K3326X- An HBOC Gene Variant

Subjects: Oncology
Contributor: Michael Tainsky, SCOTT BAUGHAN

K3326X is a rare truncating variant on the C-terminus of BRCA2.

Keywords: hereditary breast and ovarian cancer syndrome ; HBOC ; hereditary breast ovarian and pancreatic cancer syndrome ; HBOPC ; BRCA2 ; K33326X

1. The K3326X Mutation

One of the most prolifically studied and controversial C-terminal mutations of BRCA2 is K3326X, in which a lysine is mutated, resulting in early truncation of the protein with final 93 amino acids being lost. The lost domains include Thr3387, essential for the release of Rad51, the most C-terminal nuclear localization signal, and a portion of the distal Rad51-ssDNA as well as the DMC1 binding domains[1][2][3][4][5][6]. Recent evidence indicates that BRCA2 may play a key role in the repair and recovery of the cell from stalled replication forks and that exon 27, in which the K3326X mutation occurs, is essential in this function[7][8][9][10][11].

2. K3326X in Gynecologic Cancers

K3326X is well-investigated with regard to gynecologic cancer risk. A landmark study using over 70,000 cancer cases and 80,000 controls showed increased risk of breast (OR = 1.28), invasive ovarian (OR = 1.26), serous ovarian (OR = 1.46), and ER-negative breast cancer (OR = 1.5) in K3326X mutation carriers. Additionally, for individuals with a second mutation in BRCA1, there was an increased risk of ovarian cancer, showcasing the potential for additional pleiotropic events with this variant[12]. As Arbustini and colleagues note in a follow-up to this article, these new data change the knowledge paradigm regarding K3326X. They recommend expanded counseling for women with K3326X and hypothesize that these patients may be good candidates for PARP inhibitor therapy due to BRCA2 dysfunction[13]. The case study of an Italian family further implicated the K3326X variant in the development of early onset cancer and recommends that the K3326X mutation be evaluated with other pathogenic mutations[14]. Data from mouse models further supports this idea: mice lacking the final exon of BRCA2 are viable but show increased tumor incidence compared to normal littermates[15]. Finally, a 2017 cohort study found higher than expected prevalence of the K3326X allele in individuals with a history of familial cancer and a personal history of ovarian cancer (OR = 4.95, p = 0.01)[16]. Citing additional data from the Ovarian Cancer Association Consortium, the authors hypothesize that BRCA2 K3326X is likely a low risk allele for ovarian cancer with an OR of 1.22–9.3.

3. K3326X in Pancreatic Cancer

In addition to gynecological cancers, BRCA2 K3326X was investigated in the context of familial pancreatic cancer though the study of 250 patients with sporadic pancreatic cancer, 114 patients with familial pancreatic adenocarcinoma, 115 spouses of patients with pancreatic cancer as an additional environmental control, and a second control group of 125 patients with no cancer history undergoing cholecystectomy for other reasons. In individuals with familial pancreatic cancer, K3326X was present at a much higher frequency of 5.6%, compared to 1.2% in controls (OR = 4.84, 95% CI 1.27–18.55, $p < 0.01$). There was no association between the mutation and sporadic pancreatic cancer (OR: 2.37, 95% CI 0.61–9.27)[17]. Similarly, K3326X was investigated in a case control study of 5626 control subjects and 2935 sporadic cases of pancreatic ductal adenocarcinoma. The authors found an association with the K3326X variant with an OR = 1.78 (95% CI = 1.26–2.52, $p = 1.19 \times 10^{-3}$). The odds ratio remained significant when controlling for family history. K3326X was not associated with pancreatic ductal adenocarcinoma with onset before 50 years of age (OR = 1.87, 95% CI = 0.93–3.74, p = 0.08)[18].

