Tab. 5. *BRCA1* in-frame deletions detected in Czech patients.

cDNA level	Protein level	Number of families	NFE frequency (gnom)	[reference]: additional information // ¥ (co-ocurrence with deleterious BRCA1 mutation) // § (detected in cancer free controls)	LOVD-IARC class [4] // our class (if different or not specified)
c.1824_1826delGAA	**p.Lys608del**	1	0.0036%	[32]: second Nucler Localisation Signal (NLS2: AA 607–614); PROVEAN score: −11,436 (deleterious, cutoff: −2,5)	class 3; VUS
c.1846_1848delTCT	**p.Ser616del**	3	0,0016%	[43]: phosphorylation sites in BRCA1: S615, **S1616,** S617; PROVEAN score: −12,666 (deleterious, cutoff: −2,5) // **¥** [70]	class 3; VUS
c.3418_3420delATG	**p.Ser1140del**	1	0.0047%	PROVEAN score: −9,704 (deleterious, cutoff: −2,5) // **¥** [70]	class 3; VUS
c.3785_3787delCAT	**p.Ser1262del**	1	–	PROVEAN score: −2,176 (neutral, cutoff: −2,5)	– // class 3; VUS

cDNA – complementary DNA, NFE – non-Finnish European, LOVD – Leiden Open Variation Database, IARC – International Agency for Research on Cancer, VUS – variants of uncertain clinical significance

Tab. 6. *BRCA1* missense alterations detected in Czech patients classified as benign, likely benign and uncertain significance.

cDNA level (HGVS nomencl)	Protein	Number of families	NFE freq. (gnom)	Prior	Align-GVGD	Splice prediction: average (MaxEnt /NNSPLICE/SSF) // Ω mRNA analysis	[reference]: other information // ¥ (co-ocurrence with deleterious BRCA1 mutation) // § (detected in cancer free controls)	LOVD-IARC class [4] // our class (if different or not specified)
c.10T>C	**p.Ser4Pro**	1	–	0,02	C0	no effect		– // class 3, VUS
c.74C>T	**p.Pro25Leu**	1	–	0,29	C15			– // class 3, VUS
c.113A>G	**p.Lys38Arg**	1	–	0,3	C0	new donor site?		– // class 3, VUS
c.133A>C	**p.Lys45Gln**	1	0.0027%	0,04	C0	splice: -1,5%, // **Ω** (our study): no alteration	[34]: benign	class 1, benign
c.314A>G	**p.Tyr105Cys**	NC	0.013%	0,02	C15	no effect	[59]: multifactorial likelihood ratio – neutral; [34]: neutral // **¥** [70]	class 1, benign
c.513A>G	**p.Ile171Met**	1	–	0,02	C0	no effect		novel // class 2, likely benign
c.536A>G	**p.Tyr179Cys**	6	0.036%	0,02	C35	no effect / **Ω** [67]: no change	[34]: neutral // [39]: **OR 1,68** (95% CI 0.82–3.45) // **¥** [70]	class 1; benign
c.591C>T	**p.Cys197Cys**	NC	0.17%	0,04	–	– // **Ω** [27]: parcial enhancing Δ9 and Δ9_10	**§** (our study)	class 1; benign
c.603T>A	**p.Asp201Glu**	1	– // **novel**	0,02	C0	no effect		– //class 2, likely benign
c.697G>A	**p.Val233Ile**	2	– // **novel**	0,02	C0	no effect		– //class 2, likely benign
c.736T>G	**p.Leu246Val**	NC	0.061%	0,02	C0	no effect / **Ω** [60]: no aberration	[34]: neutral // **¥** [38]	class 1, benign
c.755G>A	**p.Arg252His**	1	0.00079 %	0,02	C0	no effect / **Ω** [60]: no aberration		class 2, likely benign
c.827C>G	**p.Thr276Arg**	3	0.0055%	0,02	C0	no effect		class 2, likely benign
c.1016A>G	**p.Lys339Arg**	1	–	0,02	C0	weakens the likelihood of cryptic splicing		class 2, likely benign
c.1067A>G	**p.Gln356Arg**	NC	6.57%	0,02	C0	no effect / **Ω** [60]: no aberration	[39]: **OR 0,99** (95% CI 0.95–1.03) // **¥** [38] and (our study) // **§** (our study)	class 1, benign
c.1333G>C	**p.Glu445Gln**	2	0.0045%	0,02	C0			class 2, likely benign
c.1441C>G	**p.Leu481Val**	NC	–	0,02	C0	no effect	// **¥** (our study)	– // class 2, likely benign
c.1456T>C	**p.Phe486Leu**	NC	0.036%	0,02	C0	no effect / **Ω** [60]: no aberration	[34]: neutral // **¥** [38]	class 1, benign
c.1486C>T	**p.Arg496Cys**	3	0.030%	0,02	C0	no effect / **Ω** [60]: no aberration	[34]: neutral // **¥** [38]	class 1, benign
c.1487G>A	**p.Arg496His**	5	0.085%	0,02	C0	no effect / **Ω** [60]: no aberration	[39]: **OR 1,05** (95% CI 0.77–1.43) // **¥** [70]	class 1, benign

