sequencing. Data analysis was performed using the Casava 1.8 software package (Illumina, San Diego, CA). Alignment to the human reference genome (NCBI37/hg19) was performed using the Efficient Large-Scale Alignment of Nucleotide Databases (ELAND) module (Illumina, San Diego, CA) with defined parameters to produce a build that was then processed by the Casava variant calling module to detect SNVs, insertions and deletions. Summary files were generated that contained information on sequencing depth and genotype probabilities for every site called (including coverage and consensus information). Quality filters were applied to the data during compilation into Casava and the variant output included SNVs insertions and deletions. Mean sequencing coverage was 54-fold.

CAP#1 Complete Genomics

DNA was sent to Complete Genomics in Mountain View, California, for whole genome sequencing, using a nanoarray-based short-read sequencing-by-ligation technology,[1] which included an adaption of the pairwise end sequencing approach. The resulting mate-paired reads with a particular gapped structure were aligned to the NCBI Build 37/hg19 human

reference genome. Complete Genomics performed the sequencing data QC, alignment, and variant calling. The variant output included SNVs as well as small insertions and deletions. Mean sequencing coverage was 44-fold.

Generation of Concordant Variants for CAP#1

The variant call files (VCF) from the above-described exome and genome sequencing of CAP#1 were comparatively analyzed to generate a set of concordant variants for PT. First, a customized BED file comprised of gene coding regions and flanking intronic sequences was generated from RefSeq (release 54). The customized BED file was used in conjunction with the VariantFiltration tool (GATK version 1.5) to filter exclude variants outside of the BED file target region to generate refined VCF files. The resulting refined VCF files for each exome or genome were used as inputs into the GATK CombineVariants and VariantEval tools to generate concordant variant files that included either the intersection of SNVs or insertions or deletions (ranging in size from 1-150 base pairs in length).

CAP#2 and CAP#3 Illumina Whole Exome Sequencing

DNA was sent to the Emory Genetics Laboratory (EGL) in Atlanta, Georgia. Exome capture was performed using the SureSelect Clinical Research Exome (Agilent Technologies, Santa Clara, California) according to the manufacturer's instructions and was followed by sequencing on the Illumina HiSeq 2500 in rapid run mode with paired-end sequencing chemistry to generate 2 X 100 bp paired end reads. Read data in FASTQ file format were aligned to the NCBI37/hg19 (GRCh37.62) human reference genome assembly using NextGENe (SoftGenetics, State College, Pennsylvania) and variants were called using NextGENe to generate a VCF file comprised of coding region variants +/- 10 bp into the flanking introns. Further analysis was performed using an EGL internal developed bioinformatics pipeline that annotates variants using several internal and external databases. Mean sequencing coverage was 148-fold.

CAP#2 and CAP#3 Illumina Whole Genome Sequencing

DNA was sent to the Illumina Clinical Services Laboratory in San Diego, California. A whole genome library was prepared by randomly shearing genomic DNA and sequencing was performed with a fragment library size of 300 nucleotides. To enrich the purified size-selected fragment library, the library was subjected to using whole genome amplification and re-purification prior to sequencing. Data analysis was performed using the Casava 1.8 software package. Alignment to the human reference genome (NCBI37/hg19) (GRCh37.62) was performed using the ELAND module with defined parameters to produce a build that was then processed by the Casava variant calling module to detect SNVs, insertions and deletions. Summary files were generated that contained information on sequencing depth and genotype probabilities for every site called (including coverage and consensus information). Quality filters were applied to the data during compilation into Casava and the variant output included SNVs insertions and deletions. Mean sequencing coverage was 29-fold.

Generation of Concordant Variants for CAP#2 and CAP#3.

The BEDTools suite was used for comparative analysis of the VCF files generated from the exome and whole genome sequencing described above. The comparative analysis focused on coding region variants +/- 10 bp into the flanking introns and compared SNVs and insertions and deletions with a size range of 1~100 bp.

CAP#1 Regrown Cell Line

This cell line was regrown and DNA was sent to Yale University (New Haven, Connecticut) for resequencing using their standard laboratory processes.

