Original Article

Comparison of Ion Personal Genome Machine Platforms for the Detection of Variants in *BRCA1* and *BRCA2*

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Purpose
Transition to next generation sequencing (NGS) for *BRCA1/BRCA2* analysis in clinical laboratories is ongoing but different platforms and/or data analysis pipelines give different results resulting in difficulties in implementation. We have evaluated the Ion Personal Genome Machine (PGM) Platforms (Ion PGM, Ion PGM Dx, Thermo Fisher Scientific) for the analysis of *BRCA1/2*.

Materials and Methods
The results of Ion PGM with OTG-snpcaller, a pipeline based on Torrent mapping alignment program and Genome Analysis Toolkit, from 75 clinical samples and 14 reference DNA samples were compared with Sanger sequencing for *BRCA1/BRCA2*. Ten clinical samples and 14 reference DNA samples were additionally sequenced by Ion PGM Dx with Torrent Suite.

Results
Fifty types of variants including 18 pathogenic or variants of unknown significance were identified from 75 clinical samples and known variants of the reference samples were confirmed by Sanger sequencing and/or NGS. One false-negative results were present for Ion PGM/OTG-snpcaller for an indel variant misidentified as a single nucleotide variant. However, eight discordant results were present for Ion PGM Dx/Torrent Suite with both false-positive and -negative results. A 40-bp deletion, a 4-bp deletion and a 1-bp deletion variant was not called and a false-positive deletion was identified. Four other variants were misidentified as another variant.

Conclusion
Ion PGM/OTG-snpcaller showed acceptable performance with good concordance with Sanger sequencing. However, Ion PGM Dx/Torrent Suite showed many discrepant results not suitable for use in a clinical laboratory, requiring further optimization of the data analysis for calling variants.

Key words
High-throughput nucleotide sequencing, Ion PGM, Ion PGM Dx, Genes, *BRCA1*, *BRCA2*

Introduction

About 10% of the breast cancers are known to be hereditary and the most commonly associated gene with hereditary breast and ovarian cancer syndromes (HBOC) are *BRCA1* and *BRCA2* [1,2]. The detection of genetic variants in HBOC patients and the carriers have enormous impact on the management and decision for further therapeutic options [3].

Thus, performing *BRCA1* and *BRCA2* analysis is recommended in patients suspected of HBOC [4]. According to the National Comprehensive Cancer Network guidelines, genetic risk assessment and genetic testing is required in patients with pedigrees suggestive of or known genetic predisposition [4]. Sanger sequencing has been the gold standard for *BRCA1/BRCA2* variant analysis for HBOC [5,6]. However, due to the relatively large size of the *BRCA1* and *BRCA2* genes and the lack of mutational hotspots, perform-

ing Sanger sequencing in multiple samples is often time consuming and expensive [7]; thus, the implementation of next generation sequencing (NGS) for *BRCA1/BRCA2* analysis is being considered in diagnostic settings [8-10]. Many studies have evaluated the use of NGS for the detection of *BRCA1* or *BRCA2* variants in HBOC [11-16] and some recent studies have evaluated the analysis of *BRCA1* or *BRCA2* with NGS in the clinical laboratory setting [13,15-17]. In this study, we have assessed the results of NGS assays for *BRCA1* and *BRCA2* variants performed by an amplicon based method with Ion Torrent Personal Genome Machine (PGM) (Thermo Fisher Scientific, Waltham, MA) and Ion PGM Dx (Thermo Fisher Scientific), in consideration of implementing the platforms in a diagnostic laboratory. Since different platforms and data analysis pipelines give different results, platforms available for diagnostic use were considered. Ion PGM Dx is a class II medical device approved by the U.S. Food and Drug Administration used in conjunction with the instrument-specific reagents and data analysis software, Torrent Suite. Ion Torrent systems measures the pH to detect polymerization events and is known to be prone to homopolymer errors [18,19], thus OTG-snpcaller, an optimized pipeline based on Torrent Mapping Alignment Program (TMAP) and Genome Analysis Tool kit (GATK) [20] for single nucleotide polymorphism calling from Ion Torrent data [21] were used as the analysis pipeline with the Ion PGM data. The results of *BRCA1* and *BRCA2* analysis with Ion PGM/OTG-snpcaller and Ion PGM Dx/Torrent Suite were compared to those from Sanger sequencing. Since there are no studies with the Ion PGM Dx platform, we have evaluated the results of Ion PGM and Ion PGM Dx for *BRCA1/ BRCA2* analysis with clinical and reference samples.

