Evaluation of the role of KPNA2 mutations in breast cancer prognosis using bioinformatics datasets

Layla Alnoumas1, Lisa van den Driest1, Zoe Apczynski1, Alison Lannigan2, Caroline H. Johnson3, Nicholas J.W. Rattray1* and Zahra Rattray1*

Abstract
Breast cancer, comprising of several sub-phenotypes, is a leading cause of female cancer-related mortality in the UK and accounts for 15% of all cancer cases. Chemoresistant sub phenotypes of breast cancer remain a particular challenge. However, the rapidly-growing availability of clinical datasets, presents the scope to underpin a data-driven precision medicine-based approach exploring new targets for diagnostic and therapeutic interventions. We report the application of a bioinformatics-based approach probing the expression and prognostic role of Karyopherin-2 alpha (KPNA2) in breast cancer prognosis. Aberrant KPNA2 overexpression is directly correlated with aggressive tumour phenotypes and poor patient survival outcomes. We examined the existing clinical data available on a range of commonly occurring mutations of KPNA2 and their correlation with patient survival.

Our analysis of clinical gene expression datasets show that KPNA2 is frequently amplified in breast cancer, with differences in expression levels observed as a function of patient age and clinicopathologic parameters. We also found that aberrant KPNA2 overexpression is directly correlated with poor patient prognosis, warranting further investigation of KPNA2 as an actionable target for patient stratification or the design of novel chemotherapy agents.

In the era of big data, the wealth of datasets available in the public domain can be used to underpin proof of concept studies evaluating the biomolecular pathways implicated in chemotherapy resistance in breast cancer.

Keywords: Breast cancer, KPNA2, Karyopherins, mRNA expression, Mutational signatures

Introduction
Breast cancer is the most commonly-diagnosed, and leading cause of cancer-related mortality worldwide among women with an estimate of 2.3 million new cases in 2020 [1, 2]. Breast cancer represents a heterogeneous group of diseases classified across several sub-phenotypes according to their anatomical location and gene expression profile.

Despite significant advancements in developing new treatments for breast cancer, the incidence of breast cancer in women continues to rise proportionally with age, posing a significant global public health challenge [3]. Current standard of care in breast cancer treatment involves surgery, radiotherapy, endocrine-based therapies, chemotherapies or biologicals, or a combination of these therapeutic interventions. From a diagnostic perspective, mammography remains one of the main approaches for detecting breast cancer. However, patients are often diagnosed during later stages of breast cancer with the potential to adversely impact patient clinical prognosis and outcomes. Therefore,
the recent years have seen a significant growth in novel surrogate biomarker research for diagnostic, prognostic and therapeutic interventions. Current routine stratification for breast cancer treatment is based on the hormonal status (oestrogen, progesterone and human epidermal growth receptor-2) or more recently, genetic biomolecular signatures classifying breast cancers according to intrinsic subtypes (e.g. basal, and luminal A and B) [4].

Karyopherin alpha 2 (KPNA2), a member of the Karyopherin family and an adaptor protein, is a component of the nuclear import pathway machinery involved in the nucleocytoplasmic transport of molecules involved in cell division, transcription, and DNA repair. Aberrant amplification of KPNA2 expression in cancer has been implicated in the pathogenic mis-localization of substrate proteins, resulting in tumorigenesis and conferring an aggressive sub-phenotype [5]. KPNA2 over-expression has been correlated with poor patient outcomes in a number of malignancies including glioblastoma [6], colon [7], hepatocellular carcinoma [8], ovarian [9] and breast [10–12] cancers. In breast cancer, KPNA2 expression is correlated with a lower abundance of DNA repair proteins including CHK1, UBC9, PIA51, BRCA1, RAD51 and γH2AX in cell nuclei [12]. Moreover, the incidence of KPNA2 overexpression has correlated with oestrogen receptor-negative (ER-) status [12, 13] and rapidly proliferating subtypes, specifically basal-like tumours [14].

With increasing reports of KPNA2 involvement in several cancer types [6, 7, 9, 15] and significant advancements in precision medicine technologies, coupled to extensive biobanking and electronic curation of patient metadata, the scope exists to interrogate the correlation between KPNA2 expression, breast cancer phenotype and patient prognosis.

