Review: The usability of gene panels in the diagnosis of hereditary breast cancer

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Abstract

Worldwide many women are diagnosed with hereditary breast cancer. Breast cancer is characterized by a high mortality rate and therefore detection of hereditary breast cancer is important. The traditional method of mutation screening is Sanger sequencing with which first the *BRCA1* and *BRCA2* genes are analyzed for mutations and is proceeded to less likely genes when nothing is found. But mutated *BRCA1* and *BRCA2* account only for a small part as the cause of hereditary breast cancer and thus testing can be very time consuming. Since the emergence of Next Generation Sequencing a new type of testing is introduced called gene panel testing. With gene panel testing a lot of genes related to hereditary breast cancer can be tested simultaneously. Many companies provide different panels for screening hereditary breast cancer. However, the utility of gene panel testing is not clear. The clinical significance of finding variants in the tested genes is questioned. Also little is known about the accuracy of detection and the gain of diagnoses compared to traditional testing. Furthermore, the increase of findings of which the association with breast cancer risk is unknown, which can result in confusion for the physician and patient is unclear. Because gene panel testing is already much used, it is important to get more clarity about these key questions. Therefore, in this review we will focus on the usability of gene panels in the molecular diagnosis of hereditary breast cancer. An overview will be given on genes related to high breast cancer risk, the analytical performance, gain of diagnoses and gain of variants of unknown clinical significance. Also, briefly will be talked about new found variants or genes that are associated to breast cancer which is the result of collecting data of gene panel testing.

Keywords: *gene panels, hereditary breast cancer, high penetrance genes, Sanger sequencing, NGS*

Introduction

Breast cancer is the second deadliest type of cancer in women. It is characterized by carcinoma development in the tissue of the breast commonly in women and rare in men. Of all types of cancer, breast cancer in women accounts on top for the most new cases of cancer with a rate of 29% in the United States.[1]

Hereditary breast cancer makes up for a part (5-10%) of the prevalence of breast cancer. Genetic testing for genes associated with hereditary breast cancer plays a big and important role in screening for cancer and prevention of

the development of cancer.[2]

The traditional way of genetic testing is Sanger sequencing. With Sanger sequencing the sequence of genes associated with heritable breast cancer can be checked for mutations. But because it is very time consuming and has high costs, genetic testing with Sanger sequencing is only limited to test one or two genes. The commonly tested genes for mutations are *BRCA1* and *BRCA2*. Mutations in these genes account for 20-25% as the cause of familial breast cancer. The remaining 75-80% of familial breast cancer is caused by other mutated genes Only when no mutations are found in *BRCA1* and *BRCA2* testing will proceed in finding mutations in less likely genes, which can prolong testing.[3]

With the discovery of Next Generation Sequencing (NGS) it is possible to sequence large pieces of DNA at the same time much faster, more efficient and at lower cost. This led recently to the emergence of gene panel testing which uses NGS. With gene panel testing individuals can be tested for the presence of mutations in the two common genes *BRCA1*/*BRCA2* and simultaneously tested for mutations in other genes that are also associated with hereditary breast cancer. [4]

Nowadays there are a lot of different gene panels available ranging from testing 2 genes to over 100 genes.[5] But the clinical utility of these gene panels is questionable. For a lot of genes the association of findings in these genes and the risk of developing breast cancer is not clear. Also the performance on analytical level, the gain of diagnosis and amount of results that are of unknown significance is unclear.

This review will focus on how useful gene panels are in the molecular diagnosis of hereditary breast cancer. First (a), the types of gene panels will be discussed with the focus on which genes are related with a high risk of development of breast cancer and so of the most clinical significance. Second (b), the analytical performance and the gain of diagnoses compared with the traditional Sanger method will be discussed. Third (c) the amount of variants of unknown clinical significance in genes related to hereditary breast cancer will be reviewed. Finally, (d) it will be discussed if the results from the use of gene panels has resulted in new data like relations of newly found mutated genes and breast cancer.

Gene panels and genes of clinical significance

Gene panels and gene selection

There are nowadays a lot of different gene panel tests for the detection of hereditary breast cancer offered by many companies, see **table 1**. There are big differences between the panels with respect to the number of genes that are genetically tested.[4,5] Each gene panel has a different selection of genes that are used in genetic screening of heritable breast cancer. How come there are such different selections of genes?

Mutations in genes that are possibly related to heritable breast cancer are divided in different types. The first type consists of mutations in high-penetrance genes. Mutations in these genes are highly related to heritable breast cancer and much information from research is known. There are guidelines available for prevention and treatment of breast cancer associated with these genes.[6] Mutations in these genes give a more than fourfold increase in breast cancer risk.[7]

Table 1. Examples of Multigene Testing Panels for Breast Cancer.