4. K3326X in Environmental Cancers

BRCA2 K3326X has been intensively studied in relation to environmental cancers, with a large-scale study of over 43,000 cancer patients and over 370,000 controls reported increased risk of small cell lung cancer (OR = 2.06) and squamous cell skin cancer (OR = 1.69), indicating that individuals with this SNP are vulnerable to cancers with environmental genotoxic risk factors[19]. This study did not find an association between K3326X and upper-aero digestive tract cancers (oral cavity, oropharynx, larynx/hypopharynx, and esophagus) among Icelandic subjects. This is in contrast to an earlier study that found associated risk between K3326 and upper-aero digestive tract cancers among European, Latin American, and Indian populations[20]. The association between K3326X and cutaneous squamous cell carcinoma was confirmed by a recent large meta-analysis which embodied six international cohorts including 19,149 squamous cell carcinoma cases and 680,049 controls[21]. Evidence for the relationship of BRCA2 K3326X and environmental cancers holds up well across diverse populations. A study conducted of 190 Turkmens and 1373 controls found an increased prevalence of the variant in esophageal squamous cell carcinoma with an OR of 3.38 (95% CI = 1.97–6.91, p = 0.0002)[22]. In a study of Chinese esophageal cancer, the K3326X mutation was detected in just one case, suggesting that K3326X is uncommon in Henan and Hong Kong ESCC patients (wide variation exists for this mutation across populations)[23]. A genome-wide association study of 159 cases and 2707 controls including the genotyping of 1476 non-synonymous SNPs in 871 candidate genes found increased prevalence of BRCA2 K3326X in lung cancer of unspecified type (OR = 1.72, 95% CI 1.15–2.57, p = 0.0075)[24]. BRCA2 K3326X was assessed in a case control study of 2634 breast cancer cases from familial cancer clinics and 1996 non-cancer population controls. BRCA2 K3326X was overrepresented in cases with an OR of 1.53 (95% CI 1.00–2.34, p = 0.047) [25]. Additionally, a large-scale study of European populations for lung cancer risk assessed 11,348 cases and 15,861 controls from the 1000 genomes project, with a follow up confirmation of an additional 10,246 cases and 38,295 controls. The authors found BRCA2 K3326X to be significantly associated with lung cancer (OR = 2.47, $p = 4.74 \times 10^{-20}$)[26]. In this study, BRCA2 K3326X was more significantly associated with lung cancer of the squamous variety than lung adenocarcinoma (OR = 2.47, $p = 4.74 \times 10^{-20}$ and OR = 1.47, $p = 4.66 \times 10^{-4}$, respectively). The authors note that the association with squamous cancers and BRCA2 mutations is reflective of the higher mutation frequency in squamous cancers compared to adenocarcinoma. The association was not present in nonsmokers who made up a smaller portion of the cases, a limitation of the study. Finally, the study investigated the hypothesis that the K3326X variant was in linkage disequilibrium with another deleterious variant, but found no evidence of another causative mutation in those cases.

The conclusions of the above studies are briefly compiled in Table 1.

Table 1. K3326X cancer associations: The studies of BRCA2-K3326X presented in this review are summarized here, in reference order, and with cancer type, p-value, study population size, and recommendations from the authors of the study. All studies found significant odds ratios above 1 but less than 5. The authors varied in their assessment of the variant but most recommended further investigation.

Reference	Cancer Type, [Odds Ratio]	p-Value	Population (Number)	Recommendation
[12][13]	Breast (1.28)	5.9×10^{-6},	70,000 cases	Expanded counseling
	Invasive ovarian (1.26)	3.8×10^{-3},	80,000 controls	
	Serous ovarian (1.46)	3.4×10^{-5},	-	
	ER-negative breast (1.5)	4.1×10^{-5}	-	
[14]	Early onset	N/A	Small (case study)	Further evaluation
[16]	Ovarian cancer (4.95)	<0.01	48	Treat as low risk pathogenic
[17]	Familial pancreatic (4.84)	<0.01	114 familial pancreatic cancer 115 environmental control 125 controls	None

Reference	Cancer Type, [Odds Ratio]	*p*-Value	Population (Number)	Recommendation
[18]	Pancreatic ductal adenocarcinoma (1.78)	0.0012	2935 cases 5626 controls	None
[19]	Small cell lung (2.06) Squamous skin (1.69) Lung cancer (1.54) All cancers (1.23)	9×10^{-4}, 4.2×10^{-4}, 1.2×10^{-4}, 1.6×10^{-5}	43,641 cases 370,971 controls	Vulnerability to environmental cancers
[20]	Upper aero digestive tract (2.53)	3×10^{-10}	5942 cases 8086 controls	Warrants further investigation
[21]	Cutaneous Squamous cell carcinoma (2.29)	1.0×10^{-6}	Meta-analysis of 19,149 cases, 680,049 controls	Variant likely implicated in skin cancer development
[22]	Esophageal squamous cell carcinoma (3.38)	0.0002	190 cases 1373 controls	None
[23]	Esophageal cancer	N/A	2276 cases 2058 controls	Rare variant in Henan and Hong Kong ESCC patients
[24]	Lung Cancer (1.72)	0.0075	1529 cases 2707 controls	Low penetrance alleles contribute to risk
[25]	Breast Cancer (1.53)	0.047	2634 cases 1996 controls	Variant is not neutral, should be included in SNP panels for evaluating risk
[26]	Squamous lung cancer (2.47)	4.7×10^{-20}	10,246 cases	K3326X may have a direct effect on lung cancer development

