Investigating germline susceptibility to renal cell carcinoma within the Canadian population.

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Background

There are large, unexplained, variations in the incidence of renal cell carcinoma (RCC) across the globe. Germline genetic variation contributes strongly to individual differences in susceptibility to cancer, however genetic risk factors for RCC are still poorly understood, indicating the need for large-scale studies investigating susceptibility to RCC among different populations. Large-scale studies investigating pathogenic germline variations in RCC have mostly been limited to individual populations (European, Japanese, etc.), which have shown contrasting results in which genes harbor the most germline pathogenic variants associated to RCC. Additionally, many datasets are limited for studying differences between RCC subtypes due to sample size. This highlights the need to investigate genetic susceptibility to RCC among additional populations to further understand which risk-factors may be driving differences in RCC rates, and which pathogenic variants are associated to each subtype. We conducted an investigation into the genetic susceptibility to RCC within the Canadian population and compared potential risk-genes to those identified within other populations.

Targeted sequencing and identification of pathogenic variants

We conducted targeted sequencing of 19 RCC-related and 27 cancer-predisposition genes in a cohort of 960 Canadian patients with RCC recruited through the Ontario Tumor Biobank. Multiplex PCR based methods (Figure 1) were used to target coding regions for general and RCC susceptibility genes. We called germline variants and identified pathogenic/likely pathogenic variants based on ClinVar classification and those predicted to be loss-of-function (LOF) mutations. Gene-based association tests were conducted between patients with RCC and non-cancer control data from the gnomAD public database (European, non-Finnish population) to identify candidate risk genes for RCC.

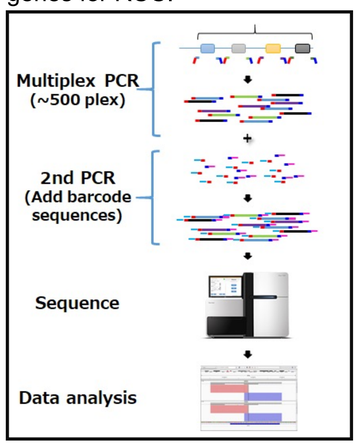

Figure 1. Workflow of multiplex PCR-based methods used to target relevant cancer-susceptibility genes for sequencing.

Patients were stratified into clear cell (ccRCC) and non-clear cell (nccRCC) groupings for the identification of risk genes for each subtype.

Lastly, we compared genes showing significant disease risk to other large population studies of RCC to identify potential differences in RCC susceptibility between Canadian, US, Japanese, European populations.

Identification of risk genes for RCC within the Canadian population

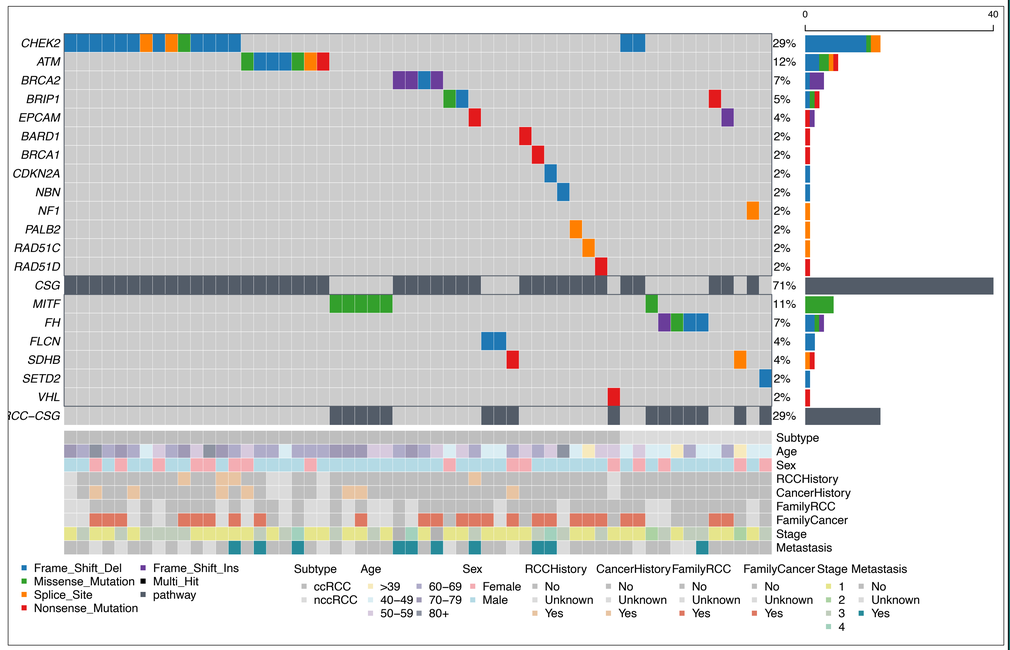

Figure 2. Summary of germline pathogenic variants identified in 68 Canadian RCC patients.