Materials and Methods

1. Samples

Clinical samples were collected from 75 breast cancer patients suspected of hereditary breast cancer for detection of *BRCA1* or *BRCA2* genetic variants as a routine clinical practice. Seventy-five consecutive samples were included in this study. In addition, 14 reference DNA samples from the National Institute of General Medical Sciences Human Genetic Cell Repository (https://catalog.coriell.org/) with known *BRCA1* or *BRCA2* pathogenic variants or variants of unknown significance (VUS) were included in this study. The sample numbers used are NA13705, NA13715, NA14090, NA14094, NA14634, NA14636, NA14637, NA14638, NA14-684, NA141-70, NA14622, NA14623, NA14624, and NA14639.

This study was approved by the Institutional Review Board of the institution (IRB-B-1508-310-302).

2. Methods

The genomic DNA was extracted by QIAamp Blood DNA mini kit (Qiagen, Valencia, CA) for the clinical samples and the reference DNA samples were obtained. For 75 clinical samples and 14 reference samples, the comparison of results of Sanger sequencing and NGS with Ion PGM was made and among the clinical and reference samples, 24 samples (10 clinical samples and 14 reference samples), were performed additionally with Ion PGM Dx for comparison.

3. Next generation sequencing

1) Library preparation

The target regions of *BRCA1* and *BRCA2* were amplified by the Ion Ampliseq *BRCA1* and *BRCA2* Panel (Thermo Fisher Scientific). The panel includes three primer pools (167 amplicons) which cover the entire coding region and 10 to 20 bp of the intronic flanking sequences of coding exons. For amplification, 4 μL of 5× Ion Ampliseq HiFi Master Mix (Thermo Fisher Scientific), 10 μL of 2× Ion Ampliseq primer pool, 20 ng of genomic DNA per reaction, and 4 μL of nuclease-free water were mixed. The temperature profile applied with the final 20 μL of the polymerase chain reaction (PCR) mixture were 99ºC for 2 minutes, 99ºC for 15 seconds, and 60ºC for 4 minutes 19 cycles, with a final hold at 10ºC. The primer sequences were partially digested and adapters and barcodes were ligated to the amplicons according to the Ion AmpliSeq Library 2.0 Kit manual. A unique adapter was applied for each library with the Ion Xpress Barcode Adapters 1 to 16 Kit (Thermo Fisher Scientific). Quantification of amplified library was performed with the Qubit ver. 2.0 fluorometer (Life Technologies, Carlsbad, CA) using the Qubit dsDNA HS Assay Kit, diluted to approximately 100 pmol/L. Ion OneTouch 2 System and the Ion OneTouch ES Instrument (Thermo Fisher Scientific) was used according to the user guide with the 200-bp chemistry kits. All barcoded samples were sequenced on the Ion PGM (Thermo Fisher Scientific) with 316 chips using 12 samples on a single chip per sequencing run.

For Ion PGM Dx, Ion PGM Dx Library Kit, Ion OneTouch Dx Template Kit, and the Ion PGM Dx Sequencing Kit was used. All barcoded samples were sequenced on the Ion PGM Dx with the Ion 318 Dx Chip using 12 samples on a single chip per sequencing run. Sequencing data were analyzed with the Ion Torrent Suite software TS 4.0.0 and contextually with Ion Reporter.

Table 1. List of pathogenic variants and variants of known significance found in clinical samples

Gene	Codon	Protein	dbSNP	BIC/Clinical importance	BIC/Clinical classification	HGMD
BRCA1	c.154C>T	p.Leu52Phe	rs80357084	Unknown	Pending	DM?
BRCA1	c.390C>A	p.Tyr130*	rs80356888	Yes	Class 5	DM
BRCA1	c.3627dupA	p.Glu1210Argfs*9	rs80357729	Yes	Class 5	DM
BRCA1	c.922_924delinsT	p.Ser308*	rs397509335	None	None	DM
BRCA1	c.2566T>C	p.Tyr856His	rs80356892	Unknown	Pending	DM?
BRCA1	c.4883T>C	p.Met1628Thr	rs4986854	Unknown	Pending	DM?
BRCA2	c.276dupA	p.Ser93fs*8	rs80359345	Yes	Class 5	DM
BRCA2	c.943T>A	p.Cys315Ser	rs80359065	Unknown	Pending	DM?
BRCA2	c.994delA	p.Ile332Phefs*7	rs80359778	Yes	Class 5	DM
BRCA2	c.2350A>G	p.Met784Val	rs11571653	Unknown	Pending	DM?
BRCA2	c.2435delA	p.Asn812Ilefs*13	rs80359329	Yes	Class 5	DM
BRCA2	c.2798_2799delCA	p.Thr993Argfs	rs80359348	Yes	Class 5	DM
BRCA2	c.3195_3198delTAAT	p.Asn1066Leufs*10	rs80359375	Yes	Class 5	DM
BRCA2	c.3744_3747delTGAG	p.Ser1248Argfs*10	rs80359403	Yes	Class 5	DM
BRCA2	c.5575_5578delATTA	p.Ile1859Lysfs	rs80359520	Yes	Class 5	None
BRCA2	c.6322C>T	p.Arg2108Cys	rs55794205	Unknown	Pending	DM?
BRCA2	c.6325G>A	p.Val2109Ile	rs79456940	Unknown	Pending	DM?
BRCA2	c.8187G>T	p.Lys2729Asn	rs80359065	Unknown	Pending	DM?

dbSNP, Short Genetic Variations database; BIC, Breast Cancer Information Core; HGMD, Human Genetic Mutation Database; DM?, likely disease causing mutation; DM, disease causing mutation.

