# Identification of a FLG-wide-type bladder cancer subtype with poor prognosis and prediction use in immune checkpoint

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## Abstract

# Background

Bladder cancer (BLCA) is one of most common urinary tract malignant tumor and immunotherapy have generated a great deal of interest in BLCA. Immune checkpoint blockade (ICB) therapy has significantly progressed the treatment of BLCA. Multiple studies have suggested that specific genetic mutations may serve as immune biomarkers for ICB therapy.

# Objective

In this study, we aimed to investigate the role of mutations genes and subtypes in prognosis and immune checkpoint prediction in BLCA.

# Method

Mutation information and expression profiles were acquired from The Cancer Genome Atlas (TCGA) database. Integrated bioinformatics analysis was carried out to explore the mutation genes of BLCA. Functional enrichment analysis Gene Ontology (GO) and Gene set enrichment analysis (GSEA) was conducted. The infiltrating immune cells and the prediction of ICB between different subtypes group were explored using immuCellAI algorithm.

# Results

The mutation genes Filaggrin (FLG) gene were identified. Following the study on its subtypes and functional enrichment analysis, Sub2 of FLG-wide type was found to have relationships with poor prognosis and immune infiltration BLCA. What's more, Sub2 of FLG-wide type may be used as a biomarker to predict the prognosis of BLCA patients receiving ICB.

# Conclusion

This research provides a new basis and ideas for guiding the clinical application of BLCA immunotherapy.

## Introduction

Bladder cancer (BLCA) is one of most common urinary tract malignant tumor and accounts for nearly 170,000 deaths annually in the world[1, 2]. It is the ninth most incident neoplasm in China and the 10th most common malignant tumor worldwide[3, 4]. BLCA has become a serious public health problem

because of its high incidence, high risk of recurrence, treatment frequently failure (e.g. intravesical bacillus Calmette-Guerin or platinum-based chemotherapy)[5, 6]. However, the treatment for BLCA has remained conservative and the curative effect has not made a breakthrough [7]. Due to the large number of recognizable antigens, BLCA might be sensitive to immunotherapy[8]. With the rapid development of immunotherapy, the immune checkpoint blockade (ICB) have become a novel treatment strategy for BLCA[9]. Immune checkpoint blockade refer to inhibitory drugs developed for immune checkpoints, which can rejuvenate immune cells and kill tumor cells again[10]. Therefore, the prediction of immune checkpoints is of clinical significance.

The Filaggrin (FLG) gene can encode a related protein that accumulates in the intermediate filaments of mammalian epidermal keratin [11, 12]. Previous studies have shown variability in the frequency of FLG variants [13]. The mutation of FLG is associated with a variety of skin diseases[14], and it is mutated in a variety of cancers such as head and neck cancer, prostate cancer, urinary cancer and bronchus and lung cancer[15]. It is suggested that FLG gene mutation is one of the risk factors for cancer,

the associations between FLG loss-of-function mutations and cancer were in subgroup analyses[16]. Besides, studies have shown that BLCA is a highly mutated tumor type[17]. However, the association between the mutation of FLG and BLCA need further dissection.

In present study, we aimed to investigate the prognosis and immune checkpoint prediction role of gene subtype in BLCA. Mutation information and expression profiles were acquired from The Cancer Genome Atlas (TCGA) database. Integrated bioinformatics analysis was carried out to explore the mutation genes of BLCA and FLG were identified. Following the study on its subtype and functional enrichment analysis, their relationships with prognosis and immune infiltration were also evaluated. Finally, we ensure the important role of FLG subtype in ICB. This research provides a new basis and ideas for guiding the clinical use of BLCA immunotherapy.

## Method And Materials

# Data source collection and processing

Transcriptome data and clinical information of BLCA patients were downloaded from the TCGA data portal (http://cancergenome.nih.gov/). The “maftools” R package was used to analyze the Mutation Annotation Format (MAF) file and visualize the somatic mutation data [18].

# Identification of gene expression-based subtypes

Unsupervised clustering was performed using the R package “ConsensusClusterPlus” for class discovery based on the comparison of gene expression profile[19, 20]. 80% item resampling, 50 resamplings and a maximum evaluated K of 10 were selected for clustering. The cumulative distribution function (CDF) and consensus heatmap were used to assess the optimal K [21].

# Differential expression analysis

The differently expressed mRNAs presented as heatmap and volcano plot were screened using GDCRNATools package, with the criterion of |log 2(fold change [FC]| > 1 and FDR < 0.05).

# Functional enrichment analysis

To assess the function of differential genes between subtypes in BLCA, Gene Ontology (GO) annotation analyses were performed by using the cluster profiler package of R software[22]. P-value < 0.05 was set as the cut-off criterion. Gene set enrichment analysis (GSEA) was conducted to examine critical pathways represented under different conditions[23]. The ridgeline plot was presented by using clusterProfiler.

# Immune infiltration analysis and Immunotherapy prediction

The infiltrating immune cells and the prediction of ICB between different subtypes group were explored using immuCellAI algorithm[24].

