ORIGINAL ARTICLE
ANALYSIS OF COMMON SOMATIC MUTATIONS IN COLORECTAL CARCINOMA AND ASSOCIATED DYSREGULATED PATHWAYS

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Background: Identification of gene targets and biological pathways involved in colorectal carcinoma (CRC) is essential for better management of patients. Our study aims to highlight common somatic mutations in colorectal carcinoma and to identify dysregulated pathways and gene enrichment based on KRAS and BRAF interaction network analysis. Methods: By using cancer browser tool in COSMIC database, mutation frequencies of the top 20 mutated genes listed for colorectal adenocarcinoma were identified. The most frequent variants of selected genes were explored with ClinVar database which led to identification of protein change along with its cytogenetic location, variant type, variant length and the associated single nucleotide polymorphism (SNP). These identified SNPs were searched in Pakistani database using 1000genome in an attempt to identify common polymorphisms. Using the database ClinicalTrial.gov the number of clinical trials based upon these selected mutations was explored. Enrichment and protein interaction (PI) analysis of KRAS and BRAF was carried out to reveal significant biological pathways associated with these genes. Results: In cumulative data, among all variants about 57% of substitution mutations are observed to be G>A including mutations in KRAS, Tp53, SMAD4, PI3K and NRAS. The mutations of KRAS (c.35G>A), TP53 (c.524G>A) and APC (c.4348C>T) were found to be pathogenic with single nucleotide variation and variant length of 1bp. Searching 1000genome database revealed that 100% of alleles found in East Asian population studied were ‘C’(frequency=1). Significant biological pathways (<0.05) identified by our search include Trk receptor signalling mediated by the MAPK pathway, signalling to p38 via RIT and RIN, signalling to ERKs, Frs2-mediated activation, ARMS-mediated activation and prolonged ERK activation events. Conclusion: Our study highlights the role of genetic profiling in CRC, with emphasis on mutations which may define treatment outcome. Targeting several collateral pathways simultaneously may be further explored to improve colorectal cancer therapeutics.

Keywords: Genetic mutations; Biological Pathways; in-silico; KRAS; BRAF


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INTRODUCTION
Globally, colorectal cancer (CRC) forms a primary cause of cancer associated morbidity and mortality.¹ Asian CRC has been reported to have the highest proportions of both incident cases as well as mortality cases among all ages and genders.² In Pakistan, the prevalence of colorectal cancer has been reported to be about five percent.³ Unfortunately, about 20% of newly diagnosed CRC patients have metastasis at presentation resulting in increased mortality.⁴ The main molecular pathways implicated in CRC include the Chromosome Instability (CIN) Pathway signifying sporadic colon cancer, and the Microsatellite Instability (MSI) Pathway, involving mutations in mismatch repair (MMR) genes. MSI pathway represents hereditary non-polyposis colon cancer as well as some sporadic cases.¹¹ Together, 80% of CRCs are found to have mutations in CIN pathway which defines adenoma carcinoma sequence emerging through mutations of several genes including KRAS, TP53, APC and Trp53 genes.¹² Studies have shown that KRAS mutation is present in about 40% of sporadic CRCs.¹³ However, the KRAS mutation alone cannot lead to malignant transformation. Additional driver mutations, like APC mutation, play a pivotal role in triggering neoplastic changes. Mutated KRAS sends a downstream...
signal to B-type RAF proto-oncogene (BRAF) kinase which results in triggering the mitogen-activated protein kinase (MAPK) signaling cascade.\textsuperscript{14} Furthermore, KRAS mutations in colorectal carcinoma have been reported to be associated with mutations in genes encoding catalytic subunits of PI3K. The PI3K mutations are arise late in adenoma carcinoma sequence and result in proliferation of colorectal cancer cells by evasion of apoptosis.\textsuperscript{15} Since a plethora of genes play a role in causation of CRC a deep insight of molecular aberrations is required to improve patient management. While the world has stepped towards precision medicine, a basic genetic profile is nonexistent in Pakistan. Since our population specific data is lacking, bioinformatics analysis may enable us to discover therapeutically significant mutations and biological pathways. Deeper understanding of genetic alterations in colorectal carcinoma and the functional consequences of these mutations can lead to improved therapeutic approach and better patient management.