2019 Re-Analysis

With the 2019 survey year, based on feedback from participating laboratories and internal discussions, it has become apparent that reference assembly GRCh37 used for variant calling has become outdated specifically in mapping of pseudogenes and calling at ambiguous genomic loci with multiple mapping, therefore for the 2020 survey year all CAP#1, #2, and #3 Illumina genome and

exome data for the three cell lines were re-aligned and SNV and indel variants re-called using hs37d5 reference assembly (which is composed of the integrated reference sequence from the GRCh37 primary assembly, the Revised Cambridge Reference Sequence (rCRS) mitochondrial sequence, human herpesvirus 4 type 1 sequence, and concatenated decoy sequences) and was based on Burrows-Wheeler Aligner - Maximum Exact Matches (BWA-MEM) alignment,[2] GATK best practices workflow for SNV and short indel calling.[3] Variant calls were annotated using snpEff[4] and confirmed for each survey using Alamut Visual (Sophia Genetics). These re-aligned variant calls were used for 2020 survey selection.

Supplemental Methods References

1. Drmanac R, Sparks AB, Callow MJ, et al. Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays. *Science.* 2010, 327:78-81.

2. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25(14):1754-1760.

3. DePristo MA, Banks E, Poplin R et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet*. 2011;43(5):491-498.

4. Cingolani P, Platts A, Wang le L et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. *Fly (Austin)*. 2012;6(2):80-92.

Supplemental Table 1: Number of participants testing each gene per mailing and number of mailings in which each gene was offered.

Gene	No. of mailings gene offered	No. of participants testing gene		
		Mean	Minimum	Maximum
AP3B1	10	66	29	122
HRAS	10	67	42	117
KRAS	10	53	37	110
RET	10	68	42	114
DSP	9	60	40	86
ELANE	9	40	24	88
KCNH2	9	64	33	109
NPHP1	9	53	30	106
POLG	9	42	27	63
RYR2	9	57	33	98
SCN1B	9	67	29	115
SERPINA1	9	59	21	103
SPRED1	9	56	30	105
TSC1	9	75	43	120
WAS	9	61	27	107
APC	8	82	41	132
BBS1	8	65	33	105
BBS2	8	47	26	107
CDKL5	8	56	29	100
CFTR	8	46	29	87
FANCC	8	63	29	102
FBN2	8	40	25	71
FGFR3	8	55	28	106
IL7R	8	53	23	97
KCNQ1	8	57	36	92
MAP2K1	8	51	33	106
MLH1	8	80	49	136
MSH6	8	87	57	135
MYH7	8	48	27	77
PKHD1	8	41	25	97
PLOD1	8	44	28	94
SGCG	8	42	33	56
SLC25A19	8	36	30	46
TCF4	8	50	31	87
TSC2	8	74	44	110
TTN	8	64	36	107
APOB	7	52	21	102

ARX	7	49	26	102
ATM	7	85	42	140
BRCA1	7	75	46	138
BRCA2	7	86	53	129
BTK	7	48	22	111
CACNA1C	7	62	28	94
CCDC40	7	44	28	76
CDH1	7	68	41	111
COL1A1	7	52	31	100
COL1A2	7	55	28	106
COL5A1	7	48	25	71
CTRC	7	36	26	56
FOXRED1	7	43	26	66
GCK	7	48	24	75
GFM1	7	30	21	41
GJB1	7	38	19	80
GYS2	7	46	20	81
KCNE1	7	40	12	74
LMNA	7	52	36	101
MEN1	7	48	32	59
MSH2	7	80	38	125
NKX2-5	7	33	20	42
PLP1	7	45	24	95
PTEN	7	94	52	136
PTPN11	7	51	37	77
RBM20	7	64	32	103
SBDS	7	47	38	61
SLC26A2	7	51	24	108
STK11	7	72	46	130
TP53	7	82	53	125
TPM1	7	54	34	94
VHL	7	54	44	64
ADA	6	47	24	74
AHI1	6	50	32	82
ALK	6	78	39	115
ALPL	6	44	24	73
ASPA	6	60	24	99
ASPM	6	57	25	96
BAP1	6	56	28	104
BBS10	6	39	24	64
BRAF	6	53	36	108
CACNA2D1	6	33	22	43
CC2D2A	6	44	22	66
CDH23	6	39	23	62
CDK4	6	52	32	64