From: D. F. Easton et al, "Gene-panel sequencing and the prediction of breast-cancer risk.," *N. Engl. J. Med.*, vol. 372, no. 23, pp. 2243–57, Jun. 2015

Company	Test	Website	Genes Included†
Ambry Genetics	BreastNext	www.ambrygen.com/tests/breastnext	*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, NF1, PALB2, PTEN, RAD50, RAD51C, RAD51D, TP53*
BreastHealth UK	BreastGene	www.breasthealthuk.com/screening-services/genetic-testing/breastgene	*ATM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, PALB2, PTEN, STK11, TP53*
Centogene	Breast Ovarian Cancer Panel	www.centogene.com/centogene/centogene-test-catalogue.php	*ATM, BARD1, BRIP1, CDH1, CHEK2, MEN1, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS1, PMS2, RAD50, RAD51C, RAD51D, XRCC2*
Emory Genetics Laboratory	High Risk Breast Cancer Panel	http://geneticslab.emory.edu/tests/MM201	*PTEN, STK11, TP53*
Fulgent Diagnostics	Breast Ovarian Cancer NGS Panel	http://fulgentdiagnostics.com/test/breast-ovarian-cancer-ngs-panel/	*APC, ATM, ATR, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, CTNNB1, EPCAM, FANCC, HOXB13, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PALLD, PMS2, PTEN, RAD50, RAD51, RAD51C, RAD51D, SMAD4, STK11, TP53, VHL, XRCC2, XRCC3*
GeneDx	OncoGeneDx	www.genedx.com/test-catalog/available-tests/breastovarian-cancer-panel	*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FANCC, MLH1, MSH2, MSH6, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11, TP53, XRCC2*
Illumina	TruSight Cancer	www.illumina.com/clinical/translational_genomics/panels/kits.html	94 Genes plus 287 SNPs reported to be associated with risk of breast cancer
Invitae	Hereditary Breast Cancer, High-Risk Panel	www.invitae.com/en/physician/panel-detail/PNL0009/	*BRCA1, BRCA2, CDH1, PALB2, PTEN, STK11, TP53*
Myriad Genetics†	myRisk	www.myriad.com/products-services/hereditary-cancers/myrisk-hereditary-cancer/	*ATM, BARD1, BRCA2, BRIP1, CDH1, CHEK2, NBN, PALB2, PTEN, RAD51C,* STK11, *TP53*
CD Genomics	Genetic Testing for the Cancer Susceptibility	www.cd-genomics.com/Genetic-Testing-for-the-Cancer-Susceptibility.html	Not specified
University of Washington†	BROCA – Cancer Risk Panel	http://web.labmed.washington.edu/tests/genetics/BROCA	*AKT1, ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FAM175A, GEN1, MRE11A, MUTYH, NBN, PALB2, PIK3CA, PTEN, RAD50, RAD51C, RAD51D, STK11, TP53, XRCC2*

* SNP denotes single-nucleotide polymorphism.

† For Myriad Genetics and the University of Washington, only genes for which breast-cancer risk is given as an indication are listed. For a complete list, see Table S1 in the Supplementary Appendix. In several cases, the panels include additional genes, and several companies also offer larger panels. Thus, even if the primary purpose of the test is prediction of the risk of breast cancer, results will often be available (and need to be interpreted) for a larger set of genes than those listed here.

The second type are mutations in moderate penetrance genes. Whereas mutations in high penetrance genes are associated with high risk of phenotypic development, mutations in moderate penetrance genes have a moderate risk of development. Mutations in these genes mostly have two to four fold increase in risk. For a lot of moderate penetrance genes the risks and clinical significance are not well established, but for some moderate genes there are.[7][8] The last type are variants of uncertain significance (VUS). A VUS is a variant in the sequence of high penetrance genes or moderate penetrance genes of which the impact on the function of the protein is unknown. Therefore, the increase of risk of developing breast cancer is also unknown. [6][9]

Providers of gene panels tests for heritable breast cancer detection use a diverse selection of genes. Some gene panels have only a small selection of the high penetrance genes, whether other gene panels include a lot of well defined and non-well defined moderate penetrance genes. Some gene panels also in-

clude genes of other types of cancer. The use of moderate penetrance genes in gene panels makes interpretation of the results very difficult.[10][11] Findings of these mutations in genes makes it hard to counsel the patient. The association of mutations in these genes (see **table 1**) and breast cancer are not clear. The lack of evidence and information about risks are not well studied. Also findings of mutations in genes related to other types of cancer makes counselling more difficult.