We identified germline pathogenic variants in 7.1% of patients (68/960), with no significant difference in the overall number of germline mutations between ccRCC (8.8%, 52/759) and nccRCC (8.0%, 16/201) patients. The most frequently mutated gene was CHEK2, with 69% of CHEK2 mutations being the c.1100delC variant (11/16 patients), which is an established breast cancer susceptibility allele, and has also been suggested to increase the risk of developing other cancers. Within ccRCC, germline pathogenic variants were most commonly found in CHEK2 (14), ATM (7), BRCA2 (6), and MITF (5). In patients with nccRCC, the most commonly mutated genes were FH (4) and CHEK2 (2). When investigating association to disease compared to the control population, CHEK2, ATM and MITF genes showed significant association to ccRCC. Additionally, FH showed significant association to nccRCC (Table 1).

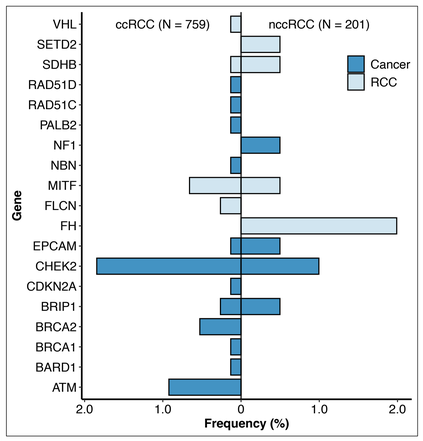

Figure 3. Frequency of PVs identified within cancer susceptibility genes and RCC susceptibility genes in the Canadian cohort.

Gene	No. of subjects with pathogenic variants (%) ccRCC patients (N=759)	gnomAD Control	P-value	OR (95% CI)
MITF	5 (0.66)	3 (0.00)	1.44× 10−9	212.3 (4.0-6.8)
CHEK2	14 (1.84)	474 (0.40)	3.94× 10−5	4.8 (1.0-2.1)
ATM	7 (0.92)	259 (0.22)	0.016	4.5 (0.7-2.2)
Gene	**No. of subjects with pathogenic variants (%) nccRCC patients (N=201)**	**gnomAD Control**	**P-value**	**OR (95% CI)**
FH	4 (2.0)	12 (0.01)	6.14× 10−9	215.1 (4.2-6.4)
SETD2	1 (0.50)	3 (0.00)	0.003	219.6 (3.1-7.2)
MITF	1 (0.50)	3 (0.00)	0.003	217.9 (3.0-7.2)
SDHB	1 (0.50)	20 (0.02)	0.033	43.1 (1.6-5.1)

Table 1. Genes showing significant disease risk for ccRCC and nccRCC compared to the gnomAD non-cancer control group.

Differences in RCC risk genes between populations

We also conducted a burden analysis comparing genes showing significant disease risk to other large studies investigating susceptibility to RCC. We saw no significant difference in germline burden between Canadians and the European population (Yngvadottir et al.), which reflects the largely European-ancestry of the Canadian population. However, when compared to the Japanese population (Sekine et al.), Canadians had significantly more carriers of germline pathogenic variants in MITF and CHEK2, whereas the Japanese population had significantly higher burden in TP53.

Gene	No. of subjects with pathogenic variants (%) Canadian patients (N=960)	Japanese patients (N=1632)	P-value
TP53	0 (0)	11 (0.72)	0.028
MITF	6 (0.63)	0 (0)	0.014
CHEK2	16 (1.67)	7 (0.46)	0.016
Gene	**No. of subjects with pathogenic variants (%) Canadian patients (N=960)**	**US patients (N=254)**	**P-value**
FH	4 (0.42)	7 (2.76)	0.007

Table 2. Gene burden analysis of significant RCC-risk genes between populations.

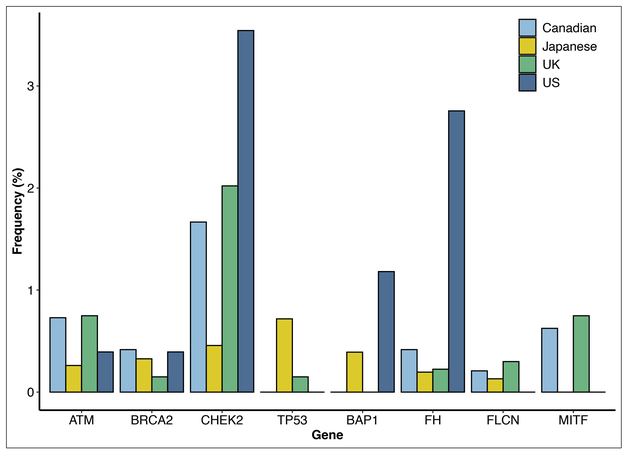

Figure 3. Frequency of germline pathogenic variants within large studies investigating RCC within Canadian, Japanese, European (UK), and US populations.

Conclusions and Future Direction

This study serves as the first investigation into renal cancer susceptibility within the Canadian population. We demonstrate that germline pathogenic variants in CHEK2, ATM, MITF and FH genes may be associated to risk of RCC within Canadians. Additionally, the prevalence of germline pathogenic variants in the Canadian population appears similar to that of the European population, indicating that studies of RCC-susceptibility within the European population may be able to apply for Canadians as well. These findings also provide further insight into differences in RCC-susceptibility around the world, and that investigation of germline risk genes in additional populations are needed to fully understand the heterogeneous susceptibility to RCC.