CEP290	6	66	27	101
CHD7	6	36	24	49
CHEK2	6	92	39	134
CLN3	6	55	31	112
CLN5	6	44	34	60
COL4A5	6	42	21	95
DSC2	6	36	26	44
EPCAM	6	78	50	123
EYA1	6	43	19	92
EYS	6	38	24	59
FBN1	6	48	38	66
FGFR1	6	45	26	96
FGFR2	6	54	29	107
FMR1	6	41	26	87
GAA	6	90	39	109
GALT	6	40	31	49
GATM	6	33	24	43
GPR98	6	38	28	56
GRIA3	6	69	27	102
HSPB1	6	37	23	68
IL2RG	6	33	22	50
JAK3	6	46	28	91
JUP	6	43	25	84
KCNJ2	6	48	31	70
KCNJ5	6	36	30	42
LAMA2	6	50	36	87
LDLR	6	42	20	101
LIAS	6	44	28	100
MBD5	6	56	32	97
MED12	6	42	31	55
MET	6	69	46	108
MTM1	6	60	21	104
MUTYH	6	60	36	76
MYBPC3	6	47	35	76
NBN	6	66	43	79
NEFL	6	45	29	86
NHEJ1	6	37	24	58
NHP2	6	41	20	96
OPHN1	6	47	28	66
PAX2	6	34	19	60
PCDH19	6	53	26	107
PEX6	6	51	24	95
PHOX2B	6	50	29	97
PKD2	6	36	27	54
PKP2	6	36	26	45

PRKDC	6	55	22	103
RAC2	6	33	20	46
RAG1	6	32	23	43
RANGRF	6	34	27	41
RPGRIP1L	6	52	27	107
RUNX1	6	58	40	110
SCN1A	6	51	27	106
SCN2B	6	42	21	88
SDHB	6	62	41	111
SDHC	6	50	41	57
SDHD	6	59	39	108
SLC25A22	6	41	33	56
SLC2A1	6	48	28	95
SMAD3	6	43	29	54
SMAD4	6	72	63	83
SMPD1	6	46	26	100
SNTA1	6	36	24	47
SOS1	6	40	33	48
SPAST	6	37	19	69
SPINK1	6	34	20	44
TCAP	6	50	33	97
TGFBR1	6	43	34	55
TGFBR2	6	71	37	114
TNNC1	6	49	31	95
TTR	6	41	34	50
UBE3A	6	37	30	45
VPS13B	6	59	30	105
XIAP	6	48	29	95
ABCB11	5	41	21	86
ACADM	5	62	29	109
ACADVL	5	69	25	106
ACTC1	5	51	32	100
ACTN2	5	59	34	97
ADSL	5	39	25	63
ATL1	5	40	20	63
ATP1A2	5	35	22	44
ATP6AP2	5	35	25	42
ATP7B	5	62	29	101
ATP8B1	5	39	21	68
ATRX	5	49	30	110
BBS4	5	31	25	44
BBS5	5	38	22	62
BLM	5	53	32	73
BMPR1A	5	109	65	128
BRIP1	5	76	57	133

CACNA1S	5	38	20	69
CAV3	5	75	39	101
CDKN2A	5	64	40	127
COL3A1	5	46	37	51
COL5A2	5	66	25	100
CSRP3	5	38	32	45
DHCR7	5	50	27	101
DMD	5	71	40	109
DNAI1	5	53	19	102
DOCK8	5	47	26	81
DSG2	5	47	37	64
ELOVL4	5	32	25	43
EVC	5	43	23	63
FKRP	5	64	39	94
FKTN	5	45	41	50
GABRG2	5	47	31	106
GATA2	5	69	40	100
GBA	5	32	21	46
GDAP1	5	30	19	42
GJB2	5	55	26	113
GLA	5	45	36	56
GPC3	5	46	38	52
HEXA	5	58	23	98
HNF1A	5	38	26	49
HNF1B	5	44	38	57
HSPD1	5	43	19	86
INF2	5	62	31	103
JAG1	5	59	32	101
KCND3	5	34	30	41
KCNQ2	5	40	31	58
KIF5A	5	31	27	41
KIF7	5	42	21	89
LYST	5	37	24	52
MAP2K2	5	60	31	98
MCOLN1	5	46	27	97
MECP2	5	60	31	107
MEFV	5	51	23	83
MFN2	5	39	25	64
MKS1	5	37	26	47
MOGS	5	51	24	100
MPV17	5	34	19	46
MPZ	5	33	26	41
MYL3	5	49	32	92
MYO7A	5	59	31	111
NEXN	5	36	32	41