Genes of clinical significance

Some genes are studied extensively for their role in the development of hereditary breast cancer. Clinical guidelines have been defined and algorithms to predict pathogenicity of mutations found exist.[11] Likely pathogenic and pathogenic mutations in these genes are related to a high risk of developing breast cancer. These genes are *BRCA1, BRCA2, PTEN, TP53, STK11, CDH1, CHEK2* and *PALB2.*[11][12]

BRCA1 and *BRCA2*

The two major contributors to developing breast cancer are the *BRCA1* (breast cancer 1) gene and *BRCA2* (breast cancer 2) gene. These two are the main genes that are used in screening for hereditary breast cancer. Pathogenic mutations in the *BRCA1* and *BRCA2* genes are highly related to breast cancer, and carriers of a pathogenic mutation in those genes have a high risk of carcinoma development in breast tissue.[13] The *BRCA1* and *BRCA2* gene are involved in DNA damage repair (see **figure 1**). They play a role in repairing double stranded DNA breaks, maintenance of genomic stability and cycle checkpoint control.[14] Both genes encode a protein which forms a DNA-repair complex with each other, RAD51 and BARD in response to DNA strand breaks. Pathogenic mutations in the *BRCA1* and *BRCA2* genes can result in a non-functional protein which can cause chromosomal instability. This is the basis of accumulating mutations and aneuploidy which can lead to cancer.[15] Pathogenic mutations in the *BRCA1* and *BRCA2* gene are both autosomal dominantly inherited. Individuals with a pathogenic mutation in *BRCA1* have a 65–80% lifetime risk of developing breast cancer. Individuals with a mutated *BRCA2* gene have a lifetime risk of 45–85%. Additionally, pathogenic mutations in the *BRCA1* gene also strongly increases the lifetime risk of developing ovarian cancer (37–62%). This risk is lower in carriers of a mutated *BRCA2* gene (11–23%).[16][17]

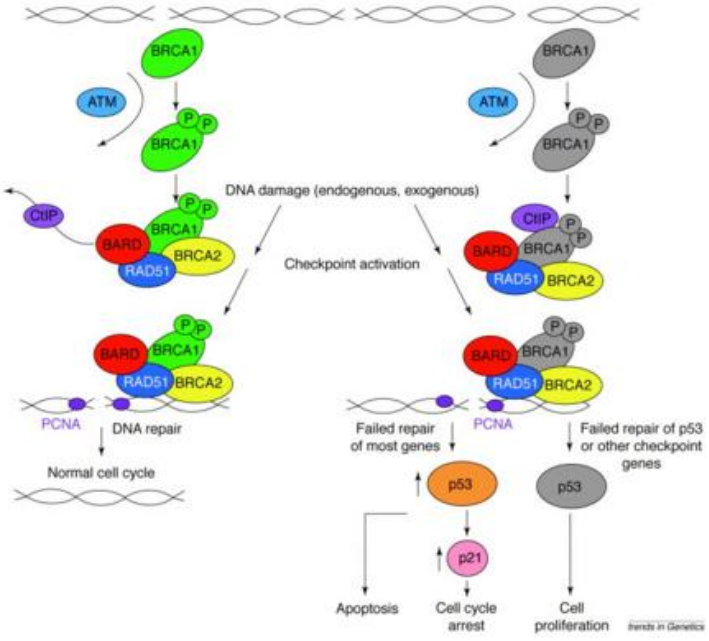

Figure 1. Overview of the functions of BRCA1 and BRCA2 in response to DNA damage. Left: In response to double stranded DNA breaks BRCA1 gets activated. BRCA1 forms a DNA-repair complex together with BRCA2, RAD51 and BARD. The complex locates to the damaged site and initiates repair of the DNA. **Right**: Mutations in BRCA1 (or BRCA2) leads to a non-functional DNA repair complex which can result in accumulation of DNA damage that can lead to cancer .

From: Piri L Welcsh et al., 'Insights into the Functions of BRCA1 and BRCA2', Trends in Genetics, 16 (2000), 69–74

PTEN

Mutations in the *PTEN* (Phosphatase And Tensin Homolog) gene are associated with a high risk of development of breast cancer. *PTEN* is a tumor suppressor gene which encodes for a phosphatase protein. It plays a major role in suppressing cell proliferation and survival by inactivating the PI 3-kinase-dependent signalling pathway.[18] Loss of *PTEN* function is the cause of Cowden syndrome which is characterized by multiple tumor growth. Germline mutations in *PTEN* are autosomal dominantly inherited. Carriers of a pathogenic mutation in the gene have a lifetime risk of developing breast cancer of 85 percent.[19]