c.1511G>A	**p.Arg504His**	1	0.0032%	0,02	C0	no effect	[59]: multifactorial likelihood ratio - neutral; [32]**: first Nucler Localisation Signal (aa 503–508)**	class 1, benign // class 3 – VUS
c.1616C>T	**p.Thr539Met**	1	0.0032%	0,02	C0	no effect		class 2, likely benign
c.1648A>C	**p.Asn550His**	2	0.036%	0,02	C0	no effect / **Ω** [60]: no aberration	[34]: neutral // **¥** [70]	class 1, benign
c.1865C>T	**p.Ala622Val**	3	0.036%	0,02	C0	no effect / **Ω** [60]: no aberration	[59]: multifactorial likelihood ratio – neutral; [34]: neutral	class 1, benign
c.2077G>A	**p.Asp693Asn**	NC	7.63%	0,02	C0	no effect/ **Ω** [60]: no aberration	[34]: neutral // **¥** [38] // **§** (our study)	class 1, benign
c.2123C>A	**p.Ser708Tyr**	2	0.0055%	0,02	C0	no effect / **Ω** [60]: no aberration		class 2, likely benign
c.2128A>G	**p.Thr710Ala**	1	–	0,02	C0	no effect		– // class 2, likely benign
c.2167A>G	**p.Asn723Asp**	1	–	0,02	C0	no effect / **Ω** [60]: no aberration	[59]: multifactorial likelihood ratio - neutral; [34]: neutral // **¥** [70]	class 1, benign
c.2315T>C	**p.Val772Ala**	1	0.019%	0,02	C0	no effect / **Ω** [60]: no aberration	[39]: **OR 1,1** (95% CI 0.55–2.17) // [34]: neutral // **¥** [70] // **§** (our study)	class 1, benign
c.2412G>C	**p.Gln804His**	2	0.011%	0,02	C0	no effect	[59]: multifactorial likelihood ratio – neutral; [34]: neutral	class 1, benign
c.2477C>A	**p.Thr826Lys**	2	0.032%	0,02	C0	no effect	[34]: neutral	class 1, benign
c.2503C>T	**p.His835Tyr**	3	0.0018%	0,02	C0	no effect / **Ω** [60]: no aberration		– // class 2, likely benign
c.2521C>T	**p.Arg841Trp**	9	0.24%	0,02	C15	no effect / **Ω** [60]: no aberration	[39]: **OR 0,81** (95% CI 0.66–1.00) // [34]: neutral // **¥** [38]	class 1, benign
c.2596C>T	**p.Arg866Cys**	2	0.024%	0,02	**C65**	no effect / **Ω** [60]: no aberration	[59]: multifactorial likelihood ratio – neutral // **¥** [70]	class 1, benign
c.2612C>T	**p.Pro871Leu**	NC	33.52%	0,02	C0	slightly enhancing cryptic splice? / **Ω** [60]: no aberration	[34]: neutral // **¥** [38] // **§** (our study)	class 1, benign
c.2666C>T	**p.Ser889Phe**	1	0.0018%	0,02	C0	no effect		– // class 2, likely benign
c.2732G>A	**p.Gly911Glu**	1	–	0,02	C0	no effect		– // class 2, likely benign
c.2884G>A	**p.Glu962Lys**	1	–	0,02	C0	no effect		– // class 2, likely benign
c.3024G>A	**p.Met1008Ile**	4	0.031%	0,02	C0	no effect / **Ω** [60]: no aberration	[39]: **OR 0,91** (95% CI 0.53–1.57) // [34]: neutral // **¥** [70]	class 1, benign
c.3055A>G	**p.Ile1019Val**	1	0.00090 %	0,02	C0	no effect		class 2, likely benign
c.3082C>T	**p.Arg1028Cys**	1	0.0032%	0,02	C15	slightly enhancing cryptic	[59]: multifactorial likelihood ratio –	– // class 2, likely

						donor splice?	neutral	benign
c.3113A>G	**p.Glu1038Gly**	NC	32.67%	0,02	C0	no effect	[34]: neutral // **¥** [38] // **§** (our study)	class 1, benign
c.3119G>A	**p.Ser1040Asn**	NC	2.03%	0,02	C0	no effect	[39]: **OR 0,95** (95% CI 0.89–1.02) // [34]: neutral // **¥** [38] // **§** (our study)	class 1, benign
c.3190A>T	**p.Ser1064Cys**	4	–	0,02	C0	no effect		– // class 2, likely benign
c.3296C>T	**p.Pro1099Leu**	NC	0.024%	0,02	C0	no effect / **Ω** [60]: no aberration	[34]: neutral // **¥** [70]	class 1, benign
c.3302G>A	**p.Ser1101Asn**	1	0.025%	0,02	C0	no effect / **Ω** [60]: no aberration	[59]: multifactorial likelihood ratio - neutral; [34]: neutral	class 1, benign
c.3454G>A	**p.Asp1152Asn**	10	0.0079%	0,02	C0	no effect		class 2, likely benign
c.3541G>A	**p.Val1181Ile**	2	0.00090 %	0,02	C0	no effect		class 2, likely benign
c.3548A>G	**p.Lys1183Arg**	NC	32.57%	0,02	C0	no effect / **Ω** [60]: no aberration	[34]: neutral // **¥** [38] // **§** (our study)	class 1, benign
c.3607C>G	**p.Arg1203Gly**	1	–	0,02	C0	no effect	[34]: neutral	– // class 2, likely
c.3640G>A	**p.Glu1214Lys**	3	0.013%	0,02	C0	no effect / **Ω** [60]: no aberration	[59]: multifactorial likelihood ratio - neutral; [34]: neutral	class 1, benign
c.3708 T>G	**p.Asn1236Lys**	1	0.037%	0,02	C0	no effect / **Ω** [60]: no aberration		class 3, VUS // class 2, likely benign
c.3711A>G	**p.Ile1237Met**	1	0.00090 %	0,02	C0	no effect		class 2, likely benign
c.3713C>T	**p.Pro1238Leu**	NC	0.0095%	0,02	C0	no effect / **Ω** [60]: no aberration	[59]: multifactorial likelihood ratio – neutral; [34]: neutral	class 1, benign
c.3748G>A	**p.Glu1250Lys**	NC	0.036%	0,02	C0	no effect / **Ω** [60]: no aberration	[34]: neutral // **¥** [70]	class 1, benign
c.3823A>G	**p.Ile1275Val**	NC	0.0071%	0,3	C0	reducing the likelihood of cryptic splicing / **Ω** [60]: no aberration	[59]: multifactorial likelihood ratio – neutral; [34]: neutral	class 1, benign
c.3914A>T	**p.Asp1305Val**	1	–	0,02	C0	no effect		class 3, VUS // class 2, likely benign
c.4036G>A	**p.Glu1346Lys**	3	0.0095%	0,02	C0	no effect / **Ω** [60]: no aberration		class 2, likely benign
c.4039A>G	**p.Arg1347Gly**	NC	0.69%	0,02	C0	no effect / **Ω** [60]: no aberration	[34]: neutral // **¥** [38]	class 1, benign
c.4132G>A	**p.Val1378Ile**	2	0.0080%	0,02	C0	no effect		class 2, likely benign
c.4333C>A	**p.Pro1445Thr**	1	0.00090 %	0,02	C0	no effect		– // class 2, likely
c.4342A>G	**p.Ser1448Gly**	1	0.0027%	0,02	C0	no effect		class 2, likely benign
c.4529T>C	**p.Met1510Thr**	1	– // **novel**	0,02	C0	slightly enhancing the likelihood of acceptor		// class 3, VUS