NF2	5	57	28	118
NLRP3	5	52	29	94
NPHP4	5	54	27	96
OTC	5	48	30	102
PALB2	5	93	61	137
PCSK9	5	33	28	43
POLD1	5	51	38	62
POLE	5	73	38	114
PRKAR1A	5	67	39	97
PRSS1	5	35	26	48
RAD51C	5	60	32	91
RAD51D	5	64	54	74
RAF1	5	56	37	105
RAG2	5	50	29	106
RB1	5	58	34	109
RPGRIP1	5	38	18	96
SCN2A	5	43	31	66
SCN5A	5	64	34	111
SDHAF2	5	48	37	56
SGCD	5	52	37	89
SLC26A4	5	50	28	100
SLMAP	5	38	23	90
SOX9	5	34	24	42
SPG7	5	61	26	91
STXBP1	5	44	30	67
TCTN2	5	44	25	98
TIMM8A	5	52	26	86
TMEM216	5	37	24	46
TMEM67	5	53	29	99
TNNI3	5	37	26	49
TNNT2	5	48	26	107
TRIM32	5	36	26	46
TRPM4	5	43	21	90
TTC21B	5	42	27	81
USH2A	5	56	26	98
VPS33B	5	43	28	86
WDR62	5	43	29	82
WT1	5	84	61	108
ABCA4	4	48	20	66
ACTA2	4	57	39	87
ACTN4	4	54	28	86
BBS9	4	31	23	43
CEBPA	4	43	38	47
CENPJ	4	57	25	95
CLCN5	4	28	20	41

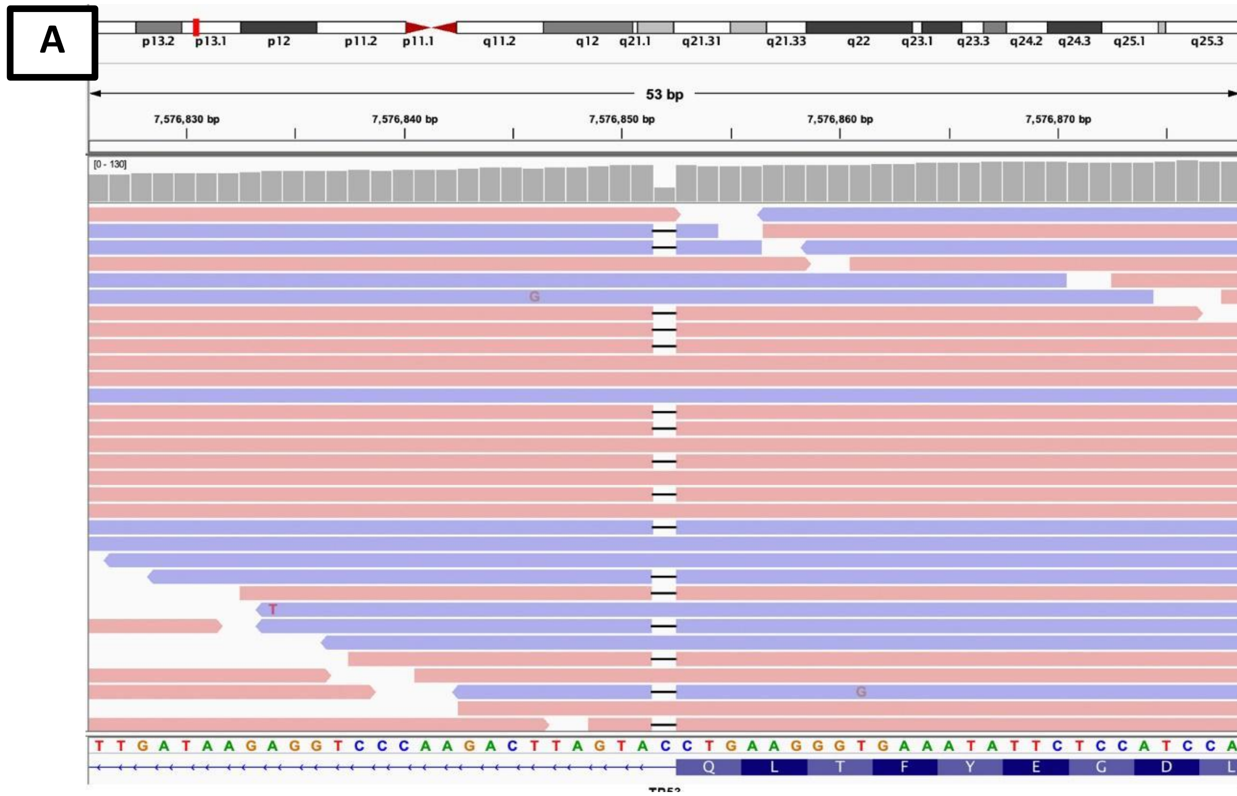
DNAH5	4	47	26	63
FH	4	53	44	63
GARS	4	43	18	85
GBE1	4	59	36	91
GPR56	4	46	31	70
HNF4A	4	30	20	40
IKBKAP	4	44	34	57
LAMB2	4	41	28	63
LAMP2	4	40	34	44
LDB3	4	55	40	89
MKKS	4	48	25	89
MYH11	4	59	38	107
MYH6	4	51	32	96
NAGLU	4	51	28	94
NDUFS1	4	32	26	39
NOTCH2	4	32	27	39
NPHS1	4	38	31	43
NPHS2	4	60	33	93
NR2E3	4	36	24	45
NRAS	4	61	38	110
OTOF	4	35	25	46
PDX1	4	29	23	35
PQBP1	4	30	24	36
PRKAG2	4	39	30	47
RAD21	4	58	29	84
RPL35A	4	31	20	41
RPL5	4	28	19	39
RPS10	4	30	19	40
SFTPB	4	28	20	40
SMARCA4	4	79	44	104
SMARCB1	4	60	38	104
STAT3	4	73	32	101
TAZ	4	59	40	103
TCTN3	4	27	22	37
TECTA	4	51	25	105
TMEM237	4	44	24	87
TMEM43	4	41	35	48
TWIST1	4	37	26	51
VCL	4	53	38	85
AARS2	3	44	29	57
ACTG1	3	79	43	103
ACVR2B	3	44	31	55
ANO5	3	54	40	79
AR	3	35	26	44
BARD1	3	61	56	68