Dysregulation of mRNA expression levels of KPNA2 in human breast cancer and its association with breast cancer prognosis has not been further investigated. A cohort by AlShareeda et al. correlated tumours over-expressing KPNA2 with poor patient prognosis and a larger tumour size [12]. Other recent studies have evaluated significant KPNA2 expression in breast cancer compared to normal samples [16–18]. In this study, we extensively investigate the effect of KPNA2 in breast cancer patients on specific prognosis outcomes using a range of bioinformatics tools. We analyzed the mRNA expression patterns and mutations of KPNA2 in patients with breast cancer from the vast number of gene expression data available within the public domain, to identify expression patterns and the potential prognostic value of KPNA2 in human breast cancer.

Materials and methods

Data retrieval

**cBioPortal** (https://www.cbioportal.org/) is an open access resource for cancer genomics that was originally developed by Memorial Sloan Kettering Cancer Center [19]. In this study cBioPortal was used to query the incidence and types of KPNA2 mutations occurring in breast cancer as a function of tumour clinicopathologic parameters.

**COSMIC** (Catalogue of Somatic Mutations in Cancer (www.sanger.ac.uk)) is a tool for studying the influence of somatic mutations in all cancers and assessing drug-gability of targets incorporation with chEMBL, which is maintained by the European Molecular Biology Laboratory. Using this resource, we identified over 500 KPNA2-related mutations, specifying the amino acid point mutation position and mutation type, and their classification as missense or insertion.

**Oncomine** (https://www.oncomine.org/resource/login.html) Analysis of KPNA2 mRNA expression patterns was conducted using the following parameter selections: Gene- KPNA2, differential analysis- cancer vs. normal analysis, cancer type-breast cancer; and data type- mRNA. A two-fold change, a P-value corresponding to 1E-4 and a top 10% gene rank were selected as thresholds for this analysis. The same parameters were applied to the analysis of gene co-expression analyses. All statistical analyses and parameters were directly exported from Oncomine.

**Prognoscan** (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html) [20] is a resource for performing meta-analysis of the prognostic role of mutations occurring in cancer through incorporating gene expression studies from multiple sources such as the Gene Expression Omnibus (GEO—www.ncbi.nlm.nih.gov/gds) and reports from individual labs [21]. Prognoscan combines expression data with clinical outcomes, which enables the evaluation of potential biomarkers and their role in cancer prognosis. In this study Prognoscan was used to assess the correlation between KPNA2 mRNA expression levels and patient prognostic endpoints for breast cancer. Output generated and exported from Prognoscan include P-values (Cox), hazard ratios and confidence intervals across breast cancer datasets available. Data available for the 201088_at KPNA2 reporter was selected for the generation of Forest plots.

**Kaplan–Meier Plotter (Kmplot)** (http://kmplot.com/analysis/index.php?P=service) [22] uses gene expression data from GEO datasets and through integration will clinical data, generates Kaplan–Meier plots across multiple prognostic outcomes. Using this tool it is possible to restrict the selection to patients with specific breast cancer sub-phenotypes, enabling the selection of
inclusion and exclusion criteria. For the purposes of this study, the prognostic value of KPNA2 was studied across all breast cancer types, and as a function of each intrinsic molecular subtype (St. Gallen definitions were used) [23]. For all survival analyses, the auto select best cut-off was used to display the $P$-value (log-rank) and false-discovery rate (FDR) for each plot and the probe ID (201088_at) of KPNA2 reporter was selected for all searches.

**Breast Cancer Gene-Expression Miner v4.6** [24] ([http://bcgenex.ico.unicancer.fr/BC-GEM/GEM-Accueil.php?js=1](http://bcgenex.ico.unicancer.fr/BC-GEM/GEM-Accueil.php?js=1)) is a breast cancer statistical mining tool providing information on gene expression and prognostic implications of gene expression profiles in breast cancer. Moreover, the correlation between multiple genes, and their association with breast cancer can be elucidated using this tool [25]. Briefly, KPNA2 expression patterns in all breast cancers were examined (RNA-seq, all platforms) and endpoint events (overall survival, disease-free survival) classified according to sub-phenotypes. Gene ontology and exhaustive gene correlations were also studied across all breast cancer groups as a function of intrinsic molecular subtype and hormone receptor expression profile.

**Statistical analysis**
Comparisons of KPNA2 mRNA expression levels performed between breast cancer and healthy breast tissue (fold-change) was performed in Oncomine using a t-test. For comparisons between breast cancer patient subsets in Geneminer, a Welch test was used to compare differences in KPNA2 mRNA expression. To analyze the prognostic value of KPNA2 using Kaplan–Meier plot (KMPlot), $P$-values from log-rank analysis were used to compare prognostic endpoints between patient cohorts using in-built algorithms on the webpage. Prognostic data obtained from PrognoScan was selected according to the calculated Cox $P$-values and corresponding Hazard ratios (95% confidence interval) for various endpoints (overall survival, disease-free survival, disease-free metastatic survival, and relapse-free survival) that were subsequently plotted and visualized with a Forest plot. Unless otherwise stated, a $P < 0.05$ was deemed as statistically significant for all comparisons.