**MATERIAL AND METHODS**

The study has been carried out after approval from Ethics Review Committee of Ziauddin University (2861120SHPAT). The selection of genes studied in our research was based upon ‘The Catalogue of Somatic Mutations in Cancer’ (COSMIC) database, which is a standardized repository containing somatic mutation data from diverse sources. By using cancer browser tool in COSMIC database, we selected large intestinal tumours and further chose tissue histology as adenocarcinoma. The list of genes presented upon our search showed how many tumors had been examined in each gene and the mutation frequency of the top 20 mutated genes for large intestinal adenocarcinoma. On applying additional filters of ‘pathogenic’ mutations in ‘tumor samples’ we identified the variants of the top 20 mutated genes. For each gene, using the total samples with mutations, we explored the variants of each gene. The most frequent substitution mutations were identified. Moreover, the most frequent variants of the top four genes among the list of 20 mutated genes were selected to explore ClinVar database which led to identification of protein change along with its cytogenic location, variant type, variant length and the associated single nucleotide polymorphism (SNP). These identified SNPs were searched in Pakistani database using 1000genome in an attempt to identify common polymorphisms. This was followed by exploring the number of clinical trials based upon these selected mutations using the database ClinicalTrial.gov. We applied filter to include clinical trials which are recruiting, active but not recruiting or completed.

Based on the significant therapeutic implications, BRAF and KRAS were selected for Enrichment and protein–protein interaction (PPI) network analysis by using Functional enrichment Analysis tool (FunRich) version 3.1.3 March 2017.\textsuperscript{16} Hypergeometric test, BH and Bonferroni test were applied in FunRich software. By using the Hypergeometric test and p-value correction with the BH method and Bonferroni tests, significant interactions and pathways associated with datasets were identified. After Bonferroni correction, the statistical cut-off of enrichment analyses was kept as $p<0.05$. The biological pathways showing significant association with these KRAS and BRAF were identified.

**RESULTS**

COSMIC database revealed that out of 54229 large intestinal tumors 47443 were carcinomas. Out of these large intestinal carcinomas, 46924 were adenocarcinoma. The Cancer browser page of COSMIC database presented a list of top 20 mutated genes in which the tumor was examined. The most frequent substitution mutations in each gene with their corresponding position and amino acid mutations was observed (Table-1). It was found that the most common type of mutation is missense substitution which constitutes about 86% of all mutations. In cumulative data, among all variants about 57% of substitution mutations are observed to be G>A including mutations in KRAS, Tp53, SMAD4, PI3K and NRAS. We narrowed down our search to mutations of the top 4 genes (Table-1) and researched ClinVar database. The mutations of KRAS (c.35G>A), TP53 (c.524G>A) and APC (c.4348C>T) were found to be pathogenic with Single nucleotide variation and variant length of 1bp. BRAF (c.1799T>A) was found to be interpreted as ‘likely pathogenic’ with variant type Indel and 2bp variant length. The KRAS c.35G>A on searching ClinVar database revealed G12D protein change at cytogenic location of 12p12.1. The associated dbSNP reports rs121913529 at position chr:12:25245350 GRCh38.p12. On exploring Tp53 c.524G>A protein change of R175H R136H, R43H and R16H at cytogenic location of 17p13.1 was revealed. The associated dbSNP reports rs28934578 at position chr17:7675088 GRCh38.p12. For APC, the protein change based on nonsense mutations was found to include R1432*, R1450*, R1167*, R1290*, R1422*, R1425*, R1349*, R1359*, R1409*, R1460*, R1324*, R1391* and R1468*. The dbSNP revealed rs121913322 at position chr5:112839942(GRCh38.p12). Upon researching BRAF c.1799T>A on ClinVar database, the protein change of V600E is found at cytogenic location of 7q34. The dbSNP revealed rs121913377 is revealed to be at position chr7:140753335-140753336(GRCh38.p12). On searching 1000genome database, it was observed that 100 % of alleles found in East Asian population studied (EAS) are ‘C’(frequency =1). The variant rs121913529 (KRAS) has alleles C/A/G/T with ancestral C and
The highest population MAF of <0.01. This variant overlaps 4 transcripts and is associated with 34 phenotypes. For variant rs28934578 (TP53) has C/A/T alleles with ancestral C and highest population MAF of <0.01. This variant overlaps 13 transcripts and is associated with 11 phenotypes. The variant rs12191332 (APC) has alleles C/G/T with ancestral C and highest population MAF of <0.01. This variant overlaps 5 transcripts and is associated with 5 phenotypes. The variant rs121913377 (BRAF) has alleles CA/AT/TT with ancestral CA. This variant overlaps 4 transcripts and is associated with 3 phenotypes.