TP53

TP53 (Tumor Protein P53) is a tumor suppressor gene. The gene encodes a protein which has a couple of functions related to DNA damage. First, it activates proteins of the DNA repair system. Second, when there is DNA damage in the cell the P53 proteins plays a role in growth arrest of de damaged cell. Furthermore it plays a role in initiating apoptosis in cells with DNA damage. Loss of *TP53* is strongly associated with tumor forming.[20] Mutations in the gene are autosomal dominantly inherited. Individuals with Li-Fraumeni syndrome have a germline mutation in this gene which manifests in formation of tumors in different tissues.[21] Individuals with a mutated *TP53* gene have an estimated risk of 30% to get breast cancer before the age of 30.[22] The lifetime risk of developing breast cancer is 50-90%. [20]

STK11

The *STK11*(serine/threonine kinase 11) gene is a tumor suppressor gene. The gene encodes a protein that is involved in cell cycle control and apoptosis. Mutations in this gene can result in Peutz-Jeghers syndrome. Individuals with germline mutations in *STK11* have a lifetime risk of 50-90% of developing breast cancer. [20]

CDH1

The gene *CDH1* (Cadherin 1, Type 1, E-Cadherin (Epithelial)) is a tumor suppressor gene. In its normal role the gene encodes a cadherin protein which plays an important role in cell adhesion, especially in cell to cell adhesion of epithelia cells. The cadherin protein interacts with actin of the cytoskeleton which is important in regulating cell migration, growth and differentiation.[23]–[25] Mutations in *CDH1* can cause the loss of active cadherin protein. This can result in loss of cell to cell adhesion which may induce cancer invasion and metastasis. Furthermore the loss of function of *CDH1* can lead to a increased cell proliferation.[26] Pathogenic mutations are highly correlated with breast cancer. Individuals with a pathogenic mutation in *CDH1* have a lifetime risk of 30-50% of developing breast cancer. [27]

CHEK2

The tumor suppressor gene *CHEK2* (checkpoint kinase 2) encodes for a protein that responds to double-stranded DNA breaks. It is involved in blocking cell proliferation by inhibiting the cell cycle protein CDC25.[28] It also plays a role in DNA repair and apoptosis by interacting with BRCA1 and p53 proteins. Pathogenic mutations in the *CHEK2* gene are linked to develop cancer including breast cancer.[29] Carriers of a mutated *CHEK2* gene with a family history of breast cancer are associated to have a 42% risk of developing breast cancer by the age of 70.

PALB2

Mutations in the gene *PALB2* (Partner And Localizer Of BRCA2) are highly related to development of breast cancer.

The gene plays a role in DNA repair of double stranded DNA breaks.[30] PALB2 protein forms a complex together with BRCA1, BRCA2 and RAD51. PALB2 functions as an adaptor between the two BRCA proteins. The whole complex acts as a DNA repair complex to repair breaks in DNA. [31] Without a functional PALB2 adaptor a non-functional DNA repair complex will be formed which results in genomic instability and tumorgenesis.[27][28] Mutations are inherited in an autosomal dominant pattern. The lifetime risk of developing breast cancer is thought to be around 33-58%.[33]

The analytical performance and detection gain of gene panel testing

By traditional Sanger sequencing the number of individuals who are positively tested of carrying pathogenic mutations in *BRCA1* and *BRCA2* is 20-25%.[3] The use of gene panels should enable to get a gain in detection of individuals carrying high risk breast cancer mutations, by finding pathogenic mutations in non-*BRCA1/2* genes. To fully replace the traditional Sanger sequencing for the detection of hereditary breast cancer, the gene panel testing needs also to perform equally on analytical level as the Sanger sequencing. The analytical quality of gene panel testing compared to the traditional Sanger method has been studies as well as the gain of discovered pathogenic mutations.

In the study of *Chong et al.*(2014)[34] research was done to test the analytical quality of gene panel testing. They used a 6-gene panel (see **table 2**) test consisting of high risk genes which are associated to breast cancer (*BRCA1, BRCA2, PTEN, TP53, CDH1* and *STK11*). They tested 250 samples of which the mutations in these six genes were already defined by traditional Sanger sequencing. The total defined mutations of the six genes of all samples were 3025 variants. The results of gene panel testing of the samples resulted in 100% detection of these variants, which means it had a great analytical quality.