## Results

# Overview of mutation information in BLCA patients

We download BLCA gene mutation data from the TCGA database and analyzed the somatic mutation information. Figure 1 showed the overall mutation status of BLCA. As shown in Figure 1A, missense mutation is the most common mutation types with a frequency far higher than that of others. Single nucleotide polymorphism (SNP) represented the largest fraction in the variant type than insertion or deletion (Figure 1B). The most common single nucleotide variant (SNV) was C > T, followed by C > G and C > A (Figure 1C). The median of per variants samples was 148 (Figure 1D) and variants classification summary was shown in Figure 1E. Figure 1F demonstrated the sample status of TOP 20 mutant genes, including TP53, TTN, FLG, etc. There are 412 samples and 392 samples have mutations in this dataset.

# Prognostic FLG wild-type splited into two subtypes

Next, we conducted a prognostic analysis of the TOP 20 mutant genes and found that FLG gene mutations are strongly related to the prognosis of patients (Figure 2A). The survival time of FLG wild-type patients is significantly shorter than that of FLG mutant patients. Patients (BLCA) who harbored FLG mutations showed a better prognosis. Figure 2B suggested the mutation information of FLG gene in BLCA, the main mutation forms are: missense mutation and nonsense mutation. Based on the above results, we have made it clear that the research object in BLCA was FLG wild-type samples. We combined the BLCA transcriptome data in the TCGA database to explore whether FLG wild-type patients with high mortality rates have different types. We used the ConsensusClusterPlus software to divide the patient samples into two categories (Sub1 and Sub2) (Figure 2C), and further analyzed the prognosis of the two types of patients. The results showed that the survival time of Sub2 patients was significantly less than that of Sub1 patients (Figure 2D). At the same time, it could be seen that the mortality rate of Sub2 was as high as 90% (Figure 2E). The death rate of Sub1 was 46%, while the death rate of Sub2 was 90%. In this analysis, we found that there are two different types of patients in FLG wild-type patients. The

prognosis of Sub2 patients is worse than that of Sub1 patients. Sub2 of FLG -wild type in BLCA is usually associated with poorer outcome.

# The 2 subtypes of FLG wild-type differed in biological functions

We used transcriptome data to explore the biological significance of two different subtypes of patients. The differently expressed genes (DEGs) between the Sub1 and Sub2 were analyzed (|log2FC| > 1 and FDR < 0.05). A total of 778 up-regulated and 972 down-regulated DEGs were identified (Figure 3A-B). The results of GO functional enrichment analysis of DEGs showed that related pathways such as inflammation, chemokine production, and T cell activation were significantly enriched (Figure 3C). The biological process (BP) pathway was further analyzed by the GSEA method, and the results also showed that T cells plays a key role in different types of tumor microenvironments. In addition, the results suggests that the pathways related to immunity and inflammation are significantly down-regulated in Sub2, and the immune response involved in T cell activation is suppressed (Figure 3D).

# Association of 2 subtypes of FLG wild-type with infiltrated immune Cells

Infiltrating immune cells in tumor are a hallmark of immune surveillance and a necessary part of complex microenvironment regulating tumor progression [25]. It is known that T cells play a key role in mediating the tumor immune microenvironment, and different subtypes of T cells have different divisions of labor[26]. Therefore, the ImmuCellAI software was carried out to analyze the T cell subtypes of FLG wild-type patients with different types. As seen in Figure 4, the results showed that the CD4 naive, central memory T (Tcm), NKT were significantly down-regulated in Sub2, while Th1 cells were significantly up-regulated in Sub2. CD4+ T cells can directly eliminate tumor cells through cytolysis or indirectly regulate the tumor immune microenvironment to target tumor cells [27]. NKT cells are a type of T cell subgroup with specific NK cell markers in immune cells. After activation, they can directly act as anti-tumor effector cells to play a killing effect, and they can also activate other immune effector cells, such as NK cells, to indirectly achieve anti-tumor effect. NKT cells play an important role in anti-tumor immunity, acquired immune response and immune regulation[28].

# Sub2 of FLG wild-type patients respond worse to ICB

ICB is beneficial to restore T lymphocyte activity and break through the physical barrier of tumor immune microenvironment to promote T cell homing. Therefore, it can activate anti-tumor immunity and improving the effect of immunotherapy. Based on the results of the current study, enhanced tumor immunogenicity predicts improved clinical response to ICB. Finally, we used immuCellAI to perform immune check-up prediction on FLG wild-type patients of different types. As shown in Figure 5, the reaction rate of Sub1 was 33.7%, while the reaction rate of Sub2 was 10%. The results showed that Sub2

patients had a worse response to ICB. The results indicated that the detection of Sub2 FLG wild-type might be used in the clinical immunotherapy.