Upon searching for the identification of these SNPs in our local population it was further highlighted that the patient data is lacking in 1000 genome. To identify clinical trials based on common mutations of CRC, we searched ClinicalTrials.gov database which revealed a total of 5689 clinical trials for CRC. To further narrow our search we applied filter to include clinical trials which are either recruiting, active but not recruiting or completed. The KRAS based clinical trials are found to be mostly carried out in European region with 91 out of the 192 registered trials (Figure 1a). Out of the total of 107 BRAF clinical trials, 55 are identified to be in Europe while 52 in South America (Figure 1b). Interventional studies based on APC and TP53 have also been reported though they do not appear to have significant therapeutic implications (Figure 1c & d).

**Figure 1:** Geographical distribution of number of clinical trials conducted around the world based upon mutation of a=KRAS, b=BRAF, c=APC, d=TP53. The number of trials shown in figure include the clinical trials which are recruiting, are active but not recruiting and the trials which are complete.

**Table 1:** Genetic variants associated with colorectal carcinoma as reported by COSMIC database

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variants (n)</th>
<th>Legacy</th>
<th>Position</th>
<th>CBS Mutation</th>
<th>AA Mutation</th>
<th>%Age</th>
<th>Substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS</td>
<td>99</td>
<td>COSM521</td>
<td>12</td>
<td>c.35G&gt;A</td>
<td>p.G12D</td>
<td>35.88</td>
<td>Missense</td>
</tr>
<tr>
<td>BRAF</td>
<td>142</td>
<td>COSM476</td>
<td>600</td>
<td>c.1799T&gt;A</td>
<td>p.V600E</td>
<td>57.65</td>
<td>Missense</td>
</tr>
<tr>
<td>TP53</td>
<td>253</td>
<td>COSM10468</td>
<td>175</td>
<td>c.524G&gt;A</td>
<td>p.R175H</td>
<td>6.75</td>
<td>Missense</td>
</tr>
<tr>
<td>APC</td>
<td>1026</td>
<td>COSM13127</td>
<td>1450</td>
<td>c.3438C&gt;T</td>
<td>p.R1480*</td>
<td>5.91</td>
<td>Nonsense</td>
</tr>
<tr>
<td>P53K</td>
<td>276</td>
<td>COSM763</td>
<td>545</td>
<td>c.1633T&gt;A</td>
<td>p.E545K</td>
<td>20.96</td>
<td>Missense</td>
</tr>
<tr>
<td>TP53</td>
<td>201</td>
<td>COSM2965</td>
<td>465</td>
<td>c.1394G&gt;A</td>
<td>p.R465K</td>
<td>5.84</td>
<td>Missense</td>
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<tr>
<td>SMAD4</td>
<td>342</td>
<td>COSM14122</td>
<td>361</td>
<td>c.1082G&gt;A</td>
<td>p.R361H</td>
<td>8.95</td>
<td>Missense</td>