						cryptic splicing		
c.4535G>T	**p.Ser1512Ile**	NC	0.37%	0,02	C0	no effect	[34]: neutral // **¥** [38]	class 1, benign
c.4546A>G	**p.Ser1516Gly**	1	– // **novel**	0,02	C0	low likelihood of new acceptor splicing		// class 3, VUS
c.4625C>G	**p.Ser1542Cys**	1	0.0081%	0,02	C0	no effect		class 3, VUS
c.4636G>A	**p.Asp1546Ans**	3	0.012%	0,02	C0	no effect / **Ω** [67]: no change	[59]: multifactorial likelihood ratio – neutral; [34 42]: neutral	class 1, benign
c.4843G>A	**p.Ala1615Thr**	1	0.00090 %	0,02	C0	no effect		class 2, likely benign
c.4956G>A	**p.Met1652Ile**	NC	1.64%	0,03	C0	no effect / **Ω** [67]: no change	[39]: **OR 1,01** (95% CI 0.94–1.09) // [59]: multifactorial likelihood ratio - neutral; [65]: no functional effect; [64]: neutral // **¥** [38] // **§** (our study)	class 1, benign
c.5005G>T	**p.Ala1669Ser**	6	0.025%	0,03	C0	slightly reducing of the likelihood of the natural acceptor splicing: -10,6%	[65]: no functional effect; [64]: neutral	class 2, likely benign
c.5176A>G	**p.Arg1726Gly**	1	0.0099%	0,03	C0	no effect	[65]: no functional effect	class 3, VUS
c.5198A>G	**p.Asp1733Gly**	4	0.0032%	0,03	C0	no effect	[65]: no functional effect; [71]: **possible influence to p53 binding**	class 3, VUS
c.5206G>C	**p.Val1736Leu**	1	– // **novel**	**0,29**	**C25**	low likelihood of new acceptor splicing		– // class 3, VUS
c.5252G>A	**p.Arg1751Gln**	2	0.0072%	0,03	C0	no effect	[39]: **OR 0,85** (95% CI 0.26–2.77) // [65]: low functional effect // [59]: multifactorial likelihood ratio – neutral; [64]: neutral // **¥** (our study)	class 1, benign
c.5281T>G	**p.Phe1761Val**	1	– // **novel**	0,03	C0	no effect		– // class 3, VUS
c.5504G>A	**p.Arg1835Gln**	1	0.00079 %	0,03	C0	no effect		– // class 3, VUS

cDNA – complementary DNA, NFE – non-Finnish European, mRNA – messenger RNA, LOVD – Leiden Open Variation Database, IARC – International Agency for Research on Cancer, NC – not counted, OR – odds ratio, CI – confidence interval, VUS – variants of uncertain clinical significance

Tab. 12. *BRCA2* missense alterations detected in Czech patients classified as benign, likely benign and uncertain significance.