# Discussion

Tumors are related to the accumulation of somatic mutations in cells as a result of carcinogens[29]. Studies have shown that BLCA is a highly mutated tumor type[17]. For example, Zhang et al. found that BLCA patients with higher tumor mutational burden (TMB) had more survival benefits[17]. The mutations of FGFR3, HRAS, KRAS, NRAS and PIK3CA in BLCA can present a companion diagnostic to define patients for targeted therapies [30, 31]. Recognizing the genetic mutations that may allow early genetic screenings for BLCA and new therapies targeting cells with these mutations[32]. The FLG gene encode a related protein that accumulates in the intermediate filaments of mammalian epidermal keratin and shown variability in the frequency variants [11–13]. FLG gene mutation is one of the risk factors for cancer, the associations between FLG loss-of-function mutations and cancer were in subgroup analyses[16]. In this study, it's the first time to found the survival time of FLG wild-type patients was significantly less than that of FLG mutant patients based on the BLCA gene mutation data in the TCGA database. Furthermore, we found that there were two different types of patients in FLG wild-type patients, sub1 and sub2. Though the sample size of sub2 is 10, the prognosis of sub2 patients was worse than that of sub1 patients, the mortality rate of Sub2 is as high as 90%. Hence, we paid attention to the biological function and pathways of sub2 in the following research.

As one of the most common urinary system cancers worldwide, BLCA ranges from unaggressive and noninvasive tumors to aggressive and invasive tumors with high disease-specific mortality[6]. However, traditional treatments did not make significantly improvement for its the 5-year survival rate[33]. Recently, newer immunotherapy have generated a great deal of interest in BLCA[34]. In this study, the results of functional enrichment analysis of differential genes showed that related pathways such as inflammation, chemokine production, and T cell activation were significantly enriched. GSEA analysis of the BP pathway suggests that the pathways related to immunity and inflammation are significantly down-regulated in Sub2, and the immune response involved in T cell activation was suppressed. Analysis of the T cell subtypes of FLG wild-type patients with different types showed that Sub2 of FLG wild-type was correlated with infiltrating levels of immune cells (CD4 naive, Tcm and NKT cell). The results above indicated that The Sub2 was related to the immune microenvironment of BLCA. The investigation of the relationship and biological mechanisms between FLG subtypes and immune microenvironment will help to better understand the role of immunotherapy in BLCA treatment.

Immune checkpoint inhibitors refer to inhibitory drugs developed for immune checkpoints, which can rejuvenate immune cells and kill tumor cells again [35]. The application of ICB, such as anti-programmed cell death (ligand) 1( anti-PD-(L)1)[36] and cytotoxic T lymphocyte-associated antigen 4 (anti-CTLA-4) [37], is becoming emerging as a novel treatment strategy for BLCA[38]. Therefore, the prediction of immune checkpoints is of great clinical significance for BLCA. Studies have shown a correlation between specific genetic mutations and the efficacy of immunotherapy[39, 40]. It was reported that TMB,

microsatellite instability, mismatch repair gene deficiency, T-cell inflamed and IFN-γ gene expression profiles (GEPs), and DNA damage response (DDR) and antigen presentation defects may serve as potential biomarkers for immune checkpoint of immunotherapy[41]. Zhang et al. identified NTRK3 as a potential prognostic biomarker associated with tumor mutation burden and immune infiltration in BLCA [17]. Lin et al. found the effect of NCOR1 mutations on immune microenvironment and efficacy of ICB in patient with BLCA[42]. In this study, we predicted the immune checkpoints of FLG wild-type patients of different types, and the results showed that Sub2 patients respond is worse to immune checkpoint. The results suggested that FLG mutilation may prove to be a novel detection and contribute to the development of immunotherapy for BLCA.

# Conclusion

In conclusion, this study systematically explored the relationship between FLG wild-type and prognosis as well as immune infiltration in BLCA. The prognostic FLG wild-type spitted into two subtypes and our data revealed that Sub2 of FLG wild-type was closely correlated with survival and immune response with further exploration. Accordingly, Sub2 of FLG wild-type may prove as a novel diagnosis method and contributed to the development of immunotherapy for BLCA. Further experiments in vivo and in vitro to investigate the underlying mechanism and clinical validation is needed.

# Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and material

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s.

### Competing interests

The authors declare that they have no conflict of interest.

### Funding

Not applicable.

### Author contributions

Liang Chen, and Xiaobo Huang designed the research. Liulin Xiong, Weinan Chen, and Lizhe An performed the experiments. Huanrui Wang, Yang Hong and Huina Wang visualized the results. Liang Chen and Liulin Xiong wrote manuscript. Xiaobo Huang provided experimental resource. All authors reviewed and approved the final manuscript.