**Results**

**KPNA2 mutations in breast cancer**
Genetic alterations impacting KPNA2 in breast cancer were analyzed using cBioPortal and COSMIC databases. Querying a combined total of 4,065 samples across five studies in cBioportal, the frequency of KPNA2 gene alterations differed across each study queried (Table 1). The percentage of samples with somatic mutations in KPNA2 were 0.2% of the KPNA2-related duplicate mutations, corresponding to 8 missense substitutions and one in-frame deletion in patients with multiple samples (see supplementary information). Amplification of KPNA2 expression was the most frequently observed alteration across all studies examined.

As a validation step, patterns of KPNA2 expression were studied across 39,619 cancer samples in COSMIC. These analyses revealed that 341 out of 2,612 breast cancer samples contained seven KPNA2 amino acid changes characterized as missense mutations. Six of the seven mutations identified in COSMIC were identical to those found in cBioPortal. In the case of COSMIC, no deletion mutations were found in the KPNA2 sequence, but an additional missense mutation (A364V) was present (Table 2). Thus, cBioPortal is a useful tool for evaluating expression patterns in breast cancer and verifying KPNA2-associated mutations.

After confirming identified mutations within BioMuta (NIH) ([https://hive.biochemistry.gwu.edu/biomuta/proteinview/P52292](https://hive.biochemistry.gwu.edu/biomuta/proteinview/P52292)), results between all databases were unified using an international protein nomenclature based on the Human Genome Variation Society (HGVS). For descriptions of the sequence variants, see Table 3. Missense, substitution or deletion protein mutations are formatted as described by HGVS to better communicate our results for future clinical findings [32].

| Study                                | Percent of total cases (Proportion of cases available in dataset) | Frequency                          |
|--------------------------------------|------------------------------------------------------------------|------------------------------------|
| The Metastatic Breast Cancer Project [26] | 15.6% (37/237)                                                   | Mutation (1.27%, $n=3$)            |
| Metastatic Breast Cancer (INSERM) [27] | 7.87% (17/216)                                                   | Amplification (14.4%, $n=34$)      |
| Breast Cancer (Metabric) [28–30]      | 7.55% (164/2173)                                                 | Amplification (7.87%, $n=17$)      |
| TCGA Pan-cancer Atlas [31]            | 6.46% (70/1084)                                                  | Deep Deletion (0.05%, $n=1$)       |
|                                      |                                                                  | Mutation (0.55%, $n=6$)            |
|                                      |                                                                  | Amplification (5.9%, $n=64$)       |
Next, we assessed the correlation between the amplification of KPNA2 mRNA expression levels in breast cancer tumours compared to matched healthy breast tissue using Oncomine. Findings from these comparisons across breast cancer intrinsic molecular subtypes and corresponding fold-changes are presented in Table 4.

Our analysis of fold-change data show that within breast cancer datasets available on Oncomine, KPNA2 frequently was ranked in the top 7% of genes altered in breast cancer with significant fold-changes observed across all studies relative to adjacent breast cancer tissue. Across all breast cancer subtypes examined, at
least a positive two-fold increase (with a corresponding \( P \)-value < 0.05) was observed in \( KPNA2 \) mRNA expression levels between healthy and breast cancer tissue, indicating \( KPNA2 \) overexpression across various breast cancer types.

Next, we performed a search of the patterns of \( KPNA2 \) mRNA expression in breast cancer using Oncomine, cBioPortal and Geneminer toolsets. Analysis of the datasets available on these resources indicated differential \( KPNA2 \) expression levels as a function of clinicopathological parameters (Fig. 1).

Analysis of \( KPNA2 \) expression level patterns across multiple toolsets shows a varied \( KPNA2 \) expression and mutational profile as a function of clinicopathological parameters. The incidence of \( KPNA2 \) genetic alterations occurred more frequently in patients with positive ER status (Fig. 1D), whereas higher \( KPNA2 \) mRNA levels appeared in patients with negative hormone receptor status (Fig. 1A and B). Relative to normal breast-like tissue, mRNA expression levels of \( KPNA2 \) are significantly elevated across all molecular subtypes. Across Geneminer and Oncomine databases, \( KPNA2 \) amplification occurred most frequently in patients aged < 40 years in comparison to postmenopausal patients (see supplementary information, Welch’s \( P < 0.0001 \), GeneMiner). We also compared \( KPNA2 \) expression profiles across different breast cancer subtypes that included carcinoma, invasive ductal carcinoma and adenocarcinoma. \( KPNA2 \) amplification occurred in patients with invasive ductal carcinoma