cDNA level (HGVS nomenclature)	Protein	Number of families	NFE frequency (gnom)	Prior	Align-GVGD	Splice prediction: average (MaxEnt/NNSPLICE/SSF) // Ω mRNA analysis	[reference]: other information // ¥ (co-occurrence with deleterious *BRCA2* mutation) // § (detected in cancer free controls)	LOVD-IARC class [4] // our class (if different or not specified)
c.125A>G	**p.Tyr42Cys**	19	0.22%	0,02	c0	no effect	[76]: functional assays: no effect; [80]: HDR assay: as wild type; [34]: benign; [39]: **OR 1,00** (95% CI 0.80–1.25) // **§** (our study)	class 1, benign
c.161A>G	**p.Asn54Ser**	1	– // **novel**	0,02	c0	no effect		– // class 2, likely benign
c.172G>A	**p.Glu58Lys**	1	0.00090 %	0,02	c0	no effect		– // class 2, likely benign
c.198A>G	**p.Gln66Gln**	6	0.027%	0,02	c0	new acceptor site?		class 2, likely benign
c.223G>C	**p.Ala75Pro**	NC	0.036%	0,02	c0	no effect // **Ω** [72]: like wild type; [67]: no change	[34]: benign	class 1, benign
c.232C>T	**p.Pro78Ser**	2	–	0,02	**c65**	no effect		– // class 3, VUS
c.353G>A	**p.Arg118His**	2	0.0032%	0,02	c0	no effect		class 2, likely benign
c.375T>A	**p.Asp125Glu**	1	–	0,02	c0	no effect		– // class 2, likely benign
c.646G>A	**p.Ala216Thr**	1	– // **novel**	0,02	c0	no effect		– // class 2, likely benign
c.800G>A	**p.Gly267Glu**	1	0.0064%	0,02	c0	no effect		– // class 2, likely benign
c.825A>T	**p.Lys275Asn**	1	0.0018%	0,02	c0	no effect		– // class 2, likely benign
c.865A>C	**p.Asn289His**	NC	3.42%	0,02	c0	no effect	[39]: **OR 0,95** (95% CI 0.90–1.00) // **§** (our study)	class 1, benign
c.887A>G	**p.Tyr296Cys**	1	0.0057%	**0,3**	c0	**high probability of new donor site?**		class 2, likely benign // class 3, VUS
c.956A>C	**p.Asn319Thr**	1	0.0057%	0,02	c0	no effect	[76]: functional assays: no effect; [80]: HDR assay: as wild type; [34]: benign;	class 1, benign
c.978C>A	**p.Ser326Arg**	NC	0.14%	0,02	c0	no effect	[34]: benign; [61]: **OR 1,2** (95% CI 0.92 – 1.56) // **§** (our study)	class 1, benign
c.1114A>C	**p.Asn372His**	NC	27.70%	0,02	c0	no effect	[76]: functional assays: no effect; [80]: HDR assay: as wild type; [34]: benign // **§** (our study)	class 1, benign
c.1146A>T	**p.Lys382Asn**	4	–	0,02	c0	no effect		– // class 2, likely benign
c.1151C>T	**p.Ser384Phe**	NC	0.12%	0,02	c0	no effect // **Ω** [67]: no	[34]: benign; [39]: **OR 0,92** (95% CI	class 1, benign

						change	0.69–1.23); **§** (our study)	
c.1181A>C	**p.Glu394Ala**	3	0.0054%	0,02	c0	no effect		– // class 2, likely benign
c.1216G>A	**p.Ala406Thr**	1	0.0018%	0,02	c0	no effect		class 2, likely benign
c.1219C>G	**p.Gln407Glu**	1	0.0018%	0,02	c0	slightly enhancing cryptic acceptor site		class 2, likely benign
c.1292C>T	**p.Thr431Ile**	2	0.0067%	0,02	c0	no effect		– // class 2, likely benign
c.1329G>T	**p.Glu443Asp**	1	0.00090 %	0,02	c0	enhancing cryptic acceptor site		– // class 3, VUS
c.1385A>G	**p.Glu462Gly**	1	0.044%	0,02	c0	no effect	[76]: functional assays: no effect; [80]: HDR assay: as wild type; [34]: benign;	class 1, benign
c.1514T>C	**p.Ile505Thr**	3	0.090%	0,02	c0	slightly enhancing cryptic acceptor site // **Ω** [67]: no change	[39]: **OR 1,10** (95% CI 0.80–1.52)	class 2, likely benign
c.1547T>C	**p.Phe516Ser**	1	–	0,02	c0	slightly enhancing cryptic acceptor site		– // class 2, likely benign
c.1729G>T	**p.Ala577Ser**	1	– // **novel**	0,02	c0	no effect		– // class 2, likely benign
c.1792A>G	**p.Thr598Ala**	NC	0.27%	0,02	c0	no effect // **Ω** [67]: no change	**§** (our study)	class 1, benign
c.1814T>C	**p.Ile605Thr**	1	–	0,02	c0	no effect		– // class 2, likely benign
c.1817C>T	**p.Pro606Leu**	1	–	0,02	c0	no effect		– // class 2, likely benign
c.1964C>G	**p.Phe655Agr**	3	0.015%	0,02	c0	no effect	[82]: mouse embryonic stem cell-based assay: not pathogenic; [34]: benign; [39]: **OR 1,25** (95% CI 0.62–2.55)	class 1, benign
c.2091A>C	**p.Lys697Asn**	1	–	0,02	c0	no effect		– // class 2, likely benign
c.2162C>G	**p.Pro721Arg**	7	–	0,02	c0	slightly enhancing cryptic acceptor site		– // class 3, VUS
c.2320A>G	**p.Thr774Ala**	7	0.0054%	0,02	c0	no effect		– // class 2, likely benign
c.2348T>G	**p.Val783Gly**	1	0.00090 %	0,02	c0	slightly enhancing cryptic acceptor site		class 2, likely benign
c.2416G>C	**p.Asp806His**	1	0.0048%	0,02	c0	no effect	[34]: benign	class 1, benign
c.2755G>A	**p.Glu919Lys**	1	0.00090 %	0,02	c0	no effect		class 3, VUS // class 2, likely benign
c.2803G>A	**p.Asp935Asn**	NC	0.11%	0,02	c0	enhancing cryptic donor site	[34]: benign	class 1, benign