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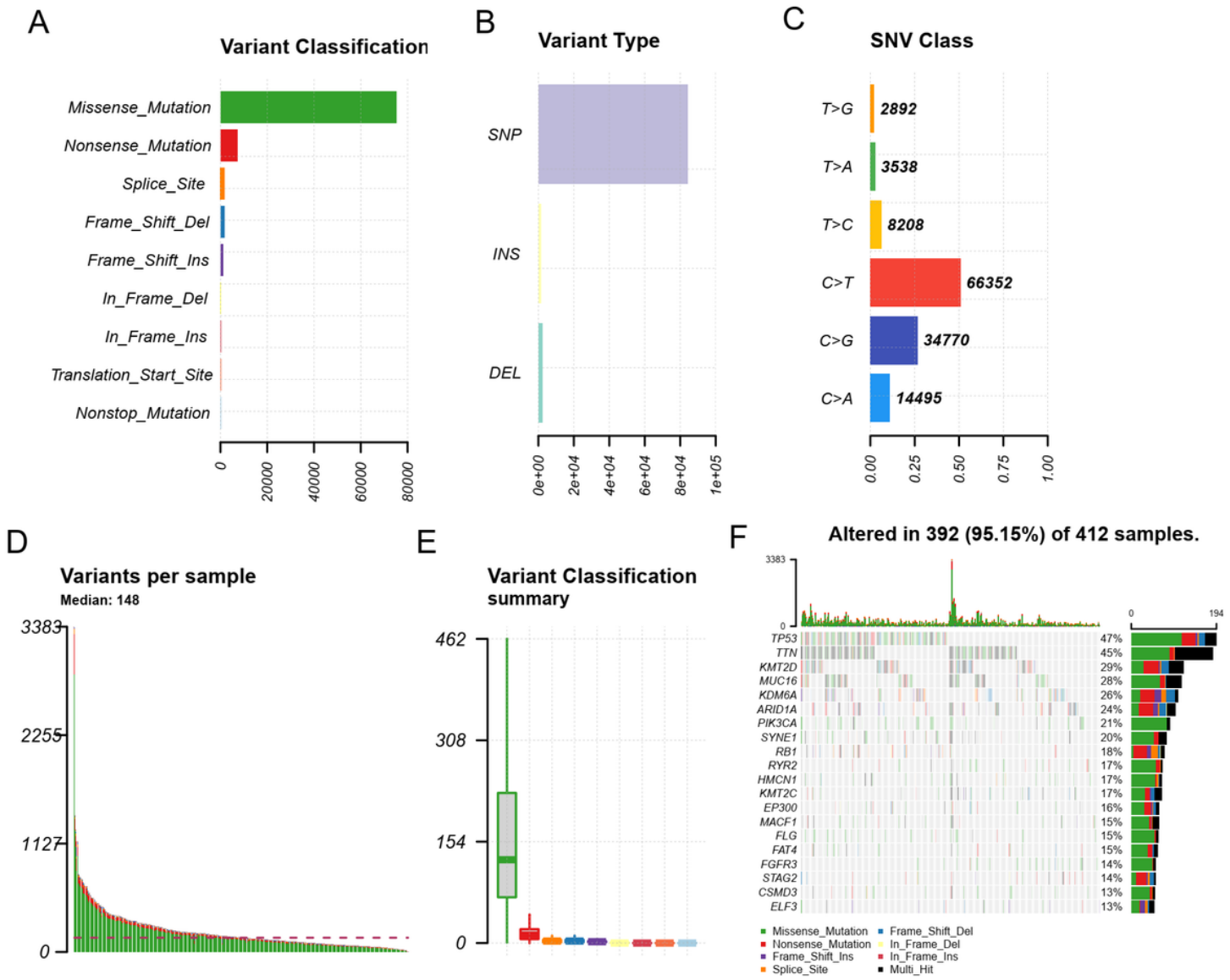
# References

1. Zuo, W., Wang, Z.Z. and Xue, J. Artesunate Induces Apoptosis of Bladder Cancer Cells by miR-16 Regulation of COX-2 Expression. International journal of molecular sciences 2014; 15(8): p. 14298
2. Patel, V., Oh, W. and Galsky, M. Treatment of muscle-invasive and advanced bladder cancer in 2020. CA: a cancer journal for clinicians 2020; 70(5): p. 404–423
3. Qu, G.Y., Liu, Z., Yang, G., et al. Development of a prognostic index and screening of prognosis related genes based on an immunogenomic landscape analysis of bladder cancer. Aging 2021; 13
4. Zhi-Peng, X.U., Huang, J.F. and Department, U. Research Progress of MicroRNA and LncRNA in Bladder Cancer. World Latest Medicine Information 2019;
5. Karim, Chamie, Mark, et al. Recurrence of high-risk bladder cancer: A population-based analysis. Cancer 2013; 119(17): p. 3219–3227
6. Lenis, A., Lec, P., Chamie, K., et al. Bladder Cancer: A Review. JAMA 2020; 324(19): p. 1980–1991
7. Tiwari, R., Ngo, N. and Lee, L. The optimal management of variant histology in muscle invasive bladder cancer. Translational andrology and urology 2020; 9(6): p. 2965–2975
8. Cao, R., Ma, B., Wang, G., et al. Identification of autophagy-related genes signature predicts chemotherapeutic and immunotherapeutic efficiency in bladder cancer (BLCA). Journal of cellular and molecular medicine 2021;
9. Lyu, Q., Lin, A., Cao, M., et al. Alterations in TP53 Are a Potential Biomarker of Bladder Cancer Patients Who Benefit From Immune Checkpoint Inhibition. Cancer control: journal of the Moffitt Cancer Center 2020; 27(1): p. 1073274820976665
10. Topalian, S., Drake, C. and Pardoll, D. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer cell 2015; 27(4): p. 450
11. Presland, R.B., Haydock, P.V., Fleckman, P., et al. Characterization of the human epidermal profilaggrin gene. Genomic organization and identification of an S-100-like calcium binding domain at the amino terminus. Journal of Biological Chemistry 1992; 267(33): p. 23772–81
12. Haydock, P.V. and Dale, B.A. Filaggrin, an intermediate filament-associated protein: structural and functional implications from the sequence of a cDNA from rat. Dna & Cell Biology 1990; 9(4): p. 251
13. Margolis, D., Mitra, N., Wubbenhorst, B., et al. Filaggrin sequencing and bioinformatics tools. Archives of dermatological research 2020; 312(2): p. 155–158