CACNB4	3	28	20	35
CAPN3	3	40	34	45
CHD8	3	47	39	60
COG4	3	35	24	44
COG6	3	35	24	44
COL6A2	3	26	21	30
COL6A3	3	25	21	29
DES	3	78	46	98
DYNC2H1	3	32	22	42
DYNC2LI1	3	21	13	31
EFEMP2	3	59	31	100
FANCA	3	54	46	59
FANCF	3	49	41	54
FANCL	3	69	52	102
FOXP3	3	29	21	34
G6PD	3	25	22	30
GDF1	3	20	18	22
HDAC8	3	70	32	101
HPS4	3	26	24	28
HPS5	3	28	18	42
ITGA7	3	36	29	48
LCA5	3	40	31	45
LTBP4	3	48	43	53
MYL2	3	43	38	48
PLN	3	36	33	41
RYR1	3	34	28	41
SCNN1G	3	27	19	40
SPG11	3	26	19	30
TMEM138	3	27	21	33
TMEM231	3	36	15	61
TPM2	3	42	30	55
TRPM1	3	32	23	45
TSEN54	3	29	24	31
TTC8	3	44	24	67
ACTA1	2	68	34	102
ACY1	2	38	36	39
AIP	2	44	37	51
AIPL1	2	34	23	44
ALDH7A1	2	40	33	47
ALG13	2	41	39	42
AMPD1	2	37	36	37
AMT	2	70	33	107
ARHGEF9	2	37	31	43
ASH1L	2	22	13	31
BAG3	2	75	49	100