2) Data analysis for NGS

For Ion PGM, we have adopted an optimized pipeline based on TMAP and GATK [20], the OTG-snpcaller for single nucleotide variant based on a previous study [21]. Briefly, the raw data from Torrent Suite 4.6 was mapped with TMAP 3.6 (https://github.com/iontorrent/TAMP) and the duplicates were removed with Remove Duplicates according to the Alignment Score Tag. To reduce the false negative results of single nucleotide variant in a gap site, Alignment Optimize Structure filtering method was incorporated. Then the variant calling was performed with GATK tool. Local mutational hotspot files were included for annotation of the variants identified.

For Ion PGM Dx, the sequence data were processed using Ion Torrent Suite software 4.0 processed on the Torrent Server (Thermo Fisher Scientific).

Sequence alignments for variants with discordant results were manually inspected with the Integrative Genomics Viewer (IGV) 2.3 [22].

4. Sanger sequencing

All clinical samples were sequenced for the entire coding regions by Sanger sequencing. B-Pure EasySeq PCR plates

for *BRCA1* and *BRCA2* (Nimagen, Nijmegen, Netherlands) were used for amplification and the PCR products were purified and sequenced with BigDye Terminator Cycle Sequencing Kit (Applied Biosystem, Foster City, CA) and products were run on 3130xl Genetic Analyzer (Applied Biosystems). The data was analyzed by Mutation Surveyor 4.0 (SoftGenetics, State College, PA) with the reference sequence NM_007294.2 for *BRCA1* and NM_000059.3 for *BRCA2*. The variants were described as recommended by the Human Genome Variation Society (HGVS) nomenclature (http://varnomen.hgvs.org).

The characterization of the variants was made primarily by Breast Cancer Information Core (BIC) from National Human Genome Institute (https://research.nhgri.nih.gov/bic/), Short Genetic Variations database (dbSNP, https://www.ncbi.nlm.nih.gov/projects/SNP/index.html), and Human Gene Mutation Database (HGMD) professional 2016.2 (Qiagen, Boston, MA) as of date September 10, 2016. BIC classifies variants into five classes: class 1 for not pathogenic/low clinical significance, class 2 for likely not pathogenic/little clinical significance, class 3 for uncertain variants, class 4 for likely pathogenic variants, and class 5 for pathogenic variants. HGMD classifies variants into disease causing mutation, likely disease causing mutation, disease associated polymorphism, disease associated polymorphism with

Table 2. List of reference Coriell DNA samples and the pathogenic/unknown significance variants in each sample

No.	ID	Gene	Coding variant	Protein variant	dbSNP	BIC/Clinical importance	BIC/Clinical classification	HGMD
1	NA13705	*BRCA1*	c.3756_3759delGTCT	p.Leu1252Serfs	rs80357868	Yes	Class 5	DM
2	NA13715	*BRCA1*	c.5266dupC	p.Gln1756Profs*74	rs80357906	Yes	Class 5	DM
3	NA14090	*BRCA1*	c.66_67delAG	p.Glu23Argfs*18	rs80357783	Yes	Class 5	DM
4	NA14094	*BRCA1*	c.1175_1214del40	p.Leu392Glnfs	rs80359874	Yes	Class 5	NA
5	NA14634	*BRCA1*	c.4065_4068delTCAA	p.Asn1355Lysfs	rs80357508	Yes	Class 5	DM
6	NA14636	*BRCA1*	c.5558dupA	p.Tyr1853*fs	rs80357629	Yes	Pending [Class 4 or 5]	DM
7	NA14637	*BRCA1*	c.4327C>T	p.Arg1443*	rs41293455	Yes	Class 5	DM
8	NA14638	*BRCA1*	c.213-11T>G	p.?	rs80358061	Yes	Pending [Class 4 or 5]	DM
9	NA14684	*BRCA1*	c.797_798delTT	p.Ser267Lysfs	rs80357789	Yes	Class 5	DM
10	NA14170	*BRCA2*	c.5946delT	p.Ser1982Argfs	rs80359549	Yes	Class 5	DM
11	NA14622	*BRCA2*	c.6275_6276delTT	p.Leu2092Profs	rs11571658	Yes	Class 5	DM
12	NA14623	*BRCA2*	c.125A>G	p.Tyr42Cys	rs4987046	Unknown	Pending	DM?
13	NA14624	*BRCA2*	c.5718_5719delCT	p.Leu1908Argfs	rs80359530	Yes	Class 5	NA
14	NA14639	*BRCA2*	c.6198_6199delTT	p.Ser2067Hisfs	rs80359564	Yes	Class 5	DM