14. Bandier, J., Ross-Hansen, K., Carlsen, B., et al. Carriers of filaggrin gene (FLG) mutations avoid professional exposure to irritants in adulthood. Contact dermatitis 2013; 69(6): p. 355–62
15. Skaaby, T., Husemoen, L., Thyssen, J., et al. Filaggrin loss-of-function mutations and incident cancer: a population-based study. The British journal of dermatology 2014; 171(6): p. 1407–14
16. Skaaby, T., Husemoen, L., Thyssen, J.P., et al. Filaggrin loss-of-function mutations and incident cancer: a population-based study. British Journal of Dermatology 2015; 171(6): p. 1407–1414
17. Zhang, Z., Yu, Y., Zhang, P., et al. Identification of NTRK3 as a potential prognostic biomarker associated with tumor mutation burden and immune infiltration in bladder cancer. BMC cancer 2021; 21(1): p. 458
18. Mayakonda, A., Lin, D., Assenov, Y., et al. Maftools: efficient and comprehensive analysis of somatic variants in cancer. Genome research 2018; 28(11): p. 1747–1756
19. Hayes, D.N. ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking. Bioinformatics 2010; 26(12): p. 1572–1573
20. Fan, Wu, Rui-Chao, et al. Molecular classification of IDH-mutant glioblastomas based on gene expression profiles. Carcinogenesis 2019;
21. Molecular subtyping reveals immune alterations in IDH wild-type lower-grade diffuse glioma. The Journal of Pathology 2020; 251(3)
22. Yu, G., Wang, L.G., Han, Y., et al. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 2012; 16(5): p. 284–7
23. Subramanian, A., Tamayo, P., Mootha, V.K., et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005; 102(43): p. 15545–50
24. Miao, Y., Zhang, Q., Lei, Q., et al. ImmuCellAI: A Unique Method for Comprehensive T-Cell Subsets Abundance Prediction and its Application in Cancer Immunotherapy. Advanced science (Weinheim, Baden-Wurttemberg, Germany) 2020; 7(7): p. 1902880
25. Kortylewski, M., Kujawski, M., Wang, T., et al. Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. Nature medicine 2005; 11(12): p. 1314–1321
26. Hao, F. and Thasler, W. Immune microenvironment modulation via the interaction of human invariant natural killer T cell (iNKT cells) and Kupffer cells (KC), dendritic cells (DC) in colorectal liver metastasis. 2017;
27. Jannie, B., Tomasz, A., Nikolina, B., et al. CD4 + T cell help in cancer immunology and immunotherapy. Nature Reviews Immunology 2018;
28. Mrakovcicsutic, I. and Pirjavecmahic, A. *NK/NKT interactions in burn-induced changes of cell mediated immunity*. in *Congress of the International Society for Burn Injuries*. 2008.
29. Dollé, M., Giese, H., Van, S.H., et al. Mutation Accumulation In Vivo and the Importance of Genome Stability in Aging and Cancer. Results & Problems in Cell Differentiation 2000; 29: p. 165