2879A>G	**p.Lys960Arg**	1	– // **novel**	**0,3**	c0	**new donor site?**		class 3, VUS
c.2926_2927 delinsAT	**p.Ser976Ile**	NC	–	–	c0	no effect		class 2, likely benign
c.2971A>G	**p.Asn991Asp**	NC	3.44%	0,02	c0	no effect	[39]: **OR 0,95** (95% CI 0.90–1.00); **§** (our study)	class 1, benign
c.3055C>G	**p.Leu1019Val**	NC	0.017%	0,02	c0	low probability of new donor site	[34]: benign; [39]: **OR 1,18** (95% CI 0.62–2.26)	class 1, benign
c.3073A>G	**p.Lys1025Glu**	2	0.0099%	0,02	c0	no effect		– // class 2, likely benign
c.3075G>T	**p.Lys1025Asn**	1	–	0,02	c0	no effect		– // class 2, likely benign
c.3211C>T	**p.His1071Tyr**	3	0.0018%	0,02	c0	no effect		– // class 2, likely benign
c.3256A>G	**p.Ile1086Val**	1	0.00082 %	**0,3**	c0	low probability of new donor site		– // class 3, VUS
c.3437A>G	**p.Glu1146Gly**	NC	0.00090 %	0,02	c0	no effect		– // class 2, likely benign
c.3515C>T	**p.Ser1172Leu**	NC	0.084%	0,02	c0	slightly enhancing cryptic acceptor site	[34]: benign // **§** (our study)	class 1, benign
c.3581G>A	**p.Gly1194Asp**	NC	0.011%	0,02	c0	no effect	[34]: benign	class 1, benign
c.3677A>T	**p.Lys1226Ile**	2	–	0,02	c0	no effect		class 3, VUS
c.3814A>G	**p.Met1272Val**	1	0.00100 %	0,02	c0	new acceptor site		– // class 2, likely benign
c.3839A>T	**p.Asp1280Val**	2	0.0062%	0,02	c0	no effect	[34]: benign	class 1, benign
c.3916G>A	**p.Val1306Ile**	1	0.0042%	0,02	c0	no effect	[34]: benign	class 1, benign
c.3941A>G	**p.Lys1314Arg**	1	0.00100 %	0,02	c0	low probability of new acceptor site	[34]: benign	class 1, benign
c.4054G>T	**p.Asp1352Tyr**	1	0.00090 %	0,02	c0	no effect	[34]: likely benign	class 2, likely benign
c.4061C>T	**p.Thr1354Met**	4	0.018%	0,02	c0	no effect	[34]: benign	class 1, benign
c.4090A>C	**p.Ile1364Leu**	1	0.0040%	0,02	c0	no effect		class 1, benign
c.4213A>C	**p.Asn1405His**	1	– // **novel**	0,02	c0	low probability of new acceptor site		– // class 2, likely benign
c.4241C>T	**p.Thr1414Met**	3	0.0056%	0,02	c0	no effect		class 1, benign
c.4258G>T	**p.Asp1420Tyr**	NC	0.78%	0,02	c0	no effect	[34]: benign [39]: **OR 0,86** (95% CI 0.77–0.96); **§** (our study)	class 1, benign
c.4301A>T	**p.Lys1434Ile**	1	0.0018%	0,02	c15	no effect	[34]: likely benign;	class 2, likely benign
c.4567G>A	**p.Gly1523Ser**	1	–	0,02	c0	no effect		– // class 3, VUS
c.4585G>A	**p.Gly1529Arg**	2	0.068%	0,02	c65	no effect	[34]: benign [39]: **OR 1,09** (95% CI 0.73–1.63) // **¥** (our study)	class 1, benign

c.4718G>A	**p.Cys1573Tyr**	1	0.0018%	0,02	c0	no effect		class 2, likely benign
c.4745C>T	**p.Thr1582Ile**	1	–	0,02	c0	no effect		– // class 2, likely benign
c.4784A>G	**p.Gln1595Pro**	1	– // **novel**	0,02	c0	no effect		– // class 2, likely benign
c.4901T>C	**p.Phe1634Ser**	2	0.0032%	0,02	c0	no effect		class 2, likely benign
c.5003C>T	**p.Ala1668Val**	1	– // **novel**	0,02	c0	no effect		– // class 3, VUS
c.5070A>C	**p.Lys1690Asn**	30	0.034%	0,02	c0	no effect	[34]: benign **§** (our study)	class 1, benign
c.5120C>T	**p.Thr1707Ile**	1	–	0,02	c0	low probability of new acceptor site		– // class 3, VUS
c.5191C>T	**p.His1731Tyr**	1	0.0018%	0,02	c0	no effect		– // class 2, likely benign
c.5312G>A	**p.Gly1771Asp**	1	0.044%	0,02	c0	no effect	[34]: benign; [39]: **OR 0,7** (95% CI 0.40–1.23)	class 1, benign
c.5350A>C	**p.Asn1784His**	1	–	0,02	c0	no effect		– // class 2, likely benign
c.5455C>T	**p.Pro1819Ser**	NC	0.025%	0,02	c0	no effect	[34]: benign	class 1, benign
c.5492T>C	**p.Ile1831Thr**	1	0.00090 %	0,02	c0	no effect		– // class 2, likely benign
c.5510T>C	**p.Phe1837Ser**	1	– // **novel**	0,02	c0	no effect		– // class 2, likely benign
c.5552T>G	**p.Ile1851Ser**	4	0.0100%	0,02	c0	slightly enhancing cryptic donor site		class 2, likely benign
c.5669T>C	**p.Met1890Thr**	1	0.00090 %	0,02	c0	no effect		class 2, likely benign
c.5704G>A	**p.Asp1902Asn**	NC	0.0024%	0,02	c0	slightly enhancing cryptic acceptor site		class 1, benign
c.5737T>C	**p.Cys1913Arg**	1	0.0040%	0,02	c0	slightly enhancing cryptic acceptor site		class 2, likely benign
c.5744C>T	**p.Thr1915Met**	NC	2.96%	0,02	c0	no effect	[39]: **OR 1,00** (95% CI 0.94–1.06); **§** (our study)	class 1, benign
c.5785A>G	**p.Ile1929Val**	1	0.011%	0,02	c0	no effect	[34]: benign	class 1, benign
c.5882G>A	**p.Ser1961Asn**	1	0.00090 %	0,02	c0	low probability of new acceptor site		class 2, likely benign
c.5885T>C	**p.Ile1962Thr**	1	0.0024%	0,02	c0	no effect		class 2, likely benign
c.5896C>T	**p.His1966Tyr**	2	0.0024%	0,02	c0	no effect		conflicting: class 3, VUS; class 2, likely benign
c.5960A>G	**p.Gln1987Arg**	1	– // **novel**	0,02	c0	reducing probability of cryptic splice		– // class 3, VUS