dbSNP, Short Genetic Variations database; BIC, Breast Cancer Information Core; HGMD, Human Genetic Mutation Database; DM, disease causing mutation, DM?, likely disease causing mutation; NA, not available.

additional supporting functional evidence, frameshift or truncating variant with no disease association reported yet, polymorphism affecting the structure, function or expression of a gene but with no disease association reported yet.

5. Multiple ligation probe amplification

To analyze for large gene rearrangement or deletion/duplication, multiple ligation probe amplification (MLPA) was performed for all clinical samples. MLPA kits (P002 for *BRCA1* and P045 for *BRCA2*) (MRC-Holland, Amsterdam, Netherlands) were used with Veriti 96-well Thermal Cycler (Applied Biosystems) and the data was analyzed with GeneMarker 2.0 (SoftGenetics).

Results

1. Sequencing (NGS) statistics

For *BRCA1* and *BRCA2* analysis, on average, 233,185 reads per patients were obtained with a mean amplicon length of 139 base pair (bp) with Ion PGM in 89 samples. The mean sequencing depth was 1,377× (775× to 2,237×) and 96.40% of the reads were on the targeted region of *BRCA1* and *BRCA2*.

The uniformity of coverage was 97.22%. Twenty-four samples were performed concurrently with Ion PGM Dx and the mean read length was 149 bp, the mean mapped reads were 280,200 bp and on target rate was 97.80% with a mean depth of 1,796× (1,062× to 4,332×) and the mean uniformity of 96.23%.

2. Detection of variants

Pathogenic variants and VUS identified in 75 clinical samples and reference DNA samples are shown in Tables 1 and 2, respectively. MLPA results showed no deletions or duplications for *BRCA1* and *BRCA2* in clinical samples. In clinical samples, six pathogenic or VUS were found in *BRCA1* including two nonsense variants, one frameshift variant, and three missense variants. Twelve pathogenic or VUS were identified in *BRCA2* including seven frameshift and five missense variants. All pathogenic or VUS identified in the clinical samples were previously reported variants. A list of all variants (n=50) identified in clinical samples are shown in the S1 Table. In total, 19 variants were identified in *BRCA1* including 18 exonic and one intronic variants. Six synonymous, nine missense variants, two nonsense variants, and one frameshift variants were identified in the exons. In *BRCA2*, 31 variants were identified with 30 exonic variants and one intronic variant. There were seven frameshift variants, 13 missense and 10 synonymous variants. In total, 14 pathogenic/VUS variants were found in the reference DNA samples in this study, which

Table 3. Discordant results by NGS and Sanger sequencing

Gene	Sanger	Ion PGM/OTG-snpcaller	Ion PGM Dx/Torrent Suite
BRCA1	Not detected	Not detected	c.117_118delTG
BRCA1	c.922_924delinsT	c.922A>T	c.922A>T
BRCA1	c.1175_1214del40	c.1175_1214del40	Not called
BRCA1	c.3113A>G	c.3113A>G	c.3107_3112delTTAAAG
BRCA1	c.3548A>G	c.3548A>G	c.3548_3549delAA
BRCA1	c.4065_4068delTCAA	c.4065_4068delTCAA	Not called
BRCA2	c.994delA	c.994delA	Not called
BRCA2	c.3744_c.3747delAGTG	c.3744_c.3747delAGTG	c.3742_3745delTGAG