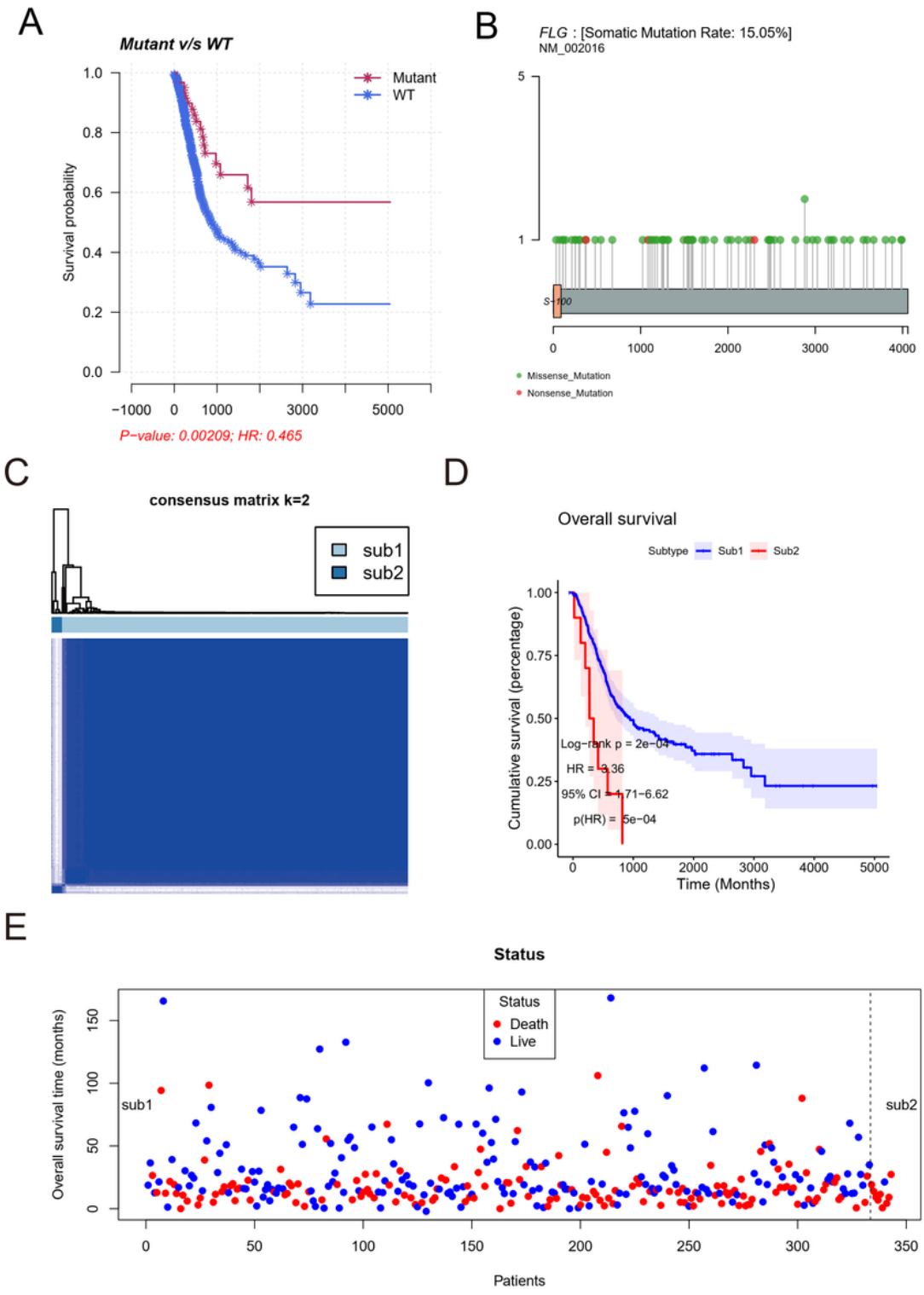
30. Kompier, L.C., Irene, L., Van, D., et al. FGFR3, HRAS, KRAS, NRAS and PIK3CA Mutations in Bladder Cancer and Their Potential as Biomarkers for Surveillance and Therapy. PloS one 2010; 5(11): p. e13821-
31. Serizawa, R.R., Ralfki?R, U., Steven, K., et al. Integrated genetic and epigenetic analysis of bladder cancer reveals an additive diagnostic value of FGFR3 mutations and hypermethylation events. International Journal of Cancer Journal International Du Cancer 2011; 129(1): p. 78–87
32. Com, C. Bladder Cancer: Researchers sequence bladder cancer gene, targets genetic mutations for therapy.
33. Cheng, W., Fu, D., Xu, F., et al. Unwrapping the genomic characteristics of urothelial bladder cancer and successes with immune checkpoint blockade therapy. Oncogenesis 2018; 7(1): p. 2
34. Vasekar, M., Degraff, D. and Joshi, M. Immunotherapy in Bladder Cancer. Current molecular pharmacology 2016; 9(3): p. 242–251
35. Day, D. and Hansen, A.R. Immune-Related Adverse Events Associated with Immune Checkpoint Inhibitors. BioDrugs 2016; 30(6): p. 571
36. Julie, R.B. and Scott, S.T.L.Q. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. New England Journal of Medicine 2012;
37. Buchbinder, E. and McDermott, D. Cytotoxic T-lymphocyte antigen-4 blockade in melanoma. Clinical therapeutics 2015; 37(4): p. 755–63
38. Mukherji, D., Jabbour, M.N., Saroufim, M., et al. Programmed Death-Ligand 1 Expression in Muscle-Invasive Bladder Cancer Cystectomy Specimens and Lymph Node Metastasis: A Reliable Treatment Selection Biomarker? Clinical Genitourinary Cancer 2016: p. 183–187
39. Riaz, N., Havel, J., Kendall, S., et al. Recurrent SERPINB3 and SERPINB4 mutations in patients who respond to anti-CTLA4 immunotherapy. Nature genetics 2016; 48(11): p. 1327–1329
40. Wang, F., Zhao, Q., Wang, Y., et al. Evaluation of POLE and POLD1 Mutations as Biomarkers for Immunotherapy Outcomes Across Multiple Cancer Types. JAMA Oncol 2019; 5(10): p. 1504–1506
41. Wang, S., He, Z., Wang, X., et al. Antigen presentation and tumor immunogenicity in cancer immunotherapy response prediction. eLife 2019; 8
42. Lin, A., Qiu, Z., Zhang, J., et al. Effect of NCOR1 Mutations on Immune Microenvironment and Efficacy of Immune Checkpoint Inhibit**ors** in Patient with Bladder Cancer. Frontiers in immunology 2021; 12: p. 630773

