Table SI. Overview of the characteristics of patients with breast cancer.

Patient	Age at initial diagnosis, years	Race	Ethnicity	Sex	ICD-10-diagnosis code	Histological type	Grade	ER	PR	HER-2	Ki-67	Molecular subtype
1	59	Caucasian	Slovak	Female	C50.9	IDC	1	Positive	Positive	Negative	Low	Luminal A
2	69	Caucasian	Slovak	Female	C50.9	IDC	3	Positive	Positive	Positive	High	Luminal B
3	39	Caucasian	Slovak	Female	C50.9	IDC	2	Positive	Positive	Negative	High	Luminal B
4	59	Caucasian	Slovak	Female	C50.9	ILC	3	Positive	Positive	Unknown	High	Luminal B
5	50	Caucasian	Slovak	Female	C50.9	LIC	2	Positive	Positive	Unknown	Low	Unknown
6	52	Caucasian	Slovak	Female	C50.9	IDC	3	Positive	Negative	Positive	High	Luminal B
7	72	Caucasian	Slovak	Female	C50.9	IDC	1	Positive	Positive	Negative	Low	Luminal A
8	49	Caucasian	Slovak	Female	C50.9	ILC	2	Positive	Positive	Negative	Low	Luminal A
9	57	Caucasian	Slovak	Female	C50.9	ILC	2	Positive	Positive	Negative	Low	Luminal A

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2; Ki-67, proliferation index.

Table SII. Summarization of identified somatic pathogenic variants.

Chr	Position	Gene	Nucleotide change	Type of mutation	Mutational effect	Coding sequence	Protein variant	SNP ID	Allele frequency	Sample	Patient
10	89717676-89717676	*PTEN*	cGg/cAg	Substitution	Missense	c.701G>A	p.Arg234Gln	rs121909235	None	1	1
17	7577511-7577511	*TP53*	cTg/cAg	Substitution	Missense	c.770T>A	p.Leu257Gln	rs28934577	None	1	1
3	178952085-178952085	*PIK3CA*	cAt/cTt	Substitution	Missense	c.3140A>T	p.His1047Leu	rs121913279	0.000008910	1	1
5	112173930-112173930	*APC*	aTc/aCc	Substitution	Missense	c.2639T>C	p.Ile880Thr	rs1400295986	None	1	1
3	178952085-178952085	*PIK3CA*	cAt/cGt	Substitution	Missense	c.3140A>G	p.His1047Arg	rs121913279	0.000008910	1.2	1
10	89692940-89692941	*PTEN*	cGg/cg	Deletion	Frameshift	c.428del	p.Gly143AlafsTer4	COSM28894	None	2.2	2
7	140481411-140481411	*BRAF*	gGa/gAa	Substitution	Missense	c.1397G>A	p.Gly466Glu	rs121913351	0.000003977	2.3	2
10	89624269-89624269	*PTEN*	Aga/Gga	Substitution	Missense	c.43A>G	p.Arg15Gly	COSM1159817	None	3	3
10	89653803-89653803	*PTEN*	gCt/gAt	Substitution	Missense	c.101C>A	p.Ala34Asp	COSM86058	None	3	3
5	112175608-112175609	*APC*	Cca/ca	Deletion	Frameshift	c.4319del	p.Pro1440HisfsTer33	rs1131691145	None	3	3
5	112175786-112175786	*APC*	Gga/Tga	Substitution	Nonsense	c.4495G>T	p.Gly1499Ter	rs756912930	None	3	3
16	68856113-68856113	*CDH1*	Cag/Tag	Substitution	Nonsense	c.1921C>T	p.Gln641Ter	rs587782750	0.000008791	4.1	4
16	68856113-68856113	*CDH1*	Cag/Tag	Substitution	Nonsense	c.1921C>T	p.Gln641Ter	rs587782750	0.000008791	4.2	4
17	7573010-7573010	*TP53*	-	-	Splice Acceptor	c.1101-2A>T	-	COSM45409	None	5.1	5
3	178952085-178952085	*PIK3CA*	cAt/cGt	Substitution	Missense	c.3140A>G	p.His1047Arg	rs121913279	0.000008910	5.2	5
1	115258736-115258736	*NRAS*	Aaa/Gaa	Substitution	Missense	c.46A>G	p.Lys16Glu	COSM6960034	None	6.3	6
17	7576873-7576873	*TP53*	Gga/Tga	Substitution	Nonsense	c.973G>T	p.Gly325Ter	rs863224500	None	6.3	6
3	178952084-178952084	*PIK3CA*	Cat/Tat	Substitution	Missense	c.3139C>T	p.His1047Tyr	rs121913281	None	7	7
7	140453136-140453136	*BRAF*	gTg/gAg	Substitution	Missense	c.1799T>A	p.Val600Glu	rs113488022	0.000003980	7	7
3	178952085-178952085	*PIK3CA*	cAt/cTt	Substitution	Missense	c.3140A>T	p.His1047Leu	rs121913279	0.000008910	7.2	7
10	89690801-89690801	*PTEN*	-	Substitution	Splice Acceptor	c.210-2A>G	-	rs1564828914	None	8	8
12	25398284-25398284	*KRAS*	gGt/gTt	Substitution	Missense	c.35G>T	p.Gly12Val	rs121913529	None	8.2	8
17	7573996-7573996	*TP53*	cTg/cCg	Substitution	Missense	c.1031T>C	p.Leu344Pro	rs121912662	None	9	9

Samples 1, 3, 7, 8 and 9 were collected before surgery; samples 4.1 and 5.1 were collected after surgery; samples 1.2, 2.2, 4.2, 5.2, 7.2 and 8.2 are formalin-fixed and paraffin-embedded samples; samples 2.3 and 6.3 were collected after adjuvant chemotherapy. Chr, chromosome.