NGS, next generation sequencing.
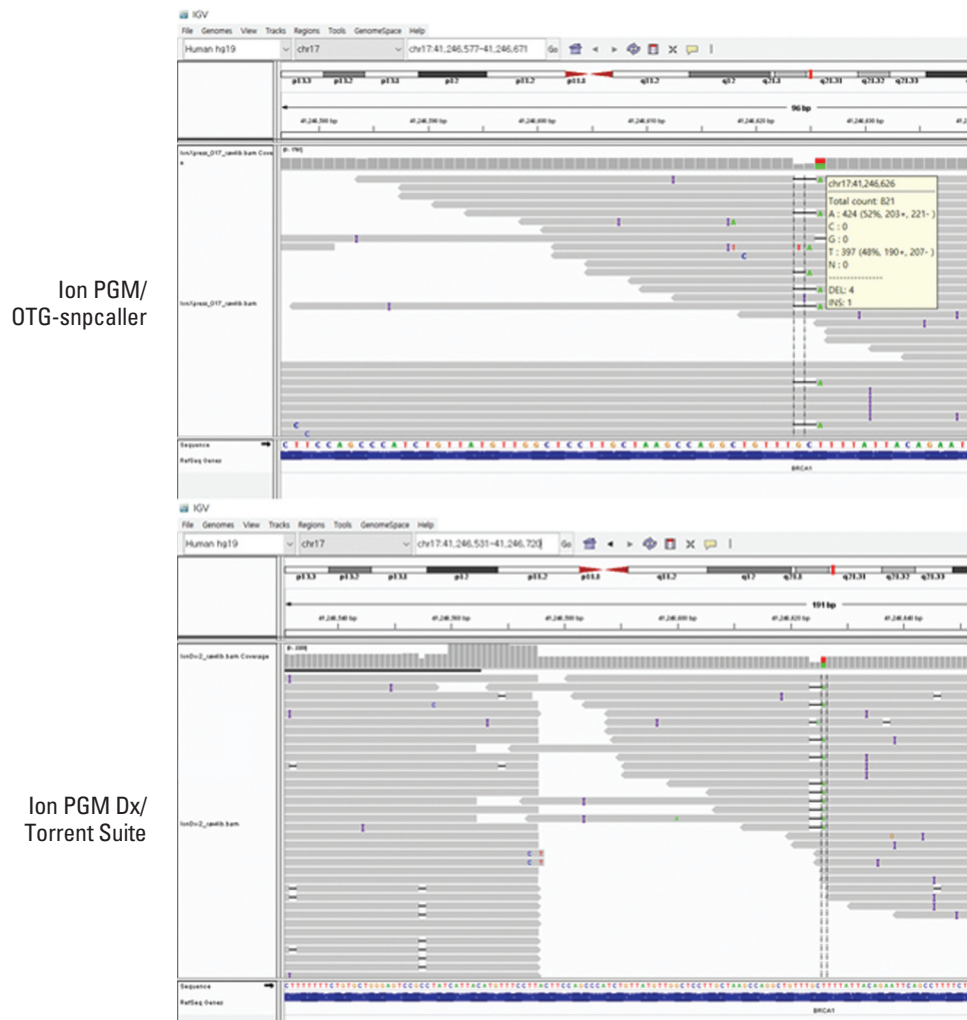
Fig. 1. Alignment of an indel variant (c.922_924delinsT) in a clinical sample viewed by Integrative Genomics Viewer, called as a single nucleotide variant, c.922C>T, by both Ion PGM/OTG-snpcaller and Ion PGM Dx/Torrent Suite.
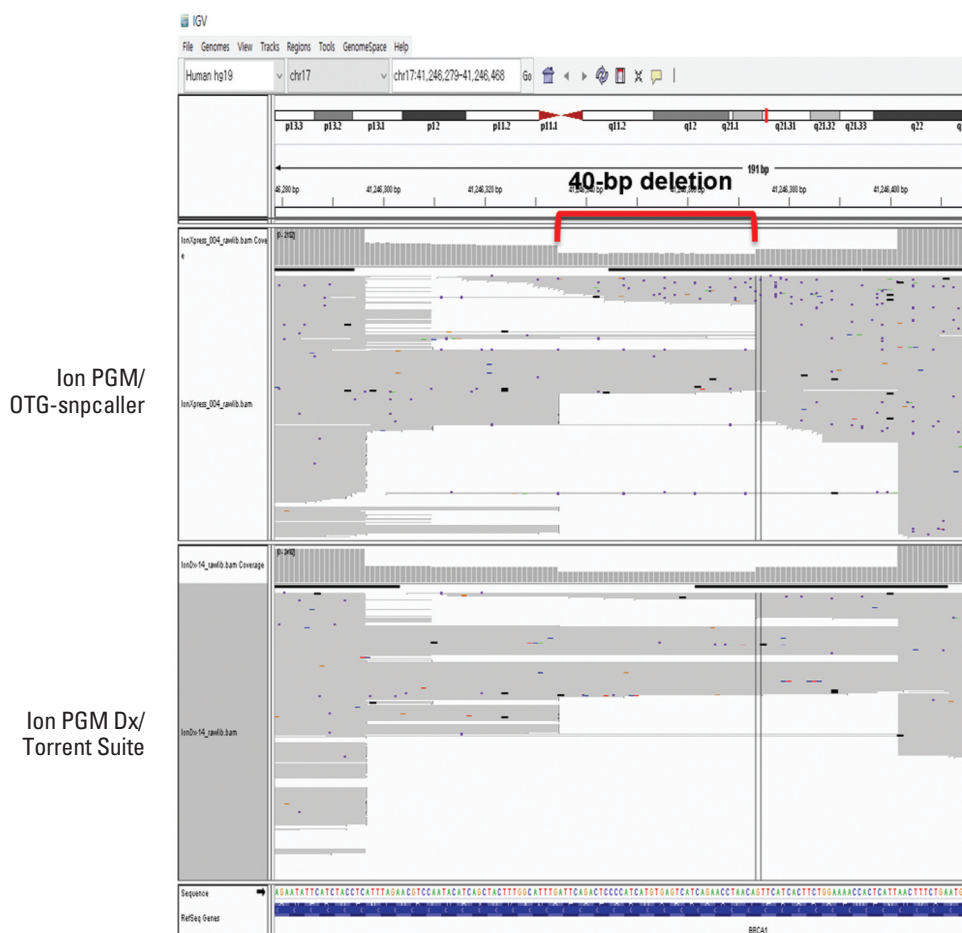
Fig. 2. (A) Alignment of a 40-bp deletion in *BRCA1* (c.1175_1214del40) in the reference Coriell DNA sample (NA14094) which was called by Ion PGM/OTG-snpcaller but not by Ion PGM Dx/Torrent Suite. *(Continued to the next page)*