![Fig. 1](image-url)  
*KPNA2* mRNA expression varies as a function of breast cancer clinicopathologic parameters. Bee swarm plots of *KPNA2* mRNA expression levels as a function of combined oestrogen (ER) and progesterone (PR) receptor status (A) and HER2 receptor status (B) across breast cancer studies obtained from Geneminer. Boxplots of *KPNA2* mRNA expression levels as a function of PAM50 molecular subtype status (C), oestrogen (D), HER2 (E), and progesterone (F) receptor status for data located on cBioPortal. Corresponding *KPNA2* mRNA levels according to Sorlie’s (G), Hu’s (H), PAM50 (I), and RSPCC (J) intrinsic molecular subtypes located in Geneminer.
and was more frequently observed in patients with oestrogen-receptor negative breast cancer. Pairwise comparisons of the relative KPNA2 mRNA expression levels were performed in Geneminer according to tumour intrinsic molecular subtype. Corresponding readout indicates differential KPNA2 expression patterns across the sub-phenotypic classifications, with normal breast-like tumours consistently exhibiting (statistically significant, \( P < 0.0001 \)) lower KPNA2 expression levels in comparison to other molecular sub-phenotypes.

**Aberrant KPNA2 expression is associated with poor breast cancer prognosis**

The prognostic value of KPNA2 in breast cancer was examined using PrognoScan and KMPlot. In PrognoScan, 25 Gene Expression Omnibus (GEO) datasets were located in total, which were divided across five categories of 10 distant metastasis-free survival (DMFS), 2 Disease-free survival (DFS), 2 Disease-specific survival (DSS), 8 Relapse-free survival (RFS), and 3 overall survival (OS). Data presented in the Forest plot consistently demonstrate a negative correlation between KPNA2 overexpression and patient survival (Fig. 2).

The number of breast cancer dataset entries extracted from PrognoScan across all KPNA2 reporters were 56 studies in total. These were further categorized into one of five categories including relapse-free survival (RFS-18), disease-free survival (DFS-5), disease-specific survival (DSS-6), overall survival (OS-8), and distant metastasis-free survival (DMFS-19). The forest plot (Fig. 2) demonstrates a direct correlation between amplification of KPNA2 expression and a poor prognosis across all endpoints.

The prognostic value of KPNA2 overexpression across various breast cancer intrinsic molecular subtypes was studied, that included basal-like, luminal A, luminal B and HER2\(^+\) malignancies. As shown in Fig. 3, elevated KPNA2 mRNA expression across all breast cancer types was associated with poorer OS (HR 1.68, CI 95% 1.35–2.08, \( P = 2.6E-6 \), Fig. 3A), RFS (HR 1.58, CI 95% 1.42–1.76, \( P < 1E-16 \), Fig. 3B), DMFS (HR 1.73, CI 95% 1.42–2.1, \( P = 3.9E-8 \), Fig. 3C) and had no statistically significant impact on PPS (HR 1.71, CI 95% 1.32–2.22, \( P = 3.8E-5 \), Fig. 3D).

Next, we examined the prognostic value of KPNA2 mRNA expression across intrinsic molecular sub-phenotypes. From the datasets examined, elevated KPNA2 mRNA levels had no significant overall prognostic impact on patients with basal carcinomas, Luminal B (except for RFS: HR 1.35, CI 95% 1.09–1.68, \( P = 0.0056 \), Fig. 3N) and HER2\(^+\) breast cancers. However, in the case of Luminal A subtype, elevated KPNA2 RNA levels were associated with poor overall survival (HR(2.03, CI 95%, 1.46–2.84, \( P = 2.2E-5 \), Fig. 3I), relapse-free survival (HR 1.73, CI 95%, 1.46–2.04, \( P = 9.6E-11 \), Fig. 3J), disease-metastatic free progression survival (HR 1.96, CI 95%, 1.46–2.62, \( P = 4.1E-6 \), Fig. 3K), and post-progression survival (HR...
Fig. 3 The prognostic value of KPNA2 mRNA expression using Kaplan–Meier plotter (KMPlot) across all breast cancers (A–D) and intrinsic molecular subtypes (E–T). Corresponding HRs for OS, RFS, DMFS, and PPS survival endpoints are presented for each breast cancer subtype. HR: Hazard ratio, BC: Breast Cancer, OS: Overall Survival, RFS: Relapse-free Survival, DMFS: Disease-Metastatic Free Progression Survival, and PPS: Post-Progression Survival.
2.18, CI 95%, 1.5–3.18, $P = 3.3E-5$, Fig. 3L). Overall, these findings show that \( KPNA2 \) overexpression in breast cancer leads to poor patient survival outcomes across multiple endpoints, demonstrating the prognostic value of \( KPNA2 \) as a potential biomarker and actionable target.