Table SIII. Identified somatic pathogenic variants of genes in ctDNA samples of patients with breast cancer.

Chr	Position	Gene	Nucleotide change	Type of mutation	Mutational effect	Coding sequence	Protein variant	Depth of coverage	VAF, %	Sample	Patient
10	89717676-89717676	*PTEN*	cGg/cAg	Substitution	Missense	c.701G>A	p.Arg234Gln	24,831	3.1	1	1
17	7577511-7577511	*TP53*	cTg/cAg	Substitution	Missense	c.770T>A	p.Leu257Gln	16,898	3.17	1	1
3	178952085-178952085	*PIK3CA*	cAt/cTt	Substitution	Missense	c.3140A>T	p.His1047Leu	16,711	3.93	1	1
5	112173930-112173930	*APC*	aTc/aCc	Substitution	Missense	c.2639T>C	p.Ile880Thr	11,720	4.34	1	1
7	140481411-140481411	*BRAF*	gGa/gAa	Substitution	Missense	c.1397G>A	p.Gly466Glu	8,848	6.37	2.3	2
10	89624269-89624269	*PTEN*	Aga/Gga	Substitution	Missense	c.43A>G	p.Arg15Gly	5,687	4.52	3	3
10	89653803-89653803	*PTEN*	gCt/gAt	Substitution	Missense	c.101C>A	p.Ala34Asp	2,261	5.75	3	3
5	112175608-112175609	*APC*	Cca/ca	Deletion	Frameshift	c.4319del	p.Pro1440HisfsTer33	6,934	7.59	3	3
5	112175786-112175786	*APC*	Gga/Tga	Substitution	Nonsense	c.4495G>T	p.Gly1499Ter	9,314	4.98	3	3
16	68856113-68856113	*CDH1*	Cag/Tag	Substitution	Nonsense	c.1921C>T	p.Gln641Ter	5,587	10.43	4.1	4
17	7573010-7573010	*TP53*	-	-	Splice Acceptor	c.1101-2A>T	-	7,667	3.46	5.1	5
1	115258736-115258736	*NRAS*	Aaa/Gaa	Substitution	Missense	c.46A>G	p.Lys16Glu	10,550	3.18	6.3	6
17	7576873-7576873	*TP53*	Gga/Tga	Substitution	Nonsense	c.973G>T	p.Gly325Ter	19,884	3.14	6.3	6
3	178952084-178952084	*PIK3CA*	Cat/Tat	Substitution	Missense	c.3139C>T	p.His1047Tyr	28,088	3.04	7	7
7	140453136-140453136	*BRAF*	gTg/gAg	Substitution	Missense	c.1799T>A	p.Val600Glu	29,762	4.03	7	7
10	89690801-89690801	*PTEN*	-	Substitution	Splice acceptor	c.210-2A>G	-	9,496	6.28	8	8
17	7573996-7573996	*TP53*	cTg/cCg	Substitution	Missense	c.1031T>C	p.Leu344Pro	5,498	3.31	9	9

Samples 1, 3, 7, 8 and 9 were collected before surgery; samples 4.1 and 5.1 were collected after surgery; samples 2.3 and 6.3 were collected after chemotherapy. Chr, chromosome; VAF, variant allele frequency; ctDNA, circulating tumor DNA.

Table SIV. Identified somatic pathogenic variants of genes in formalin-fixed and paraffin-embedded DNA samples of patients with breast cancer.

Chr	Position	Gene	Nucleotide change	Type of mutation	Mutational effect	Coding sequence	Protein variant	Depth of coverage	VAF, %	Sample	Patient
3	178952085-178952085	*PIK3CA*	cAt/cGt	Substitution	Missense	c.3140A>G	p.His1047Arg	29,865	15.67	1.2	1
10	89692940-89692941	*PTEN*	cGg/cg	Deletion	Frameshift	c.428del	p.Gly143AlafsTer4	23,778	40.66	2.2	2
16	68856113-68856113	*CDH1*	Cag/Tag	Substitution	Nonsense	c.1921C>T	p.Gln641Ter	14,569	50.53	4.2	4
3	178952085-178952085	*PIK3CA*	cAt/cGt	Substitution	Missense	c.3140A>G	p.His1047Arg	29,205	14.55	5.2	5
3	178952085-178952085	*PIK3CA*	cAt/cTt	Substitution	Missense	c.3140A>T	p.His1047Leu	40,713	26.01	7.2	7
12	25398284-25398284	*KRAS*	gGt/gTt	Substitution	Missense	c.35G>T	p.Gly12Val	12,881	8.04	8.2	8

Chr, chromosome; VAF, variant allele frequency.