included nine variants in *BRCA1* (one nonsense, one splice site, and seven frameshift variants) and five variants in *BRCA2* including one missense and four frameshift variants.

The variants identified by the NGS and Sanger sequencing were compared. The different parameters for variant calling in Ion Torrent variant caller for Ion PGM Dx are shown in S2 Table. In Ion PGM, the "Generic-PGM-Germline Low stringency" parameter was used for variant calling while the default option was used for Ion PGM Dx/Torrent Suite.

The discordant sequencing results including those from clinical and reference DNA samples are shown in Table 3. Ion PGM/OTG-snpcaller and Sanger sequencing showed only one discrepant result in *BRCA1* for c.922_924delinsT in which Ion PGM/OTG-snpcaller identified the indel as a single nucleotide variant, c.922A>T (Fig. 1). All the other pathogenic variants or VUS identified were in concordance with Sanger

sequencing. However, eight discrepant results were present between Sanger sequencing and Ion PGM Dx/Torrent Suite. One false-positive variant in *BRCA1*, c.117_118delTG was identified by Ion PGM Dx but not present by visual inspection with the IGV. Three false-negative results were present with Ion PGM Dx/Torrent Suite in which pathogenic variants of *BRCA1*, c.1175_1214del40, c.4065_4068delTCAA and a pathogenic variant of *BRCA2*, c.994delA were not called by the Ion PGM Dx/Torrent Suite (Fig. 2). Four other discordant results were present misidentifying a variant as another variant with Ion PGM Dx/Torrent Suite and of these discordant results, two variants were recurrently present in seven samples among 24 samples tested with Ion PGM Dx/Torrent Suite. Single nucleotide variants of *BRCA1*, c.3113A>G and c.3548A>G, were called as c.3107_3112delTTAAAG and c.3548_3549del-AA. The other misidentified variant was c.922_924delinsT
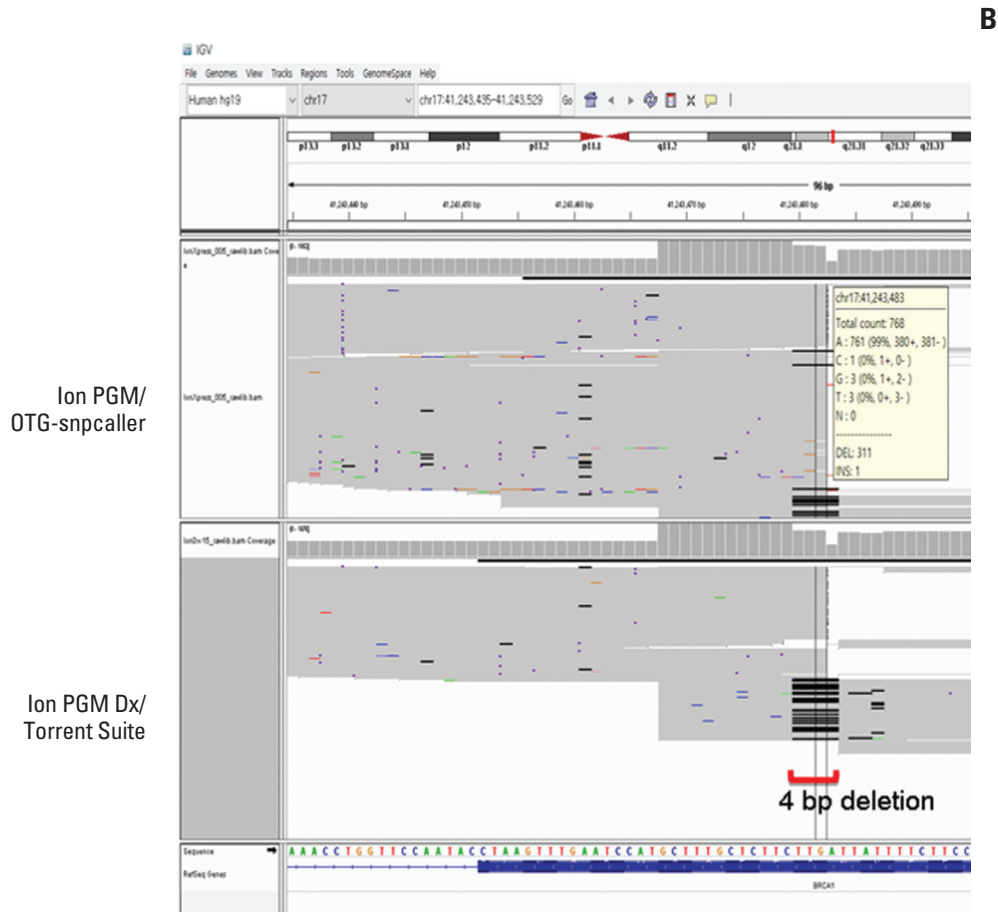
B

