The discovery of breast cancer (BC) predisposing genes, BRCA1 and BRCA2, is apparently the most impressive example of the triumph of translational medical research. BRCA1 and BRCA2 were initially identified through the linkage analysis of uniquely large cancer pedigrees, but their contribution to BC and ovarian cancer (OC) morbidity turned out to extend beyond clearly familial cancer cases: indeed, frequency of BRCA1 and BRCA2 germ-line mutations approaches close to 10% in BC and 15% in OC patients, being even higher when the affected women are selected for young age, presence of multiple tumors, specific tumor histology, etc. BRCA1 and BRCA2 play a role in cancer incidence worldwide, although significant country- and ethnicity-specific variations in frequency and spectrum of these mutations are recognized. Great efforts were invested into the estimation of BRCA1- and BRCA2-associated disease risks; based on these calculations, appropriate guidelines for BC/OC screening and prevention were formulated and subjected to clinical validation. Finally, novel drugs, which were intentionally designed to target vulnerabilities in BRCA1/2-driven cancers, recently entered clinical practice. Virtually all current standards of clinical management of healthy people and cancer patients carrying BRCA1/2 germ-line mutations rely on solid medical evidence (1-4).

BRCA1 and BRCA2 are responsible for no more than 30% of familial BC clustering. Genetic studies of BRCA1/2 mutation-negative families expectedly led to discovery of new BC genes. However, clinical utilization of these discoveries turned out to be significantly more complicated, partially due to rarity and/or uneven geographic spread of novel BC-associated mutations. The BC-predisposing role of PALB2 was reported in the year 2007, however it took almost a decade to estimate PALB2-associated risks and realize that PALB2 mutation carriers indeed require some active medical intervention (4-7). CHEK2 mutations were repeatedly proven to at least double the risk of BC disease; however appropriate clinical recommendations are only now being defined and appear to be largely based on common sense but not yet on extensive clinical evidence (4,7,8). Consideration of other genes is even more problematic. For example, BRIP1 was identified as hereditary BC gene via a highly conclusive investigation (9); however, its actual BC-predisposing role was refuted in a recent exhaustively large case-control study (10). ATM, NBS1/NBN, BLM, RECQL, etc. protein-truncating mutations were also shown to play a role in BC predisposition, but the degree of mutation-associated risks appears to be moderate, and some controversies between the studies exist (11-13).

Clinical genetic testing of women with suspicion for hereditary BC is usually limited to BRCA1 and BRCA2 gene analysis. In selected cases, genes associated with rare cancer syndromes are considered as well [for example, TP53 (Li-Fraumeni), PTEN (Cowden), CDH1 (hereditary...
gastric cancer), etc.]. Recent introduction of next generation sequencing (NGS) has changed a technological approach to genetic testing: instead of lengthy sequential gene-by-gene analysis, it is now possible to integrate all potentially significant genes into a single panel, and to obtain relevant information just within a single NGS run. Importantly, NGS is highly reliable in terms of technical validity: by no doubt, it provides perfect concordance with conventional gene testing methods (14). Furthermore, while the increase of the number of genes analyzed by Sanger sequencing results in proportional increase of the cost, adding of a few more genes to a NGS multigene panel has only marginal impact on expenses. Not surprisingly, this possibility of extended genetic testing attracts interest from industry, public and medical community. At present, approximately a dozen of multigene cancer genetic tests are marketed (7,15,16).

It is getting increasingly discussed that limitations of multigene testing are often not properly acknowledged (15-18). Actually all commercial panels mix genes with overt clinical significance together with genes whose BC-predisposing role seems at best suggestive (7,13,15,16,19). Furthermore, all modern multigene tests were developed using the genetic data obtained on cancer patients of European descent; it is self-explanatory, that distinct nations have distinct ancestors and therefore carry unique pool of pathogenic mutations, therefore the value of existing NGS panels for non-White patients remains unclear (1,12,15). Furthermore, while fairly balanced information regarding BRCA1 and BRCA2 is readily available for physicians and patients, clinical data on “novel” BC genes remain scarce and therefore are more likely to be misinterpreted.

Recent studies confirm all these cautions. Tung et al. analyzed 488 sequential BC patients using NGS panel consisting of 25 hereditary cancer genes (20). Quite reassuringly, all instances of BRCA1/2 mutation (n=30, 6.1%) were revealed in women who were either already tested BRCA1/2-positive during routine clinical examination (n=26) or met clinical criteria for BRCA1/2 testing anyway (n=4). Therefore, NGS, being a robust technical approach, is unlikely to reveal BRCA1/2 mutation carriers who would be missed by existing clinical attitudes. Mutations in other presumably BC-predisposing genes were identified in 20 women, with the highest frequency for CHEK2 (n=10), ATM (n=4) and BRIP1 (n=4). Only one carrier of PALB2 germ-line mutation was detected. It is not surprising, that in contrast to BRCA1/2, germ-line mutations in “novel” BC genes did not correlate with younger age of patients. Indeed, given that premenopausal and postmenopausal BC may have significantly different natural histories, one could expect that a subset of genes would predispose specifically to late-onset but not to early-onset BC. Similarly, lack of association with BC receptor phenotypes cannot be considered as a strong evidence against BC-predisposing role of CHEK2, ATM, BRIP1, etc. What is more alarming, it is the lack of correlation between germ-line mutations in non-BRCA1/2 genes and a family history of cancer disease, especially given that the information on patient relatives was collected and analyzed in a very careful way. Of course, moderate-penetration genes rendering approximately 2-fold excess of the risk are less likely to show familial clustering than BRCA1/2; furthermore, family studies of gender-specific cancers, like BC and OC, are compromised by the absence of these target organs in males. Therefore, this study actually had limited power to detect family history associations for moderately penetrant genes. Nevertheless, the results of the study Tung et al. (20) strongly indicate that even if multiple genes are pooled in the same NGS panel, the analysis of the results does not permit pooling and has to be done on gene-by-gene basis. This is particularly true assuming that one of the most frequently mutated gene in this study, BRIP1, is likely to have null significance for modifying BC risk (10). Similar data were obtained in the study of Thompson et al., which was published in the same issue of the Journal of Clinical Oncology (19). To our knowledge, this is the first large NGS-based case-control study in cancer research; it compared 2000 BRCA1/2 mutation-negative familial BC cases versus 1997 non-affected women. Contrary to Tung et al. (20), Thompson et al. (19) confirmed the significance of PALB2, but failed to detect high frequency of CHEK2 mutations. There is a good agreement between these two studies on limited contribution of “novel” BC genes in the disease morbidity. It is essential to keep in mind, that the selection of women with strong family history of BC, as it was done by Thompson et al. (19), may not be appropriate for the evaluation of BC-predisposing impact of moderately penetrant genes.

The invention of the next generation sequencing is an absolutely outstanding event in the modern biomedical science, whose significance is comparable with the discovery of PCR. NGS has an enormous potential for academic and translational research, and it will certainly revolutionize clinical management of hereditary and cancer diseases. Massive parallel sequencing provides excellent opportunities to improve access to the testing of medically relevant cancer genes, such as BRCA1, BRCA2, PALB2, CHEK2, TP53,
etc. However, biotech companies, physicians, diagnostic services, etc. have to be discouraged to include in routine clinical testing genes with yet unclear actionability.

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**Footnote**

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