</tr>
<tr>
<td>LRPI</td>
<td>326</td>
<td>COSM123609</td>
<td>3837</td>
<td>c.11511G&gt;A</td>
<td>p.M3837I</td>
<td>0.78</td>
<td>Missense</td>
</tr>
<tr>
<td>TCF7L2</td>
<td>118</td>
<td>COSM12406</td>
<td>451</td>
<td>c.1411T&gt;C</td>
<td>p.R471C</td>
<td>3.19</td>
<td>Missense</td>
</tr>
<tr>
<td>FAT4</td>
<td>234</td>
<td>COSM105090</td>
<td>3735</td>
<td>c.11028G&gt;T</td>
<td>p.R3735C</td>
<td>0.34</td>
<td>Missense</td>
</tr>
<tr>
<td>Nras</td>
<td>34</td>
<td>COSM564</td>
<td>12</td>
<td>c.35G&gt;A</td>
<td>p.G12D</td>
<td>16.75</td>
<td>Missense</td>
</tr>
<tr>
<td>KMT2C</td>
<td>381</td>
<td>COSM1179670</td>
<td>309</td>
<td>c.925G&gt;A</td>
<td>p.R309S</td>
<td>2.71</td>
<td>Missense</td>
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<tr>
<td>CTNNB1</td>
<td>143</td>
<td>COSM667</td>
<td>45</td>
<td>c.134C&gt;T</td>
<td>p.S45F</td>
<td>16.0</td>
<td>Missense</td>
</tr>
<tr>
<td>ATM</td>
<td>259</td>
<td>COSM21323</td>
<td>337</td>
<td>c.1099C&gt;T</td>
<td>p.R337C</td>
<td>3.03</td>
<td>Missense</td>
</tr>
<tr>
<td>RNF</td>
<td>67</td>
<td>COSM918170</td>
<td>132</td>
<td>c.394C&gt;T</td>
<td>p.R132C</td>
<td>1.37</td>
<td>Missense</td>
</tr>
<tr>
<td>KMT2D</td>
<td>179</td>
<td>COSM128786</td>
<td>145</td>
<td>c.433C&gt;T</td>
<td>p.R145*</td>
<td>1.37</td>
<td>Missense</td>
</tr>
<tr>
<td>P16</td>
<td>155</td>
<td>COSM17154</td>
<td>223</td>
<td>c.695C&gt;T</td>
<td>p.R232*</td>
<td>4.34</td>
<td>Missense</td>
</tr>
<tr>
<td>POLE</td>
<td>148</td>
<td>COSM917332</td>
<td>286</td>
<td>c.857C&gt;G</td>
<td>p.P286R</td>
<td>4.97</td>
<td>Missense</td>
</tr>
<tr>
<td>AMER1</td>
<td>129</td>
<td>COSM28714</td>
<td>353</td>
<td>c.1057C&gt;T</td>
<td>p.R353*</td>
<td>2.73</td>
<td>Nonsense</td>
</tr>
</tbody>
</table>

COSM= catalogue of somatic mutation, *= stop codon
Signalling to p38 via RIT and RIN showed interaction with each other (Fig. 2). The PPI network showed the interaction network visualization and analysis of the selected KRAS and BRAF 47 genes all of which were found to be lead genes mapped in 5-HSP related biological pathways (p<0.05) associated with each other (Table 2).

The leading biological pathways (p<0.05) associated with these interacting proteins were found to be Trk receptor signalling mediated by the MAPK pathway, signalling to p38 via Ras-like protein in tissues (RIT) and Ras-like protein in neurons (RIN) GTpases, signalling to ERKs, Frs2-mediated activation, Ankyrin-Rich Membrane Spanning Trk tyrosine receptor kinase.