Fig. 2. *(Continued from the previous page)* (B) A 4-bp deletion in *BRCA1* (c.4065_4068delTCAA) not called by Ion PGM Dx/Torrent Suite present in a reference Coriell DNA sample (NA14634). *(Continued to the next page)*

which was called as c.922A>T as in Ion PGM/OTG-snpcaller and a 4-bp deletion in *BRCA2*, c.3741_3744delTGAG, which was c.3744_3747delAGTG by Sanger sequencing.

Discussion

The implementation of NGS to a clinical laboratory requires validation and thorough evaluation. Many studies have shown comparison results of *BRCA1* and *BRCA2* with NGS to Sanger sequencing, which has been considered the golden standard for variant analysis. However, different NGS platforms and data analysis pipeline showed variable performance [12,13,17,23], which lead us to the evaluation of Ion PGM and Ion PGM Dx, which were candidates of NGS

platforms to our laboratory. Our data showed that Ion PGM/OTG-snpcaller showed comparable results to Sanger sequencing with one false negative results for an insertion/deletion variant. However, Ion PGM Dx with the supplied Torrent Suite software alone was not suitable for *BRCA1* and *BRCA2* analysis in a diagnostic setting, due to false negative and positive errors.

In total, 50 variants including 12 pathogenic variants/VUS and 38 benign variants were found in 75 clinical samples and the known pathogenic or VUS of the 14 reference Coriell DNA samples were confirmed with Sanger sequencing and NGS. Most of the variants were identified with Sanger sequencing and Ion PGM/OTG-snpcaller except for one discordant result for a pathogenic variant. An indel variant in *BRCA1*, c.922_924delinsT was identified as a single nucleotide variant (c.922A>T) with Ion PGM/OTG-snpcaller and this variant was also not correctly called by Ion PGM Dx/
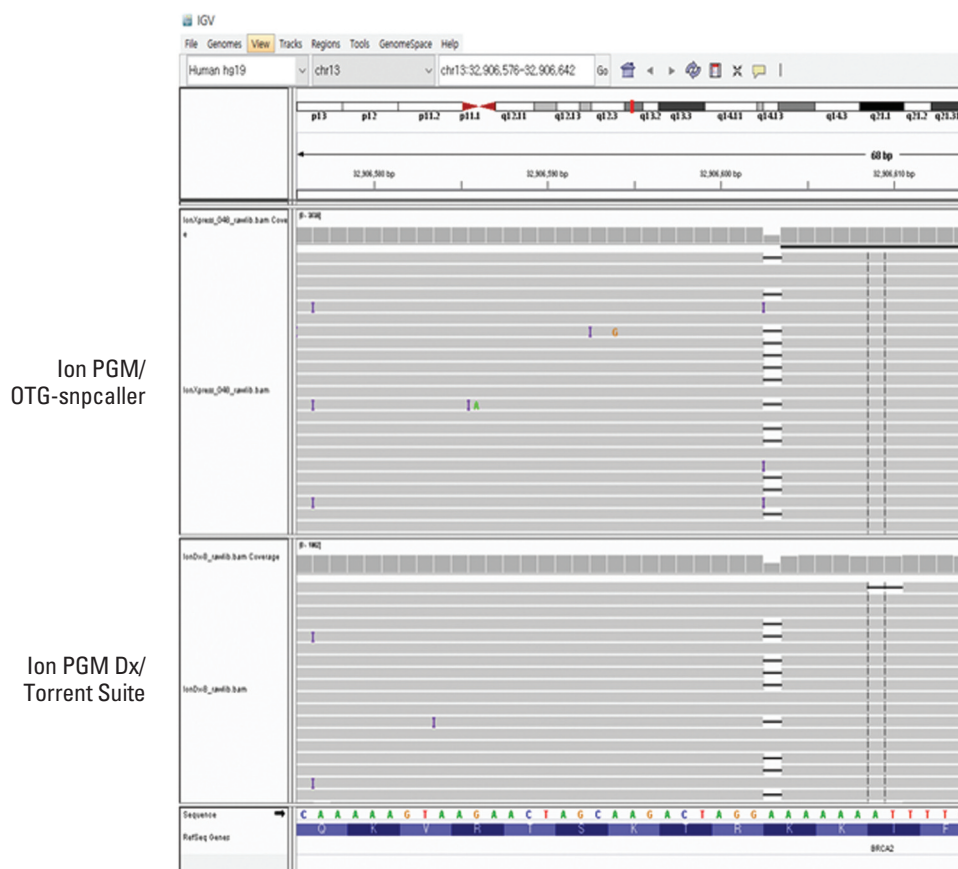
Fig. 2. *(Continued from the previous page)* (C) A 1-bp deletion in *BRCA2* (c.994delA) not called by Ion PGM Dx/Torrent Suite present in a clinical sample.