References

1. Guidugli, L.; Carreira, A.; Caputo, S.M.; Ehlen, A.; Galli, A.; Monteiro, A.N.; Neuhausen, S.L.; Hansen, T.V.; Couch, F.J.; Vreeswijk, M.P.G.; et al. Functional Assays for Analysis of Variants of Uncertain Significance inBRCA2. Hum. Mutat. 2014, 35, 151–164. [Google Scholar] [CrossRef] [PubMed]
2. Roy, R.; Chun, J.; Powell, S.N. BRCA1 and BRCA2: Different roles in a common pathway of genome protection. Nat. Rev. Cancer 2011, 12, 68–78. [Google Scholar] [CrossRef] [PubMed]
3. Martinez, J.S.; Baldeyron, C.; Carreira, A. Molding BRCA2 function through its interacting partners. Cell Cycle 2015, 14, 3389–3395. [Google Scholar] [CrossRef] [PubMed]
4. Davies, O.R.; Pellegrini, L. Interaction with the BRCA2 C terminus protects RAD51–DNA filaments from disassembly by BRC repeats. Nat. Struct. Mol. Biol. 2007, 14, 475–483. [Google Scholar] [CrossRef]
5. Rajagopalan, S.; Andreeva, A.; Rutherford, T.J.; Fersht, A.R. Mapping the physical and functional interactions between the tumor suppressors p53 and BRCA2. Proc. Natl. Acad. Sci. USA 2010, 107, 8587–8592. [Google Scholar] [CrossRef] [PubMed]
6. Martinez, J.S.; Von Nicolai, C.; Kim, T.; Ehlén, Å.; Mazin, A.V.; Kowalczykowski, S.C.; Carreira, A. BRCA2 regulates DMC1-mediated recombination through the BRC repeats. Proc. Natl. Acad. Sci. USA 2016, 113, 3515–3520. [Google Scholar] [CrossRef]
7. Costanzo, V. Brca2, Rad51 and Mre11: Performing balancing acts on replication forks. DNA Repair 2011, 10, 1060–1065. [Google Scholar] [CrossRef]
8. Kolinjivadi, A.M.; Sannino, V.; De Antoni, A.; Técher, H.; Baldi, G.; Costanzo, V. Moonlighting at replication forks - a new life for homologous recombination proteins BRCA1, BRCA2 and RAD51. FEBS Lett. 2017, 591, 1083–1100. [Google Scholar] [CrossRef]

9. Chandramouly, G.; Willis, N.; Scully, R. A protective role for BRCA2 at stalled replication forks. Breast Cancer Res. 2011, 13, 314. [Google Scholar] [CrossRef]

10. Kim, T.M.; Son, M.Y.; Dodds, S.G.; Hu, L.; Hasty, P. Deletion of BRCA2 exon 27 causes defects in response to both stalled and collapsed replication forks. Mutat. Res. Mol. Mech. Mutagen. 2014, 767, 66–72. [Google Scholar] [CrossRef]

11. Schlacher, K.; Christ, N.; Siaud, N.; Egashira, A.; Wu, H.; Jasin, M. Double-Strand Break Repair-Independent Role for BRCA2 in Blocking Stalled Replication Fork Degradation by MRE11. Cell 2011, 145, 529–542. [Google Scholar] [CrossRef] [PubMed]

12. Meeks, H.D.; Song, H.; Michailidou, K.; Bolla, M.K.; Dennis, J.; Wang, Q.; Barrowdale, D.; Frost, D.; McGuffog, L.; Ellis, S.; et al. BRCA2 Polymorphic Stop Codon K3326X and the Risk of Breast, Prostate, and Ovarian Cancers. J. Natl. Cancer Inst. 2015, 108, djv315. [Google Scholar] [CrossRef] [PubMed]

13. Arbustini, E.; Sgarella, A.; Ferrari, A.; Grasso, D.; Cassani, C.; Lucioni, M.; Di Giulio, G.; Grasso, M. RE: BRCA2 Polymorphic Stop Codon K3326X and the Risk of Breast, Prostate, and Ovarian Cancers. J. Natl. Cancer Inst. 2016, 108, djw172. [Google Scholar] [CrossRef]

14. Palmirotta, R.; Lovero, D.; Stucci, L.S.; Silvestris, E.; Quaresmini, D.; Cardascia, A.; Silvestris, F. Double Heterozygosity for BRCA1 Pathogenic Variant and BRCA2 Polymorphic Stop Codon K3326X: A Case Report in a Southern Italian Family. Int. J. Mol. Sci. 2018, 19, 285. [Google Scholar] [CrossRef] [PubMed]

15. A McAllister, K.; Bennett, L.M.; Houle, C.D.; Ward, T.; Malphurs, J.; Collins, N.K.; Cachafeiro, C.; Haseman, J.; Goulding, E.H.; Bunch, D.; et al. Cancer susceptibility of mice with a homozygous deletion in the COOH-terminal domain of the Brca2 gene. Cancer Res. 2002, 62, 990–994. [Google Scholar]