**Co-expression patterns of \( KPNA2 \) mRNA in breast cancer**

To identify the pathways impacted by aberrant \( KPNA2 \) activity, we examined the correlation in gene expression patterns between \( KPNA2 \) and other genes using Oncomine. The top positive and negatively correlated genes with \( KPNA2 \) are shown in Fig. 4. The Richardson Breast 2 study was selected to study gene co-expression patterns ($P$-value: 0.001, Fold change:2, Gene rank: 10%), with 186 located genes upregulated genes in ductal breast carcinoma.

As shown in Fig. 4, genes most frequently co-expressed with \( KPNA2 \) in ductal breast carcinoma were found to be least expressed in healthy breast tissue.

Taken together, our findings from analyses of \( KPNA2 \) expression levels, mutational signature, impact on prognostic endpoints and co-expression patterns evidence that \( KPNA2 \) is implicated in cancer progression and prognosis.
Discussion

In the present study we examined the expression patterns of KPNA2 and its prognostic significance in breast cancer as a function of clinicopathologic parameters using online bioinformatics databases. To-date, datasets from the genomic and transcriptomic-based analyses of breast cancer tumour biopsies and their corresponding metadata have been curated and deposited across multiple databases for public access as a precision medicine tool [24, 34]. To our knowledge, a comprehensive analysis of clinical datasets interrogating the frequency and patterns of KPNA2 gene alterations as a function of tumour clinicopathologic parameters has not previously been attempted.

The dysregulation and aberrant function of Karyopherin activity has previously been correlated with tumour aggressiveness and poor patient prognosis across multiple cancer types. KPNA2, a member of the Karyopherin family, is involved in the nucleocytoplasmic transport of a range of key cellular factors including DNA repair, transcription, and cell division factors [17]. Previous work has shown a direct correlation between KPNA2 overexpression and poor patient prognosis across a range of cancer types, including glioblastoma, colorectal and ovarian cancer [6, 7, 9]. Despite the involvement of the Karyopherin family in breast cancer prognosis and tumorigenesis, the distinct role of KPNA2 in breast cancer outcomes and its expression patterns within breast tumour subtypes requires further investigation.

We used datasets available from online resources to analyse the frequency of genetic alterations occurring in KPNA2 mRNA expression levels across breast cancer intrinsic molecular subtypes (Geneminer, cBioPortal, COSMIC and Oncomine), examined patterns of KPNA2 co-expression with other genes (Geneminer and Oncomine) and evaluated the prognostic implications of KPNA2 mRNA overexpression in patients with breast cancer (Prognoscan and Kaplan–Meier Plotter).

Our analysis of patterns of KPNA2 mutations in cBioPortal and COSMIC revealed that N375S is also present in the MET gene, occurs across a range of cancer types and is detected in 9% of advanced breast tumours. MET mutations indicate a tyrosine kinase mutation previously shown to be oncogenic and dysregulated in early-stage lung cancers [35]. R366H mutations are common in colon cancer and involves a defective phosphorylation pathway of Long interspersed nuclear elements (LINE-1), activating inflammatory immune responses that drive tumour development [36]. Our searches of the Geneminer and cBioPortal repositories (Fig. 1) consistently show that the most frequently-occurring KPNA2 genetic alteration in breast cancer tumours is overexpression. Furthermore, our results demonstrate that patients with hormone receptor-negative (ER/PR) status are most likely to exhibit higher KPNA2 mRNA expression levels, in comparison to patients with hormone receptor-positive breast cancers ($P<0.0001$, Fig. 1). These data were further confirmed with the inverse correlation between KPNA2 and oestrogen and progesterone receptor mRNA levels (Geneminer, supplementary information). The incidence of KPNA2 amplification was also found to be higher in younger patients with breast cancer (supplemental information), suggesting its role in breast cancer progression in this age group. Furthermore, KPNA2 mRNA expression levels were found to be significantly amplified in patients with invasive ductal carcinoma (Fig. 1B).

