Next-generation sequencing of the **BRCA1** and **BRCA2** genes for the genetic diagnostics of hereditary breast and/or ovarian cancer

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Genetic testing for hereditary breast and/or ovarian cancer (HBOC) mostly relies on laborious molecular tools that use Sanger sequencing to scan for mutations in the **BRCA1** and **BRCA2** genes. We have explored a more efficient genetic screening strategy based on next-generation sequencing (NGS) of the **BRCA1** and **BRCA2** genes in 210 HBOC patients. We first validated this approach in a cohort of 115 samples with previously known **BRCA1** and **BRCA2** mutations and polymorphisms. Genomic DNA was amplified using the Ion AmpliSeq™ BRCA1 and BRCA2 panel. The DNA Libraries were pooled, barcoded and sequenced using an Ion Torrent PGM sequencer. The combination of different robust bioinformatics tools allowed us to detect all previously known pathogenic mutations and polymorphisms in the 115 samples, without detecting spurious pathogenic calls. We then used the same assay in a discovery cohort of 95 uncharacterized HBOC patients for **BRCA1** and **BRCA2**. In addition, we describe the allelic frequencies across 210 HBOC patients of 74 unique definitively and likely pathogenic, and uncertain **BRCA1** and **BRCA2** variants, some of them not previously annotated in the public databases. Targeted NGS is ready to substitute classical molecular methods to perform genetic testing on the **BRCA1** and **BRCA2** genes, and provides a greater opportunity for more comprehensive testing for at-risk patients.

**NGS vs Sanger validation study design**

**Variant annotation**

- **All variants annotated with all information available!**
- **Variant**
- **R^2 terms**
- **Pathway**
- **Disease**
- **Conservation**
- **Structural**
- **Other**

**NGS vs Sanger validation study results**

- **Validation Cohort (n=115 HBOC patients)**
  - Sensitivity: 100%
  - Specificity: 99%
  - Positive predictive value: 91%
  - Negative predictive value: 100%

- **Discovery Cohort (n=95 HBOC patients)**
  - Diagnostic rate: 51%

**BRCA1/2 testing at Centogene**

CENTOGENE is highly experienced in the diagnosis of hereditary disorders, including cancers. We offer a comprehensive oncogenic testing portfolio, such as gene sequencing of **BRCA1/2** with expert result interpretation and a medically reviewed report within 15 working days. For patients who are tested **BRCA1/2** negative, we offer alternatively complex gene panels which include genes also linked to hereditary breast and ovarian cancer.