Torrent Suite.

Several other discrepancies were present with Ion PGM Dx/Torrent Suite, showing recurrent false positive and negative variant assignments. Three recurrent false positive variants in *BRCA1*, c.3107_3112delTTAAAG and c.3548_3549-delAA, which corresponded to c.3113A>G and c.3548A>G by Sanger sequencing were present in the variant call format (VCF), however there were no insertions or deletions manually confirmed by IGV. We have speculated that these false positive assignments occur when multiple possible variants are listed for a chromosome position which includes a hotspot variant inserted prior to analysis. Therefore, to minimize the discrepancies by Ion PGM Dx/Torrent Suite, we have deleted the two recurrent hotspot variants from the local database and those recurrently misidentified as a pathogenic variant were not reported in the VCF files. However, the manual deletions of these hotspot variants would be not advisable in populations where these variants are reported. Moreover, since incorrect hotspot IDs were matched to the variants, we have developed a simple program with python 2.7, "Filter Dx" which selects homozygous or heterozygous variants and eliminates the assigned hotspot variants when there are multiple alternative alleles, where errors were found to be prone. This program condenses the long list of variants with possible germline variants (provided at request).

False-negative results were present by Ion PGM Dx missing a 40-bp deletion in *BRCA1* (c.1175_1214del40), a 4-bp deletion in *BRCA1* (c.4065_4068delTCAA) in reference DNA samples and a 1-bp deletion in *BRCA2* (c.994delA) (Fig. 2). The 40-bp deletion have been reported as being difficult in NGS for detection and not detected in certain platforms requiring modification of data analysis pipelines [17,24,25]. Not many parameters were adjustable in the Ion PGM Dx/

Torrent Suite platform in processing the data acquired from the Ion PGM Dx, thus requiring further modification of the variant calling pipelines in Torrent Suite as suggested in previous studies for its use in the clinical laboratory setting [17,26].

As in a previous study, we have also found that Ion PGM Dx/Torrent Suite reported a variant with AGTG deletion from chr13:32912234 (c.3742_3745del), whereas a deletion of TGAG from chr13:32912236, c.3744_3747delTGAG was detected in Sanger sequencing [16] and the discrepancy in codon numbers occurs since the HGVS nomenclature requires that the most 3′ position possible of the reference sequence is arbitrarily assigned to have been changed. Thus, confirming the variants with IGV and checking the HGVS nomenclature is essential for reporting variants.

Ion Torrent platforms is known to have disadvantages in homopolymer errors, false indel detections [19,26]; however, many studies have shown that Ion Torrent platforms can be used in clinical settings when sufficient read depth can be obtained and quality control measures are implemented [8,12,13]. In our study, modifying the data analysis algorithm with TMAP and GATK (OTG-snpcaller) with Ion PGM reduced the calling errors and identified the variants not called by Ion PGM Dx/Torrent Suite, showing the importance of optimizing data analysis pipeline for implementing NGS [12,26].

The reference DNA samples allowed confirmation of pathogenic variants with the various methods, helping the evaluation of procedural evaluations as incorporated in many of the validation studies with NGS [24,25,27].

Although approved as class II medical device, the use of Ion PGM Dx with the vendor supplied Torrent Suite analysis software showed both false-negative and -positive results not suitable for use in a clinical laboratory. Further optimization of the data analysis pipeline is necessary for use of Ion PGM platforms to be used in a clinical laboratory.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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