Our search of the Oncomine database showed that at the transcriptional level relative to matched healthy breast tissue, the expression of KPNA2 was significantly upregulated in invasive lobular breast carcinoma, ductal breast carcinoma in situ, and invasive breast carcinoma. In all searches performed, KPNA2 was ranked in the top 7% of genes dysregulated in cancer across breast cancer subtypes located.

Functional assessment of KPNA2 co-expression showed that KPNA2 mRNA overexpression is directly correlated with an enrichment in genes regulating the cell cycle. SCL-interrupting locus protein (STIL), previously identified in prostate cancer [37], is a G2 phase gene involved in cell growth and development. This oncogene also activates the cell cycle-dependent protein kinase 1 (CDK1) pathway. CDK1, also co-expressed with KPNA2, promotes G2/M cell cycle transition and has previously been reported in hepatocellular carcinomas [8]. Moreover, KPNA2 overexpression in ovarian cancer was recently linked to KIF4F signalling upregulation accelerating tumour progression [38, 39].

ZW10 interacting kinesin protein (ZWINT) and Epithelial cell transforming 2 (ECT), both mitotic checkpoint proteins, have been shown to contribute to poor prognosis across multiple cancer types including glioblastoma [40]. Though previous reports show an association between ZWINT overexpression and triple-negative breast cancers, the functional role of ZWINT and ECT in breast cancer remains largely unexplored [41]. The ECT gene has been implicated in the protein assembly in cell division [42], and its dysregulation in breast cancer remains poorly understood. Another gene directly co-expressed with KPNA2 is the Cell division cycle 20 (CDC20), a late mitosis checkpoint mediator that predominantly occurs in hormone positive (ER+) breast tumours (58% ($N=870$), METABRIC study) [43]. Aberrant CDC20 overexpression has previously been implicated in pan-cancer disease progression and poor patient prognosis.
Our evaluation of the prognostic role of KPNA2, showed that across multiple prognostic endpoints (OS, RFS, DMFS and PPS) from PrognoScan and KMPlot (Fig. 3), KPNA2 overexpression was associated with poor survival outcomes. Our findings are in agreement with a previous report indicating that KPNA2 overexpression can serve as a prognostic marker across multiple cancer types and is associated with malignant transformation and poor patient survival [14, 44, 45].

To-date a limited number of reports have studied the functional role of KPNA2 in patient response to standard of care treatments and breast cancer outcomes. Our investigation primarily focused on using existing databases to inform the future rationale for exploring the biomolecular and phenotypic role of KPNA2 in breast cancer. Our integrated analyses of existing datasets indicate that KPNA2 can serve as a prognostic biomarker in breast cancer, warranting further investigation of its biomolecular role in tumour aggressiveness. We identified the functional associations and prognostic significance of KPNA2 in breast cancer, which warrants its further investigation as a promising prognostic biomarker or druggable target.

**Conclusion**

During the COVID-19 pandemic and with limitations in laboratory access clinical datasets freely available on databases have provided a tool for data mining and scoping new projects. Open access databases provide a useful toolbox for investigation the correlations between biomolecular drivers of cancer and prognostic outcomes. Here, we used outputs from such databases to explore the rationale for targeting KPNA2 as a novel druggable target. Our analyses of existing clinical datasets for expression and survival outcomes show that KPNA2 over-expression contributes to poor patient survival outcomes, further necessitating its investigation in future studies to consider its clinical utility for triple negative breast cancer subtypes.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12885-022-09969-4.

**Acknowledgements**

We acknowledge funding awarded to ZRF from Tenovus Scotland, The Royal Society of Edinburgh Research Reboot, and Engineering and Physical Sciences Research Council, EPSRC (EP/V028960/1). For the purpose of open access, the authors have applied for a CC BY copyright license to any Author Accepted Manuscript version arising from this submission. This work was also supported by the FRAME summer studentship for LVD internship, and Kuwait University for LA PhD scholarship funding. We acknowledge support to CHJ from the American Cancer Society Research Scholar Grant 134273-RSG-20-065-01-TBE and the US National Cancer Institute of the National Institutes of Health under Award Number K12CA215110. We dedicate this manuscript to all the patients with breast cancer who support ongoing efforts in developing new diagnostics and therapies of the future.

**Authors’ contributions**

Layla Alnoumas: Methodology, Data curation, Formal analysis, Investigation, Writing – review & editing. Lisa van Den Driest: Methodology, Investigation. Zoe Apczynski: Investigation. Alison Lannigan: Writing – review & editing. Caroline Johnson: Data Interpretation, Funding acquisition, Writing - review & editing. Nicholas Rattray: Supervision, Writing – review & editing. Zahra Rattray: Conceptualization, Methodology, Investigation, Supervision, Funding acquisition, Formal analysis, Writing – original draft, Writing – review & editing. All author(s) have read and approved the final manuscript.