BIN1	2	40	39	41
BSCL2	2	33	20	45
CACNA1A	2	45	44	45
CASR	2	46	43	49
CCDC78	2	61	25	96
CHAT	2	97	94	99
CHD2	2	44	43	44
CLN6	2	74	35	112
CLN8	2	48	46	50
CTSD	2	40	33	46
CYBA	2	31	18	44
CYP1B1	2	23	23	23
DICER1	2	50	43	56
DNM1	2	41	39	42
DOK7	2	37	31	42
EEF1A2	2	60	38	81
ELP4	2	34	16	51
GAMT	2	45	42	48
GDNF	2	36	34	38
GNAS	2	70	45	95
IL10RA	2	20	17	23
KCNE1L	2	26	24	27
KCNJ13	2	35	27	43
KCNT1	2	34	28	39
KCTD7	2	71	45	96
MAX	2	51	49	53
MRE11	2	54	52	55
MRPS16	2	25	20	30
NIPBL	2	84	80	88
PAH	2	38	31	45
PMS2	2	43	43	43
POT1	2	32	25	38
PRF1	2	50	49	51
RPS26	2	25	19	31
SDHA	2	49	43	55
SEPN1	2	26	25	27
SFTPC	2	38	29	46
SPG20	2	43	30	56
SPTAN1	2	68	30	105
STAT1	2	25	20	30
SUFU	2	52	51	52
TCF12	2	29	21	37
TNXB	2	28	20	35
WDR35	2	61	55	67
WRN	2	75	51	98

XPA	2	76	49	102
XPC	2	46	40	52
ZFYVE27	2	46	25	67
ABCB4	1	20	20	20
ACTB	1	30	30	30
ADGRV1	1	47	47	47
CECR1	1	22	22	22
CHRNA7	1	22	22	22
EDA	1	19	19	19
FAT4	1	12	12	12
G6PC	1	49	49	49
GALK1	1	33	33	33
GARS1	1	94	94	94
GLI3	1	33	33	33
MRE11A	1	106	106	106
PKD1	1	17	17	17
PNPO	1	25	25	25
POU3F4	1	27	27	27
RAD51	1	45	45	45
SLITRK5	1	12	12	12
STAG2	1	24	24	24
TJP2	1	28	28	28
TRPC6	1	28	28	28
UBAP1	1	28	28	28
WAC	1	12	12	12
XRCC2	1	48	48	48
XYLT1	1	36	36	36

Supplemental Figure 1: In this 2020-A dry challenge**,** an Integrated Genome Viewer (IGV) screenshot of aligned sequencing reads for a region of *TP53* gene is provided (Supplemental Figure 1A). An intronic deletion variant affecting the canonical splice site was included. The dry challenge also included a clinical scenario along with answer choices (Supplemental Figure 1B). Participants were asked to select the correct HGVS nomenclature and best classification for the variant. *NGS, next generation sequencing*



B

NGS-98 Dry Challenge (Ungraded)

The provided figure is an IGV *TP53* 3' portion of exon 9 and 5' portion of intron 9 gene sequence from peripheral blood DNA from an individual with a history of an osteosarcoma as well as multiple family members with cancer (ie, brain, leukemia) diagnosed at a young age. The genomic coordinate of the variant of interest provided by the variant caller is chr17:7576852 (hg19/GRCh37).

To display the online IGV image, select the **View Image(s)** link on the result form data entry page.