c.6100C>T	**p.Arg2034Cys**	27	0.50%	0,02	c0	slightly enhancing cryptic acceptor site	[34]: benign	class 1, benign
c.6223A>C	**p.Lys2075Gln**	2	– // **novel**	0,02	c0	no effect		– // class 3, VUS
c.6289A>G	**p.Thr2097Ala**	2	–	0,02	c0	no effect		– // class 2, likely benign
c.6317T>C	**p.Leu2106Pro**	2	0.019%	0,02	c0	no effect		class 2, likely benign
c.6347A>T	**p.His2116Leu**	1	–	0,02	c0	no effect		– // class 3, VUS
c.6338A>G	**p.Asn2113Ser**	1	0.0040%	0,02	c0	no effect	[34]: benign	class 1, benign
c.6427_6428 delinsAT	**p.Ser2143Ile**	1	– // **novel**	–	c0	no effect		– // class 3, VUS
c.6613G>A	**p.Val2205Met**	9	0.0045%	0,02	c0	no effect		class 3, VUS // class 2, likely benign
c.6650A>G	**p.Lys2217Arg**	1	–	0,02	c0	enhancing cryptic acceptor site		– // class 3, VUS
c.6706G>A	**p.Glu2236Lys**	1	0.0054%	0,02	c0	no effect		class 3, VUS
c.6806T>C	**p.Ile2269Thr**	1	0.00090 %	0,02	c0	no effect		– // class 2, likely benign
c.6821G>T	**p.Gly2274Val**	3	0.083%	0,02	c0	no effect	[34]: likely benign	class 2, likely benign
c.6829C>T	**p.Leu2277Phe**	1	–	0,02	c0	low probability of new acceptor site		– // class 2, likely benign
c.6881A>T	**p.Asp2294Val**	1	– // **novel**	0,02	c0	no effect		– // class 3, VUS
c.7012A>G	**p.Thr2338Ala**	1	– // **novel**	0,02	c0	no effect		– // class 2, likely benign
c.7057G>C	**p.Gly2353Arg**	1	0.0081%	0,02	c0	no effect	[34]: benign; [39]: **OR 1,94** (95% CI 0.58–6.46)	class 1, benign
c.7397C>T	**p.Ala2466Val**	NC	0,6%	0,02	c0	no effect // **Ω** [40]: no aberration; [67]: no change	[39]: **OR 0,64** (95% CI 0.35–1.19); [33]: HDR assay: neutral	class 1, benign
c.7402G>A	**p.Val2468Ile**	NC	–	0,02	c0	no effect		– // class 1, benign
c.7469T>C	**p.Ile2490Thr**	3	0.017%	0,03	c0	no effect	[79]: protein likelihood ratio: neutral; [39]: **OR 1,48** (95% CI 0.49–4.43)	class 1, benign // low risk allele?
c.7505G>A	**p.Arg2502His**	4	0.0063%	0,03	c0	no effect // **Ω** [67]: no change	[79]: protein likelihood ratio: neutral	conflicting: class 3: VUS; class 2, likely benign
c.7544C>T	**p.Thr2515Ile**	NC	0.096%	0,03	c0	no effect	[79]: protein likelihood ratio: neutral, [34]: benign; [39]: **OR 0,9** (95% CI 0.64–1.26)	class 1, benign
c.7633G>A	**p.Val2545Ile**	1	–	0,03	c0	no effect // **Ω** [62]: no aberration	[79]: protein likelihood ratio: uncertain	class 3, VUS