**Funding**

We acknowledge funding awarded to Zahra Rattray from Tenovus Scotland, The Royal Society of Edinburgh Research Reboot, FRAME summer studentship for LVD internship, and Kuwait University for LA PhD scholarship funding. We acknowledge support to Caroline Johnson from the American Cancer Society Research Scholar Grant 134273-RSG-20-065-01-TBE and the US National Cancer Institute of the National Institutes of Health under Award Number K12CA215110.

**Availability of data and materials**

The datasets that support the findings of this study are available from third parties, with the weblinks provided in the manuscript methodology section and can be located at the following sites: CBioportal (https://www.cbioportal.org/), see Table 1 for datasets used in this manuscript), COSMIC (www.sanger.ac.uk), Oncomine (https://www.oncomine.org/resource/login.html, see Table 4 for datasets used in this manuscript), PrognoScan (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html), Kaplan–Meier Plotter (KMplot (http://kmplot.com/analysis/index.php?P=service), Breast Cancer Gene-Expression Miner v4.6 (http://bcgenex.ico.unicanerf/BCC-GEM/GEM-Accueil.php?js=1).

**Declarations**

Ethics approval and consent to participate

All data reported in this manuscript were obtained from GSE datasets or previously-published and accessible datasets deposited on CBioportal. To our
knowledge, all these methods used to obtain data were carried out in accordance with the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare no known competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

Author details
1Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK. 2Wishaw General Hospital, NHS Lanarkshire, Scotland, UK. 3Yale School of Public Health, Yale University, New Haven, CT, USA.

Received: 18 January 2022 Accepted: 4 August 2022 Published online: 10 August 2022