In the study of *Lincoln et al.* (2015)[35] research was done to determine both the analytical performance and gain of pathogenic mutations in *BRCA1/2* and non-*BRCA1/2* genes by the use of gene panel testing compared to traditional Sanger sequencing. They tested 1105 individuals with a 29-gene panel test which consisted of genes related to mostly breast cancer and ovarian cancer) of varying penetrance (see **table 2**). The individuals were previously tested of which 92% were tested by traditional method on mutations in *BRCA1* and *BRCA2*. The other individuals were previously tested with other gene panels. From the results they observed that the data from the gene panel test compared to data from traditional tests had 100% similarity. They concluded that the analytical performance of gene panel testing was highly comparable to the traditional Sanger sequencing method. From data of mutations in non-*BRCA1/2* (genes related to breast cancer), they found in 4.5% of the individuals who were negative for mutations in *BRCA1/2* pathogenic mutations which were clinical significant.

In other studies the authors only focused on the gain of pathogenic mutations in high risk genes related to breast cancer other than *BRCA1/2.*

In the study of *Tung et al.*(2015) [36] research was done to determine the frequency of pathogenic mutations in non-*BRCA1/2* genes. They tested 2158 individuals which were divided in two

groups. Group one consisted of 1781 individuals who were referred for heritable breast cancer screening. Group two consisted of 377 individuals who previously tested negative for pathogenic *BRCA1/2* mutations. A 25-gene panel was used (see **table 2**). Most genes of the panel were related to hereditary breast cancer varying from high risk to moderate risk and a couple of genes were related to other hereditary cancers. In group one the results showed that the frequency of 3.9% of mutations in genes other than *BRCA1/2* related to breast cancer was present. 9.3% of the individuals had mutations in *BRCA1/2*. In group two the frequency of 2.9% was present of mutations in breast cancer related genes other than *BRCA1/2*. No mutations were found in *BRCA1/2* which was expected.

In a similar study (*Couch et al.,* 2015) [37] they did also research on the frequency of mutations in predisposition genes of hereditable breast cancer. They used samples of 1824 patients who were diagnosed with triple-negative breast cancer to determine the frequency of mutations in 17 predisposition genes for their gene panel testing, including *BRCA1* and *BRCA2* (see **table 2**). Triple-negative breast cancer is a type of breast cancer that is characterized by the tumor lacking expression of HER2, estrogen receptors and progesterone receptors.[38] The results showed that 14.2% of all the triple-negative breast cancer patients had deleterious mutations. Of the patients 8.5% had mutations in *BRCA1* and 2.7% of the patients in *BRCA2*. 3.7% of the patients had deleterious mutations in the other 14 genes.

A gain in pathogenic mutations in genes other than *BRCA1/2* was also found in the study of *LaDuca et al*. (2014) [39]. The aim of the study was to determine the usability of gene panels in diagnosing hereditary cancer. They wanted to know whether the use of gene panels can play a role in detection of heritable cancer that otherwise would be missed by traditional testing, and so would result in more diagnoses. In the study they tested four different gene panels of which each panel was designed for the detection of a different hereditable cancer. They used 2079 patients which were divided in four groups based on clinical history to test one of the gene panels. The majority of the patients (93.8%) had a personal history of cancer. 4.8% of the patients were unaffected, but had family who were affected by cancer. There was no information present about the cancer history of the other patients. Of the 2079 patients 874 were tested with a breast cancer gene panel consisting of 15 genes (see **table 2**). *BRCA1* and *BRCA2* were not included. The results of the gene panel testing for the detection of breast cancer showed that 7.4% of the patients had pathogenic mutations in the tested genes. This meant that 7.4% were diagnosed with a high risk of developing heritable breast cancer that would normally be missed by traditional testing.

Variants of unknown clinical significance

With the use of gene panels a gain of detected pathogenic mutations in genes related to hereditable breast cancer is observed. But screening multiple genes at the same also increases the amount of variants of unknown clinical significance (VUS). These variants are generally single nucleotide polymorphisms (SNP's). This can be missense mutations, insertions, deletions, splice mutations or nonsense mutations.[40][41] The impact of these variants on protein function are unknown because the variant is not

Table 2. Overview of the used gene panels, number of patients and results of the studies.