# Figures




## Figure 1

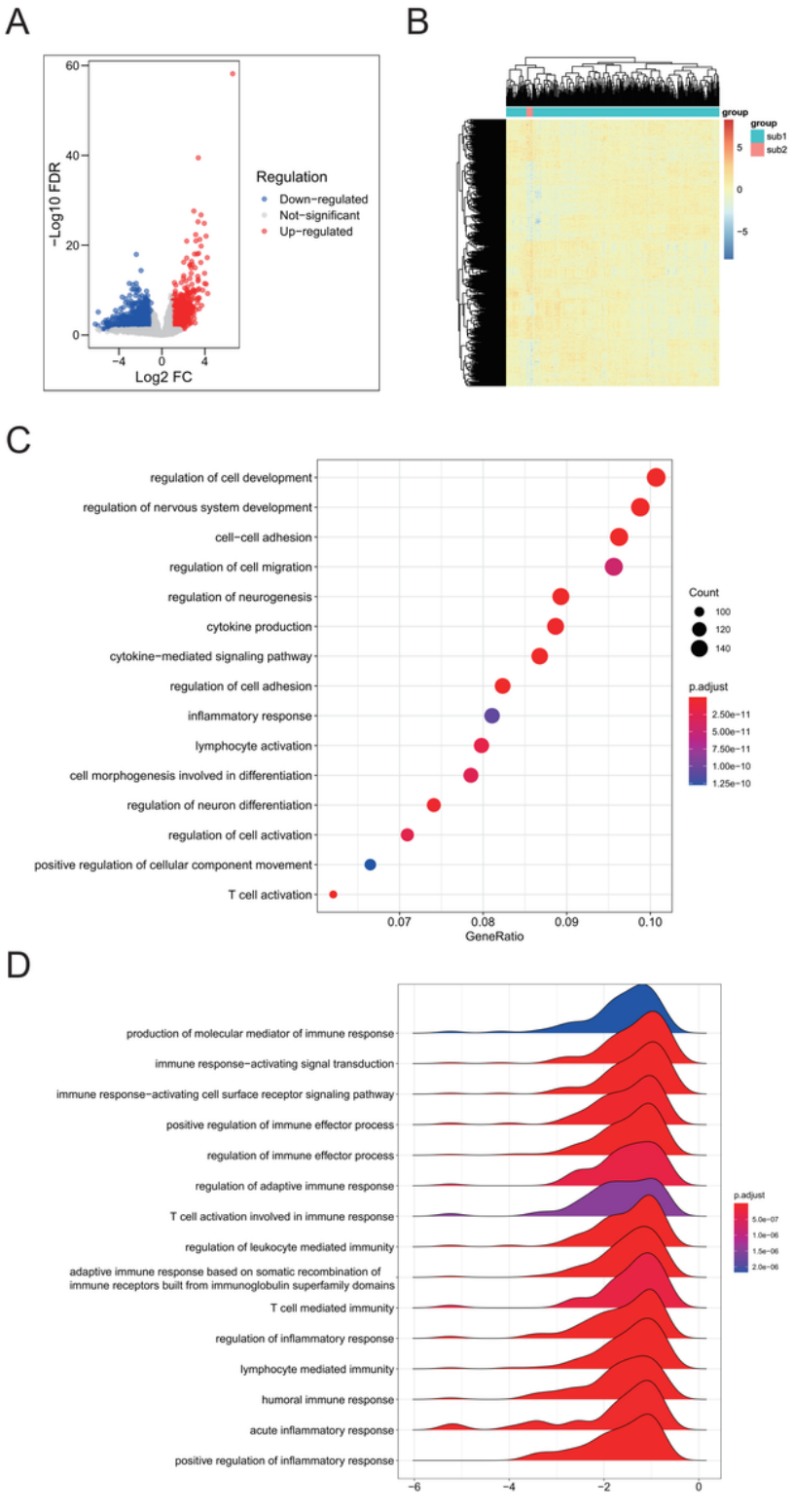
Overview of mutation information in BLCA patients (A-C) Variant classifications (A), variant types (B) and SNV classes (C) in BLCA samples. SNP: single nucleotide polymorphism, INS: insertion, DEL: deletion, SNV: single nucleotide variants. (D) Variants per samples. (E) Variant classification summary. (F) Waterfall plot showing the mutation information for top 15 genes.




## Figure 2

Prognostic FLG wild-type splited into two subtypes (A) Kaplan-Meier (KM) estimates of overall survival (OS) of FLG wild-type and FLG mutant groups in BLCA patients. (B) FLG gene mutation information. (C) Identifying the subtypes of FLG wild-type. ConsensusClusterPlus software was used to divide the samples into 2 subtypes, including Sub1 and Sub2. (D) KM estimates of OS of the Sub1 and Sub2 from