c.7765C>T	**p.Pro2589Ser**	1	–	0,03	c0	no effect		– // class 3, VUS
c.7864A>G	**p.Asn2622Asp**	2	–	**0,29**	c15	slightly enhancing cryptic acceptor site		– // class 3, VUS
c.7928C>G	**p.Ala2643Gly**		0.0045%	**0,64**	c0	new donor site? // **Ω** [72]: like wild type; [67]: no change	[79]: protein likelihood ratio: neutral; [76]: functional assays: inconclusive; [80]: HDR assay: as wild type;	– // class 3, VUS
c.7997G>C	**p.Arg2666Thr**	1	–	0,03	c0	no effect	[79]: protein likelihood ratio: neutral	class 2, likely benign
c.8111C>T	**p.Ser2704Phe**	1	0.0036%	0,03	c0	no effect	[79]: protein likelihood ratio: neutral	class 2, likely benign
c.8123C>T	**p.Thr2708Ile**	1	– // **novel**	0,03	c0	no effect	**novel**	– // class 2, likely benign
c.8149G>T	**p.Ala2717Ser**	NC	0.19%	0,03	c0	slightly enhancing cryptic acceptor site	[79]: protein likelihood ratio: neutral; [33]: HDR assay: neutral; [34]: benign; [39]: **OR 0,8** (95% CI 0,62–0,96)	class 1, benign
c.8182G>A	**p.Val2728Ile**	NC	0.40%	0,03	c0	no effect // **Ω** [67]: no change	[79]: protein likelihood ratio: neutral; [33]: HDR assay: neutral	class 1, benign
c.8187G>T	**p.Lys2729Asn**	4	0.0024%	0,03	c0	enhancing cryptic acceptor site	[76]: functional assays: no effect; [79]: protein likelihood ratio: uncertain; [33]: HDR assay: neutral; [34]: benign; [39]: **OR 2,8** (95% CI 0,29–27,64)	conflicting: class 1, class 2 // **class 3, VUS: low–middle risk allele?**
c.8417C>T	**p.Ser2806Leu**	1	–	**0,29**	c25	enhancing cryptic acceptor site	potentially clinically significant?	class 3, VUS
c.8503T>C	**p.Ser2835Pro**	1	0.010%	0,03	c0	no effect // **Ω** [67]: no change	[79]: protein likelihood ratio: neutral	class 1, benign
c.8567A>C	**p.Glu2856Ala**	14	0.22%	0,03	c0	no effect	[79]: protein likelihood ratio: neutral; [34]: benign; [80]: HDR assay: as wild type, [39]: **OR 0,94** (95% CI 0.78–1.13)	conflicting: class 2, likely benign; class 1, benign
c.8648C>T	**p.Pro2883Leu**	1	– // **novel**	0,03	c0	no effect		– // class 3, VUS
c.8710C>G	**p.Leu2904Val**	1	– // **novel**	**0,29**	c15	low probability of new donor site		– // class 3, VUS
c.8722G>A	**p.Val2908Met**	1	–	0,03	c0	no effect		– // class 3, VUS
c.8723T>G	**p.Val2908Gly**	4	0.0036%	**0,66**	**c35**	no effect	[76]: functional assays: no effect; [79]: protein likelihood ratio: uncertain; [33]: HDR assay: neutral; [34]: likely benign;	class 2, likely benign
c.8850G>T	**p.Lys2950Asn**	1	0.083%	**0,66**	**c35**	no effect // **Ω** [67]: no change	[79]: protein likelihood ratio: neutral; [34]: likely benign; [39]: **OR 0,97** (95% CI 0.72–1.32); [33]: HDR assay: neutral	class 1, benign
c.8851G>A	**p.Ala2951Thr**	33	0.49%	0,03	c0	no effect // **Ω** [67]: no change	[79]: protein likelihood ratio: neutral; [80]: HDR assay: as wild type // **¥** (our study)	class 1, benign
c.8905G>A	**p.Val2969Met**	27	0.046%	0,03	c0	no effect	[79]: protein likelihood ratio: likely deleterious; [33]: HDR assay: neutral;	class 1, benign

							[34]: benign; [39]: **OR 0,87** (95% CI 0.58–1.34)	
c.9038C>T	**p.Thr3013Ile**	NC	0.045%	0,03	c0	no effect // **Ω** [67]: no change	[79]: protein likelihood ratio: neutral; [80]: HDR assay: as wild type,	conflicting: class 1; class 2, likely benign
c.9104A>C	**p.Tyr3035Ser**	2	0.012%	**0,66**	**c55**	no effect // **Ω** [72]: like wild type	[79]: protein likelihood ratio: likely deleterious; [33]: HDR assay: likely neutral; [39]: **OR 2,5** (95% CI 1,0–6,05) // **¥** (our study)	class 3, VUS // low–middle risk allele?
c.9155G>A	**p.Arg3052Gln**	3	0.0036%	**0,66**	**c35**	no effect	[79]: protein likelihood ratio: uncertain; [34]: likely benign; [39]: **OR 1,79** (95% CI 0.33–9.79)	conflicting: class 2; class 3 // low– risk allele?
c.9249A>T	**p.Lys3083Asn**	2	0.0018%	0,03	c0	no effect	[79]: protein likelihood ratio: neutral	class 2, likely benign
c.9275A>G	**p.Tyr3092Cys**	1	0.0071%	**0,81**	**c65**	slightly enhancing cryptic acceptor site // **Ω** [67]: no change	[79]: protein likelihood ratio: likely deleterious; [34]: likely benign; [80]: HDR assay: as wild type, // **¥** (LOVD database)	class 2, likely benign
c.9292T>C	**p.Tyr3098His**	2	0.026%	0,03	c0	no effect	[79]: protein likelihood ratio: uncertain; [80]: HDR assay**:** as wild type**,** [34]: benign; [39]: **OR 0,7** (95% CI 0.35–1.38)	class 1, benign
c.9449C>T	**p.Pro3150Leu**	1	–	**0,81**	**c65**	no effect	[79]: protein likelihood ratio: uncertain	class 3, VUS
c.9458G>C	**p.Gly3153Ala**	2	0.0063%	0,03	c0	slightly enhancing cryptic acceptor site	[79]: protein likelihood ratio: neutral;	class 2, likely benign
c.9509A>G	**p.Asp3170Gly**	1	0.00090 %	0,03	c0	no effect	[79]: protein likelihood ratio: uncertain; [34]: benign; [33]: HDR assay: neutral	class 1, benign
c.9628G>C	**p.Gly3210Arg**	1	– // **novel**	0,02	c0	enhancing cryptic acceptor site		– // class 3, VUS
c.9629G>T	**p.Gly3210Val**	1	– // **novel**	0,02	c0	enhancing cryptic acceptor site		– // class 3, VUS
c.9742A>G	**Met3248Val**	1	– // **novel**	0,02	c0	**new donor site?**	potentially clinically significant	– // class 3, VUS
c.9875C>T	**p.Pro3292Leu**	1	0.0054%	0,3	c0		[43]: P3292L- altering the phosphorylation of BRCA2 by ATM; **potentially clinically significant**	class 3, VUS
c.9975T>G	**p.Phe3325Leu**	1	– // **novel**	0,02	c0	no effect		– // class 2, likely benign
c.10234A>G	**p.Ile3412Val**	NC	0.16%	0,02	c0	no effect		class 1, benign

cDNA – complementary DNA, HGVS – Human Genome Variation Society, NFE – non-Finnish European, GVGD – Grantham Variation and Grantham Deviation, mRNA – messenger RNA, LOVD – Leiden Open Variation Database, IARC – International Agency for Research on Cancer, HDR – homology directed repair, NC – not counted, OR – odds ratio, CI – confidence interval, VUS – variants of uncertain clinical signifikance