Study	Number of genes	Genes included	Number of patients	Frequency of deleterious mutations (%, and number of patients)	Gain of diagnoses (%)
Chong et al.(2014)	6	*BRCA1, BRCA2, PTEN, TP53, CDH1, STK11*	250	-	-
***Lincoln et al.*(2015)**	29	*BRCA1, BRCA2, CDH1, PTEN, STK11, TP53, ATM, BRIP1, CHEK2, NBN, PALB2, RAD51C, PCAM, MLH1, MSH2, MSH6, PMS2, APC, BMPR1A, SMAD4, CDK4, CDKN2A, PALLD, MET, MEN1, RET, PTCH1, VHL, MUTYH*	1105	*BRCA1/2* 13.5 (149) *MUTYH* 1.8 (20) *ATM* 0.45 (5) *PALB2* 0.45 (5) *CHEK2* 0.27 (3) *CDK2NA* 0.09 (1) *	4.5
***Tung et al.* (2015)**	25	*BRCA1, BRCA2, TP53, CDH1, PTEN, ATM, CHEK2, STK11, RAD51C, PALB2, BARD1, BRIP1, NBN, MLH1, MSH2, MSH6, PMS2, EPCAM, RAD51D, APC, MUTYH, CDKN2A, SMAD4, CDK4, BMPR1A*	** **Gr.1 and Gr.2** 1781 and 377	**Gr.1; Gr.2** *BRCA1/2* 9.1; 0.0 (162; 0) *CHEK2* 1.6; 1.3 (29; 5) *ATM* 0.67; 0.26 (12; 1) *PALB2* 0.67; 0.26 (12; 1) *BRIP1* 0.39; 0.00 (7; 0) *BARD1* 0.34; 0.26 (6; 1) *NBN* 0.17; 0.26 (3; 1) *TP53* 0.11; 0.00 (2; 0) *CDH1* 0.00; 0.53 (0; 2) *PMS2* 0.22; 0.00 (4; 0) *MSH6* 0.11; 0.00 (2; 0) *MSH2* 0.056; 0.00 (1; 0) *MUTYH* 0.056; 0.27 (1; 1) *APC* 0.00; 0.27 (0; 1) *CDK2NA* 0.00; 0.27 (0;1)	**Gr.1 and Gr.2** 3.9 and 2.9
***Couch et al.* (2015)**	17	*BRCA1, BRCA2, PALB2, BARD1, BRIP1, RAD51C, RAD51D, RAD50, NBN, MRE11A, XRCC2, ATM, CHEK2, TP53, PTEN, STK11, CDH1*	1824	*BRCA1* 8.5 (155) *BRCA2* 2.7 (49) *PALB2* 1.2 (21) *BARD1* 0.49 (9) *BRIP1* 0.44 (8) *RAD51D* 0.38 (7) *RAD50* 0.33 (6) *RAD51C* 0.33 (6) *XRCC2* 0.16 (3) *MRET11A* 0.11 (2) *ATM* 0.11 (2) *TP53* 0.055 (1) *NBN* 0.055 (1) *PTEN* 0.055 (1)	3.7
***LaDuca et al.*(2014)**	14	*ATM, BARD1, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, PALB2, PTEN, RAD50, RAD51C, STK11, TP53*	874	*CHEK2* 2.2 (19) *ATM* 2.0 (18) *PALB2* 1.7 (15) *TP53* 0.46 (4) *PTEN* 0.34 (3) *RAD50* 0.34 (3) *RAD51C* 0.23 (2) *BRIP1* 0.11 (1) *MRE11A* 0.11 (1) *NBN* 0.11 (1)	7.4

*list not complete
** Gr.1= group 1, Gr.2= group 2

previously reported. Also, it can be unknown due only a small couple of studies indicate that the VUS is deleterious or neutral and more research is needed to confirm it. Because the effect of the mutation on protein function is unknown, it's clinically difficult to say what it means for the patient. Finding of VUS in high risk genes can make recommendations for prevention or treatment very complex and can cause confusion for the patient counseling.[40]

Increase of VUSs

In traditional Sanger sequencing for the detection of hereditary breast, *BRCA1* and *BRCA2* are first screened on containing pathogenic mutations. Because these genes are studied for more than 20 years, the found mutations are well characterized. Through the years the VUSs were detected and studied from which the pathogenicity of these VUS were determined. Nowadays there are still new VUSs found in *BRCA1* and *BRCA2*.[42] VUSs in *BRCA1* and *BRCA2* are very low. In genetic testing of these genes the prevalence of new VUSs in only 2-5%.[43] With the use of gene panel testing a lot of genes (less characterized than the *BRCA* genes) are screened which increases the amount of VUSs. However, not much is known about the rate of variants of unknown significance by using gene panel testing for hereditary breast cancer.[44]

In the study *Lincoln et al*. (2015)[35](see also page 6) research was done to test the performance and gain of found mutations of a 29-gene panel test compared to traditional Sanger sequencing. They used 1105 individuals who were referred for genetic testing for the presence of mutations in breast cancer risk genes. From the results (see **figure 2**) a large amount of VUSs were found. Among the group of the tested individuals 41% had at least one VUS present in a gene of the 29-gene panel. A percentage of 11.4% had two or more VUSs. 5% of the individuals had VUSs in *BRCA1* and *BRCA2* which is expected.[43] So compared to traditional Sanger sequencing (in which only first *BRCA1/2* are tested), with this gene panel a gain of 38% VUSs was observed. They concluded that the amount of genes added to the panel will significantly increase the rate of the amount of VUSs found compared to the amount of detected pathogenic mutations.