16. Lopes, J.L.; Dyson, G.; Levin, N.K.; Chaudhry, S.; Rosati, R.; Kalpage, H.; Wernette, C.; Petrucelli, N.; Simon, M.S.; Tainsky, M.A. Reanalysis of BRCA1/2 negative high risk ovarian cancer patients reveals novel germline risk loci and insights into missing heritability. PLoS ONE 2017, 12, e0178450. [Google Scholar] [CrossRef]

17. Martin, S.T.; Matsubayashi, H.; Rogers, C.D.; Philips, J.; Couch, F.J.; Brune, K.; Yeo, C.J.; E Kern, S.; Hruban, R.H.; Goggins, M. Increased prevalence of the BRCA2 polymorphic stop codon K3326X among individuals with familial pancreatic cancer. Oncogene 2005, 24, 3652–3656. [Google Scholar] [CrossRef]

18. Obazee, O.; Archibugi, L.; Andriulli, A.; Soucek, P.; Małecka-Panas, E.; Ivanauskas, A.; Johnson, T.; Gazouli, M.; Pausch, T.; Lawlor, R.T.; et al. Germline BRCA2 K3326X and CHEK2 I157T mutations increase risk for sporadic pancreatic ductal adenocarcinoma. Int. J. Cancer 2019, 145, 686–693. [Google Scholar] [CrossRef]

19. Rafnar, T.; Sigurjonsdottir, G.R.; Stacey, S.N.; Halldorsson, G.H.; Sulem, P.; Pardo, L.M.; Helgason, H.; Sigurdsson, S.T.; Gudjonsson, T.; Tryggvadottir, L.; et al. Association of BRCA2 K3326* With Small Cell Lung Cancer and Squamous Cell Cancer of the Skin. J. Natl. Cancer Inst. 2018, 110, 967–974. [Google Scholar] [CrossRef]

20. Delahaye-Sourdeix, M.; Anantharaman, D.; Timofeeva, M.N.; Gaborieau, V.; Chabrier, A.; Vallée, M.P.; Lagiou, P.; Holcátová, I.; Richiardi, L.; Kjaerheim, K.; et al. A Rare Truncating BRCA2 Variant and Genetic Susceptibility to Upper Aerodigestive Tract Cancer. J. Natl. Cancer Inst. 2015, 107. [Google Scholar] [CrossRef]

21. Sarin, K.Y.; Lin, Y.; Daneshjou, R.; Ziyatdinov, A.; Thorleifsson, G.; Rubin, A.; Pardo, L.M.; Wu, W.; Khavari, P.A.; Uitterlinden, A.G.; et al. Genome-wide meta-analysis identifies eight new susceptibility loci for cutaneous squamous cell carcinoma. Nat. Commun. 2020, 11, 1–8. [Google Scholar] [CrossRef] [PubMed]

22. Akbari, M.R.; Malekzadeh, R.; Lepage, P.; Roquis, D.; Sadjadi, A.R.; Aghcheli, K.; Yazdanbod, A.; Shakeri, R.; Bashiri, J.; Sotoudeh, M.; et al. Mutations in Fanconi anemia genes and the risk of esophageal cancer. Qual. Life Res. 2011, 129, 573–582. [Google Scholar] [CrossRef] [PubMed]

23. Ko, J.M.-Y.; Ning, L.; Zhao, X.; Chai, A.W.Y.; Lei, L.C.; Choi, S.S.A.; Tao, L.; Law, S.; Kwong, A.; Lee, N.P.; et al. BRCA2 loss-of-function germline mutations are associated with esophageal squamous cell carcinoma risk in Chinese. Int. J. Cancer 2020, 146, 1042–1051. [Google Scholar] [CrossRef] [PubMed]

24. Rudd, M.F.; Webb, E.L.; Matakidou, A.; Sellick, G.S.; Williams, R.D.; Bridle, H.; Eisen, T.; Houlston, R.S. Variants in the GH-IGF axis confer susceptibilityto lung cancer. Genome Res. 2006, 16, 693–701. [Google Scholar] [CrossRef] [PubMed]

25. Thompson, E.R.; Gorringe, K.; Rowley, S.M.; Li, N.; McInerny, S.; Wong-Brown, M.; Devereux, L.; Li, J.; Trainer, A.H.; Mitchell, G.; et al. Reevaluation of the BRCA2 truncating allele c.9976A > T (p.Lys3326Ter) in a familial breast cancer context. Sci. Rep. 2015, 5, 14800. [Google Scholar] [CrossRef] [PubMed]

26. Wang, Y.; McKay, J.D.; Rafnar, T.; Wang, Z.; Timofeeva, M.N.; Broderick, P.; Zong, X.; Laplana, M.; Wei, Y.; Han, Y.; et al. Rare variants of large effect in BRCA2 and CHEK2 affect risk of lung cancer. Nat. Genet. 2014, 46, 736–741.