References
1. Sporkova Z, et al. Genetic markers in triple-negative breast cancer. Clin Breast Cancer. 2018;18(5):e841–50.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jamal A, Bray F. Global cancer statistics. 2020 GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209–49. https://doi.org/10.3322/caac.21660.
3. Coughlin S. Epileptogenesis of breast cancer in women. Adv Exp Med Biol. 2019;1152:9–29.
4. Russnes HG, et al. Breast cancer molecular stratification: from intrinsic subtypes to integrative clustering. Am J Pathol. 2017;181(10):2152–62.
5. Han Y, Wang X. The emerging roles of KPNA2 in glioblastomas by regulation of c-myc. J Exp Clin Cancer Res. 2018;37(1):194.
6. Zhang Y, et al. Karyopherin alpha 2 is a novel prognostic marker and a potential therapeutic target for colon cancer. J Exp Clin Cancer Res. 2015;34:145.
7. Gao C-L, et al. Karyopherin subunit-a 2 expression accelerates cell cycle progression by upregulating CCNB2 and CDK1 in hepatocellular carcinoma. Oncot Lett. 2018;15(3):2815–20.
8. Huang L, et al. KPNA2 promotes migration and invasion in epithelial ovarian cancer cells by inducing epithelial-mesenchymal transition via Akt/ GSK-3β/Smad activation. J Cancer. 2018;9(11):157–65.
9. Ma A, et al. USP1 inhibition destabilizes KPNA2 and suppresses breast cancer metastasis. Oncogene. 2019;38(13):2405–19.
10. Noetzel E, et al. Nuclear transport receptor karyopherin-α2 promotes malignant breast cancer phenotypes in vitro. Oncogene. 2012;31(16):2101–14.
11. Alshareeda AT, et al. KPNA2 is a nuclear export protein that contributes to aberrant localisation of key proteins and poor prognosis of breast cancer. Br J Cancer. 2015;112(12):1929–37.
12. Pavlou MP, et al. Integrating meta-analysis of microarray data and targeted proteomics for biomarker identification: application in breast cancer. J Proteome Res. 2014;13(6):2897–909.
13. Gluz O, et al. Nuclear karyopherin α2 expression predicts poor survival in patients with advanced breast cancer irrespective of treatment intensity. Int J Cancer. 2008;123(6):1433–8.
14. Huang L, et al. KPNA2 promotes cell proliferation and tumorigenicity in epithelial ovarian carcinoma through upregulation of c-Myc and down-regulation of FOXO3a. Cell Death Dis. 2013;4(8):e475–e475.
15. Xu C, Liu M. Integrative bioinformatics analysis of KPNA2 in six major human cancers. Open medicine (Warsaw, Poland). 2021;16(1):498–511.
16. Muzzo H, et al. PrognoScan: a new database for meta-analysis of the prognostic value of genes. BMC Med Genomics. 2009;2(1):18.
17. Muzzo H, et al. PrognoScan: a new database for meta-analysis of the prognostic value of genes. BMC Med Genomics. 2009;2:18–18.
18. Gyorffy B, et al. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. Breast Cancer Res Treat. 2010;123(3):275–31.
19. Thomssen C, et al. St. Gallen/Vienna 2021: a brief summary of the consensus discussion on customizing therapies for women with early breast cancer. Breast Care. 2021;16(2):135–43.
20. Jézéquel P, Gouraud W, Azouz FB, Guerin-Charbonnel C, Jun P, Lasla H, Campone M. bc-GenExMiner 4.5: new mining module computes breast cancer differential gene expression analyses. Database: The Journal of Biological Databases and Curation. 2021.
21. Parry M. Introducing the metastatic breast cancer project: a novel patient-partnered initiative to accelerate understanding of MBC. ESMO open. 2018;3(7):e000452–e000452.
22. Curtis C, et al. The genomic and transcriptomic architecture of 2,000 human cancers reveals novel subgroups. Nature. 2012;486(7403):346–52.
23. Rueda OM, et al. Dynamics of breast-cancer relapse reveal late-recurring ER-positive genomic subgroups. Nature. 2019;567(7449):399–404.
24. Pereira B, et al. The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. Nat Commun. 2016;7:11479.
25. Berger AC, et al. A comprehensive pan-cancer molecular study of gynecologic and breast cancers. Cancer Cell. 2018;33(4):690–705.e9.
26. Ogunn S, et al. Standard mutation nomenclature in molecular diagnostics. J Mol Diagn. 2007;9(1):1–6.
27. Richardson AL, et al. X chromosomal abnormalities in basal-like human breast cancer. Cancer Cell. 2006;9(2):121–32.
28. Clare SE, Shaw PL. “Big Data” for breast cancer: where to look and what you will find. NPJ Breast Cancer. 2016;2(1):16031.
29. Tovar EA, Graveel CR. MET in human cancer: germline and somatic mutations. Ann Transl Med. 2017;5(10):205–205.
30. Zhang X, Zhang R, Yu J. New understanding of the relevant role of LINE-1 retrotransposition in human disease and immune modulation. Front Cell Dev Biol. 2020;8:657–657.
31. Wu X, et al. The human oncogene SLC/TAL1 interrupting locus (STIL) promotes tumor growth through MAPK/ERK, PI3K/Akt and AMPK pathways in prostate cancer. Gene. 2019;686:220–7.
32. Cui X, Wang H, Wu X, Hsu K, Jing X. Increased expression of KPNA2 promotes unfavorable prognosis in ovarian cancer patients, possibly by targeting KIF4A signaling. J Ovarian Res. 2021;14(1):71.
33. Wang J, et al. KIF4A silencing inhibits the proliferation and migration of breast cancer cells and correlates with unfavorable prognosis in breast cancer. BMC Cancer. 2014;14(1):461.
34. Tang J, et al. Genome-wide expression profiling of glioblastoma using a large combined cohort. Sci Rep. 2018;8(1):15104–12.
35. Li HN, Zheng WH, Du YY, Wang G, Dong ML, Yang ZF, Li XR. ZW10 interacting kinesin may serve as a prognostic biomarker for human breast cancer: An integrated bioinformatics analysis. Oncol Lett. 2020;19:2163–74.
36. Yang Z, Li H, Yang Z. NOX1 promotes tumor growth through MAPK/ERK, PI3K/Akt and AMPK pathways in prostate cancer. Gene. 2019;686:220–7.
37. Chen M, et al. Structure and regulation of human epithelial cell transforming 2 protein. Proc Natl Acad Sci. 2020;117(2):1027–35.
38. Alfarsi LH, et al. CDC20 expression in oestrogen receptor positive breast cancer predicts poor prognosis and lack of response to endocrine therapy. Breast Cancer Res Treat. 2019;179(3):335–44.
39. DankoF, et al. KPNA2 protein expression in invasive breast carcinoma and matched peritoneal ductal carcinoma in situ. Virchows Arch. 2007;451(5):877–81.
40. Dhal E, et al. Molecular profiling of laser-microdissected matched tumor and normal breast tissue identifies Karyopherin α2 as a potential novel prognostic marker in breast cancer. Clin Cancer Res. 2006;12(3):3950–60.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.