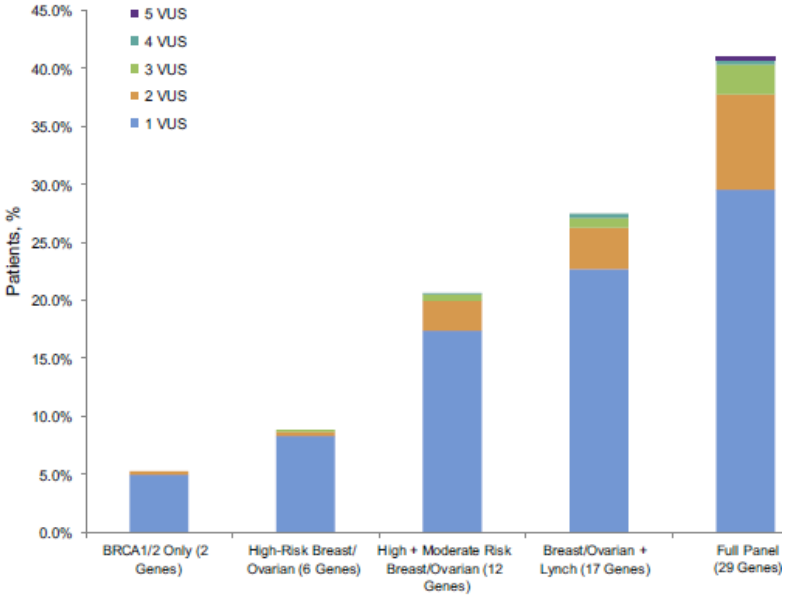

Figure 2. Prevalence of variants of unknown significance (VUSs). Cumulative fraction of clinical cases with one or more VUSs reported, irrespective of pathogenic variants observed, as the scope of testing increases.

From: S. E. Lincoln et al., "A Systematic Comparison of Traditional and Multigene Panel Testing for Hereditary Breast and Ovarian Cancer Genes in More Than 1000 Patients.," *J. Mol. Diagn.*, vol. 17, no. 5, pp. 533–44, Sep. 2015

In another research (*Tung et al.,*2015) [36] (see also page 6) similar results were observed. They tested with a 25 gene panel individuals who were referred for screening and individuals who were previously screened (*BRCA1/2* negative). From their results it was observed that 41.7% of the 1781 individuals who were referred for breast cancer screening had one or more VUSs. Of the 377 *BRCA1/2* negative individuals 41.6% had at least one VUS.

Other studies [45] with gene panels consisting of 20-30 genes associated with breast cancer also have similar percentages of VUSs. It is expected when more genes are used in panels, the percentage of detected VUSs will be much higher and so the clinical information will be more difficult to understand. This was also seen in the study *Kurian et al., 2014*

[46] in which gene panel testing with 42 genes was done. 198 women with breast cancer or germline mutations in *BRCA1* and 2 were tested. They found VUSs in a large part of the patients. Of all patients 88% had VUSs in one or more genes of the tested genes. On average 2.1 VUS/patient were present over all tested patients.

Processing of VUS data

When a genetic variant is found in one of the tested genes the variant is checked in different databases. The pathogenicity is determined by classification. The approach of classification is different between laboratories and no central approach is present.[47][48]. If no information about the variant is present, it will get classified as a variant of unknown significance. To determine the significance reclassification needs to be done. Again, the approach of reclassification is also very different between laboratories. For example, family members can get asked to participate on genetic testing in effort to check segregation, which helps determining the significance of the VUS.[49] A population frequency study can be done to check whether the VUS is present in other patients. Functional assays in vitro can be done to check the effect of the mutation on protein function. Also computer programs that use algorithms derived from analyses of many amino acid changes can be used to check the effect on protein function. [42] However, mostly the clinical significance stays unknown of the VUS due to no effective central approach and no open central databases. [46]

New findings

With gene panel testing it is possible to screen a lot of genes at the same time. This yields a lot of detected mutations in different genes associated with hereditary breast cancer ranging from high risk genes to moderate risk genes. Collecting and analyzing this data can be useful in finding more of the remaining 75-80% mutated genes (non-*BRCA1/2*) which are related to causing hereditary breast.[3]

Of the starting of gene panel testing genes were added of which was not clear if mutations in this genes were associated with a high risk of developing breast cancer, like the genes *PALB2* and *CHEK2.* By studying the collected data these gene were better characterized and now the genes are considered high risk genes in developing of breast cancer and clinical guidelines exists.[50]

Now large population studies are done to determine the effect of mutations in different genes of which the risk in developing breast cancer is not well defined. Of this the genes *BARD1* and *BRIP1* of which association with breast cancer was not well defined, more evidence is present that the genes are possible highly correlated to breast cancer.[50]

Furthermore current known high risk genes and other known genes correlated to hereditary breast cancer are getting more defined. More VUSs in these genes are detected, analysed and the pathogenicity is determined.