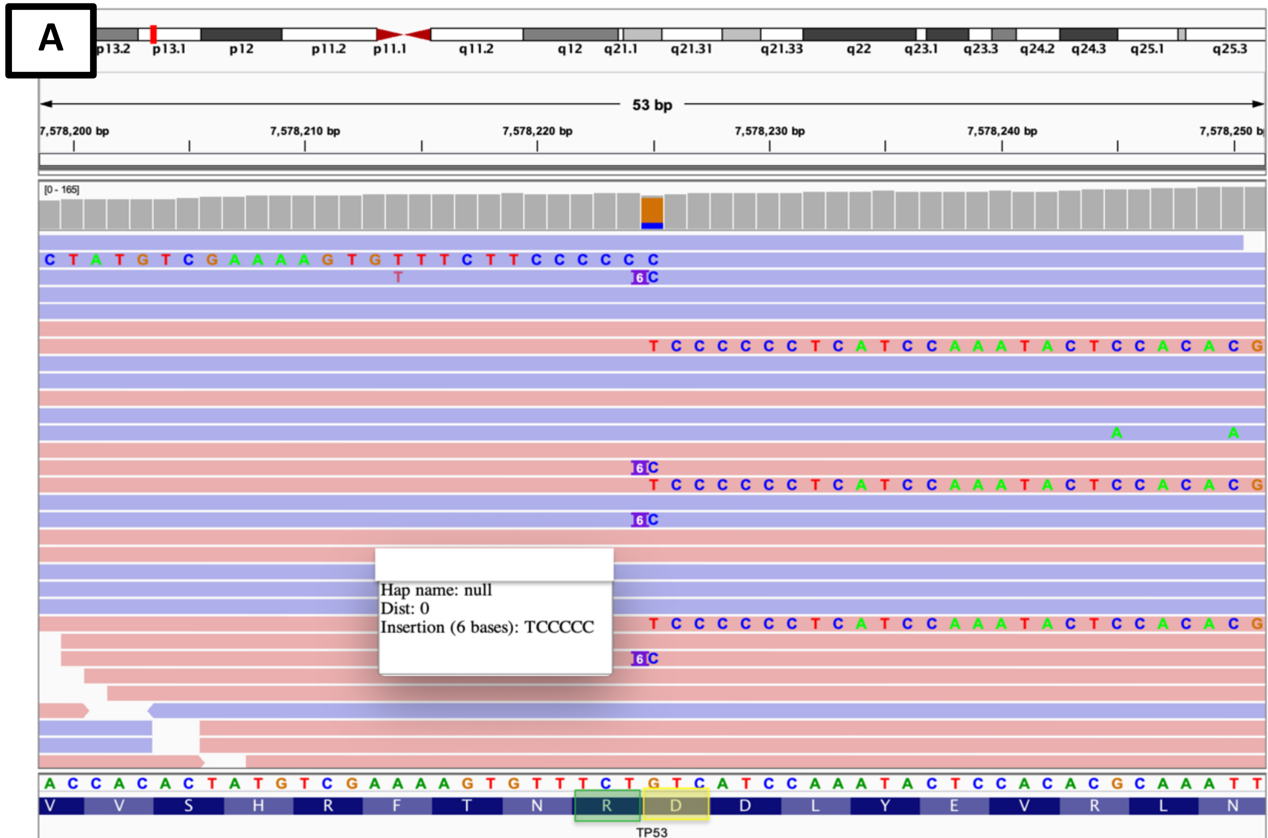
1. Using coding DNA reference sequence NM_000546.5, what is the correct HGVS nomenclature for the variant shown in this IGV figure?

   110 ○ 3365 c.993delG
   ○ 3366 IVS9+1delG
   ○ 3367 c.993+1delG
   ○ 3368 IVS9-1delC

2. What is the best classification for this variant?

   120 ○ 3369 Likely pathogenic/pathogenic
   ○ 3370 Variant of uncertain significance
   ○ 3371 Likely benign/benign

Supplemental Figure 2: In the 2020-B dry challenge, an Integrative Genomics Viewer (IGV) screenshot of aligned reads for a region of *TP53* gene was provided (Supplemental Figure 2A). It showed a single delins variant, which was represented by the variant calling pipeline as two variants. The dry challenge also included a clinical scenario along with answer choices (Supplemental Figure 2B). The participants were asked to select the best interpretation and description for this sequence change. *NGS, next generation sequencing*



B

NGS-99 Dry Challenge (Ungraded)

Peripheral blood DNA from an affected individual with a family history suspicious for Li-Fraumeni syndrome was sequenced and two *TP53* variants were called by the bioinformatics pipeline: chr17:7578224, NM_000546.5:c.624_625insGGGGGA, p.Asp208_Arg209insGlyGly and chr17: 7578225, NM_000546.5:c.624C>G, p.Asp208Glu. The figure is an IGV screen shot of representative sequence reads aligned to the hg19/GRCh37 reference genome from the region of *TP53* on chromosome 17, including the variants called by the bioinformatics pipeline. Codon D208 is highlighted in yellow and D209 is highlighted in green.

To display the online IGV image, select the **View Image(s)** link on the result form data entry page.

The best interpretation of the *TP53* sequence change in this case is:

- ◯ 3443 Two different variants: c.624_625insGGGGGA and c.624C>G, most likely *in trans*
- ◯ 3444 Two different variants: c.624_625insGGGGGA and c.624C>G, most likely *in cis*
- ◯ 3445 A single variant: c.624delinsGGGGGGA, p.Asp208delinsGluGlyGly
- ◯ 3446 A single variant: c.624delinsGGGGGA, p.Asp208Glufs*41