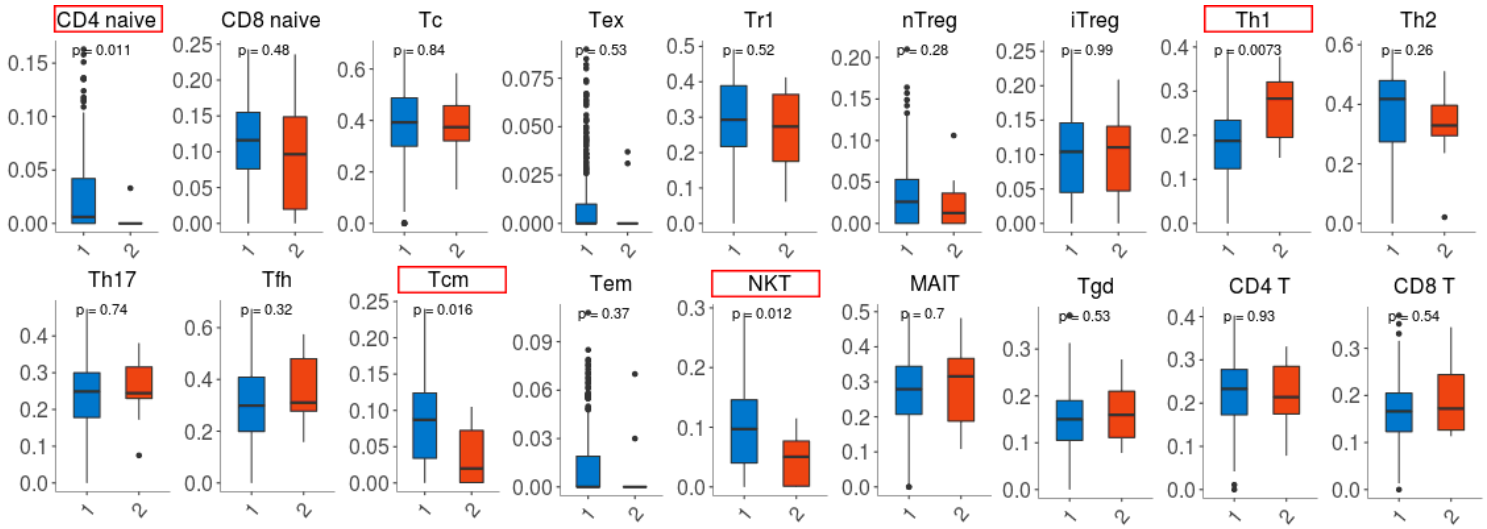
FLG wild-type in BLCA patients. (E) Overall survival time of Sub1 and Sub2 from FLG wild-type in BLCA patients.




## Figure 3

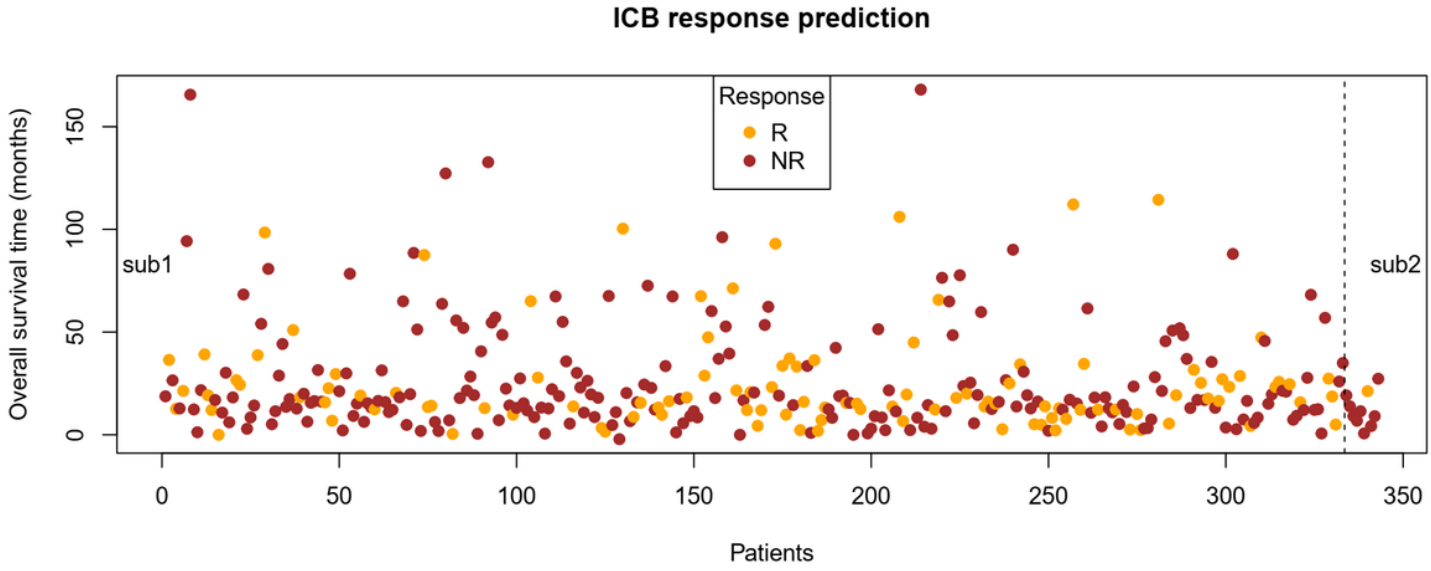
The 2 subtypes of FLG wild-type differed in biological functions (A-B) Volcano map (A) and heat map (B) showing differently expressed genes (DEGs) between the Sub1 and Sub2. (C) The bubble chart showing

top 15 enriched biological process (BP) of DEGs. (D) Ridgeline plot showing the GSEA enrichment analysis.




## Figure 4

Association of 2 subtypes of FLG wild-type with infiltrated immune Cells




## Figure 5

Predicting immune checkpoints for 2 subtypes of FLG wild-type.