Discussion

Due to the discovery of NGS the field of genetic testing is changing, including testing for hereditary breast cancer. Traditional Sanger sequencing, in which first the most likely genes (*BRCA1* and *BRCA2)* are screened and proceeded to less likely genes when nothing is detected, is gradually replaced by gene panel testing. With gene panel testing it is possible to test a lot of genes simultaneously.[4] There are currently a lot dif-

ferent gene panels available and used in detection of hereditary breast cancer. The panels are strongly varying in number of genes, which give a lot information about mutations in genes with a risk of developing breast cancer. This can increase the amount of diagnoses of patient who were referred for genetic testing. However, it's unclear how useful the gene panels are in genetic screening of hereditary breast cancer. [51]

In this review an overview was given about the utility of gene panels in testing for hereditary breast cancer. Focused was on the usability of the genes in the panels, the analytical performance, increase of diagnoses and increase of variants of unknown significance.

As mentioned there're a lot different gene panels available. Every panels consists of a different selection of genes (**table 1**). There is often a combination of high risk genes in which pathogenic mutations are related to hereditary breast cancer, well-defined moderate risk genes and non-well defined moderate risk genes. Some panels also contain genes related to different diseases. But it's questionable how useful all these genes are in gene panel testing. Pathogenic mutations in non-well defined moderate risk genes have probably a moderate chance of developing breast cancer, but because the lack of research the risk is not clear. For those there are no clinical management and guidelines available.[6] The interpretation of findings in non-well defined moderate genes is difficult. Because more studies are needed to determine the risk of findings in these genes, it seems only increased surveillance is the best option for the patient. This until guidelines are available. The use of genes in a gene panel associated to other cancer types increases incidental findings. The usability and ethics of screening genes that are not related to hereditable breast cancer is debateable. [52][53] Although much of the genes in gene panels have no clinical guidelines, there are genes that are highly related to hereditary breast cancer, have guidelines and are clinical significant. This are the genes *BRCA1, BRCA2, PTEN, TP53, STK11, CDH1, CHEK2* and *PALB2.* [11][12] The majority of these genes are tumor suppressor genes of which pathogenic mutations are autosomal dominantly inherited.

To replace traditional Sanger sequencing the new gene panel testing needs to perform on the same level. The studies *Chong et al.*(2014) and *Lincoln et al.*(2015) showed that the analytical performance of gene panel testing is 100% equal to traditional sequencing. No mutations are missed by gene panel testing compared to traditional Sanger sequencing. This means that on analytical level gene panel testing can replace Sanger sequencing.

A gain of diagnoses by gene panel testing was shown by the studies *Tung et al.*(2015), *Couch et al.* (2015), *Lincoln et al.* (2015 and *LaDuca et al*. (2014). Traditional Sanger sequencing in which only first *BRCA1/2* are screened results in 20-25% diagnoses of individuals who are referred for heredity breast cancer testing.[3] The results of the studies showed that with gene panel testing 2.9-7.4% of referred individuals for breast cancer testing have pathogenic mutations in non-*BRCA1/2* genes. This amount of diagnoses would normally be missed by traditional testing. This indicates that gene panel testing can be an efficient enrichment for genetic testing of hereditary breast cancer. But, as shown in *Lincoln et al*. (2015), *Tung et al*. (2015) and *Kurian et al*. (2014) multigene testing also strongly increases the amount of variants of unknown clinical significance (VUSs). In traditional Sanger sequencing the prevalence of new VUSs in only 2-5%.[43] The studies showed that adding more genes to the

panel will significantly increase the rate of the amount of VUSs which can lead up to 88%. The interpretation of VUSs is very difficult. VUSs can cause anxiety for the patient and overtreatment. There's a lack of guidelines for classifying VUSs and counsel methods for patients. Identifying VUSs is also difficult because of the presence of private databases and no large central databank.[46]

Overall, it can be concluded that gene panel testing can be very useful in the diagnosis of hereditary breast cancer. Gene panels in which only genes of clinical significance are included are informative for the patient, which testing results in an increase of the amount of diagnoses compared to traditional Sanger sequencing. The only concern is the strong rise of VUSs of which is important to have clinical guidelines available.

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