Tab. 13. *BRCA2* intronic variants detected in Czech patients classified as benign, likely benign and uncertain significance.

cDNA level (HGVS nomenclature)	Number of families	NFE (gnom)	Splice prediction: Alamut average (MaxEnt/NNSPLICE/SSF)	Ω mRNA analysis // ¥ (co-occurrence in trans with BRCA1 mutation) // § (detected in cancer free controls) //& (multifactorial likelihood ratio) // [reference]: other information	LOVD-IARC class [4] // our class
c.-33T>C	1	–	not predicted		– // class 2, likely benign
c.-32T>C	1	– // **novel**	+1,1		– // class 2, likely benign
c.-39-12_-39-10del	1	–	**−12,5%**		class 3, VUS
c.-11C>T	1	0.0047%	not predicted		class 1, benign
c.68-7T>A	21	0.24%	−25,6%	**Ω** [72]: Enhanced Ex 3 skipping; [83]: increased level of the delta3-transcript; [84]: risk of breast cancer **OR 1.03** (95% CI 0.86–1.24) // **§** (our study)	class 2, likely benign,
c.68-3T>G	1	–	**−99,8%**	**Ω** (our study): in-frame deletion of Ex 3 r.68_316del	– // class 3, VUS
c.317-22C>G	NC	–	not predicted	LOVD database: prevalence in normal controls in Austria	class 2, likely benign,
c.425+6A>G	1	– // **novel**	low probability of new donor site		– // class 3, VUS
c.426-37T>A	NC	–	not predicted	**Ω** [58]: like wild type	– // class 2, likely benign
c.475+22T>C	1	– // **novel**	not predicted		– // class 3, VUS
c.476-41A>T	1	– // **novel**	not predicted		– // class 3, VUS
c.476-16_476-15del	1	– // **novel**	not predicted		– // class 3, VUS
c.476-31C>T	1	– // **novel**	not predicted		– // class 3, VUS

c.516+14C>T	1	0.0063%	0.0063%	**Ω** [67]: no change; [73]: no aberration	class 1, benign
c.682-86delA	1	– // **novel**	not predicted		– // class 2, likely benign
c.682-34T>C	1	– // **novel**	not predicted		– // class 3, VUS
c.682-32A>G	NC	0.039%	not predicted		class 2, likely benign
c.6841+33A>G	NC	0.0018%	low probability of new acceptor site		– // class 3, VUS
c.6841+44dupT	1	0.00090%	slightly enhancing cryptic acceptor site		– // class 3, VUS
c.6842-29A>T	1	– // **novel**	not predicted		– // class 3, VUS
c.6937+38A>C	1	– // **novel**	enhancing cryptic acceptor and donor site		– // class 3, VUS
c.6938-35dupT	1	– // **novel**	not predicted		– // class 3, VUS
c.6938-15delA	1	–	−2,8%		– // class 3, VUS
c.7435+6G>A	1	0.0048%	+20,9%	**Ω** [58]: wild type; [40]: no aberration	class 2, likely benign
7435+54G>A	1	0.0067%	slightly enhancing cryptic acceptor site		class 1, benign
c.7617+15A>G	1	– // **novel**	not predicted		– // class 3, VUS
c.7618-22T>C	1	0.0032%	not predicted		– // class 3, VUS
c.7806-14T>C	NC	51.83%	−4,5%		class 1, benign
c.7976+23C>T	3	0.025%	not predicted	**Ω** [39]: no change	class 2, likely benign
c.7976+35C>A	1	0.023%	not predicted	**Ω** [74]: no change	class 2, likely benign

c.8332-29T>C	1	– // **novel**	not predicted		– // class 3, VUS
c.8633-28T>G	1	– // **novel**	low probability of new acceptor site		– // class 3, VUS
c.9257-16T>C	NC	0.87%	not predicted	**Ω** [19]: like wild type; [39]: no change; [58]: wild type	class 1, benign
c.9502-12T>G	2	**0.022%**	**–20,1 %**	**Ω** [85]: Ex 26 skipping r.9502_9648del p.(Asn3181_Leu3216del); [49]: weak effect: Ex 26 skipping (**8%**): role in BC is questionable	conflicting: class 2, likely benign or class 3, VUS
c.9649-37A>C	NC	0.00090%	slightly enhancing cryptic acceptor site	// **¥** (our study)	class 3, VUS // class 2, likely benign
c.9649-20C>T	NC	0.014%	+2%	**Ω** [39]: no change	class 2, likely benign

cDNA – complementary DNA, HGVS – Human Genome Variation Society, NFE – non-Finnish European , mRNA – messenger RNA, LOVD – Leiden Open Variation Database, IARC – International Agency for Research on Cancer, NC – not counted, OR – odds ratio, CI – confidence interval, VUS – variants of uncertain clinical significance