Table-2: Biological Pathways associated with KRAS and BRAF (p<0.05)

<table>
<thead>
<tr>
<th>Biological Pathway</th>
<th>Fold enrichment</th>
<th>p-value (Hypergeometric Test)</th>
<th>Bonferroni Method</th>
<th>BH method</th>
<th>Genes mapped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signalling to p38 via RIT and RIN</td>
<td>392.880075</td>
<td>0.010112963</td>
<td>0.000403783</td>
<td>KRAS; BRAF;</td>
<td></td>
</tr>
<tr>
<td>Signalling to ERKS</td>
<td>209.597134</td>
<td>0.0036684538</td>
<td>0.000736908</td>
<td>KRAS; BRAF;</td>
<td></td>
</tr>
<tr>
<td>Frs2-mediated activation</td>
<td>330.387011</td>
<td>0.003423819</td>
<td>0.00007383</td>
<td>KRAS; BRAF;</td>
<td></td>
</tr>
<tr>
<td>ARMS-mediated activation</td>
<td>349.2509717</td>
<td>0.0012902838</td>
<td>0.000405783</td>
<td>KRAS; BRAF;</td>
<td></td>
</tr>
<tr>
<td>Prolonged ERK activation events</td>
<td>314.3435283</td>
<td>0.0016623132</td>
<td>0.000405783</td>
<td>KRAS; BRAF;</td>
<td></td>
</tr>
</tbody>
</table>

**Figure-2: Protein –Protein Interaction (PPI) network of KRAS and BRAF(selected genes)**

HNRNPC= Heterogeneous nuclear ribonucleoprotein C, RAPIGD3S= Rapi GDP dissociation stimulator 1, RASSF2= Ras Association Domain Family Member 2, RASSF5= Ras Association Domain Family Member 5, FNTA= farnesyltransferase type-1 subunit alpha, FNTB= farnesyltransferase type-1 subunit beta, PIK3CG= Phosphatidylinositol-4, 5-Bisphosphate 3-Kinase Catalytic Subunit Gamma, RASSF2P= Ras guanyl releasing protein 2, SHOC2= Lexine Rich Repeat Scaffold Protein, RALGDS= Raf guanine nucleotide dissociation stimulator, RAF1= Rapidly Accelerated Fibrosarcoma/Serine-Threonine Kinase, BCL2= B-cell lymphoma 2- apoptosis regulator, CALMI= Calmodulin 1, PIK3CA= phosphatidylinositol-4, 5-Bisphosphate 3-Kinase catalytic subunit alpha, OIP5= Opa Interacting Protein 5, YWHAG= Tryptophan 5-Monoxygenase Activation Protein Gamma, YWHAH= Tryptophan 5-Monoxygenase Activation Protein Protein beta, YWHAI= Tryptophan 5-Monoxygenase Activation Protein Eta, YWHAE= Tryptophan 5-Monoxygenase Activation Protein Protein epsilon, LMK1= LIM Domain Kinase 1, HRAS= Harvey rat sarcoma viral oncogene homolog, ARAF= A-Raf protooncogene serine/threonine kinase, YWHAA= Tryptophan 5-Monoxygenase Activation Protein Theta, WHAZ= Trytophan 5-Monoxygenase Activation Protein Zeta, TMM50= translocase of Inner Mitochondrial Membrane 50, AKT1= Akt Serine/Threonine Kinase 1, RAP1A= Ras related protein 1 MAPK1= Mitogen-Activated Protein Kinase 1, HSPA1B= Heat Shock Protein Family A member 1B, PRKCE= Protein Kinase C epsilon, MAP2K1= Mitogen-Activated Protein Kinase Kinase 1, MRAF= Muscle RAS Oncogene Homolog, RHEB= Ras Homolog, MTORC1 Binding, SFRS= Stratifin, PHKB= Phospholipase Kinase Regulatory Subunit beta, MAPK3= Mitogen-Activated Protein Kinase 3, HSPD1= Heat Shock Protein Family A member 8, RAP1GAP= RAP1 GTPase Activating Protein, MAP2K2= Mitogen-Activated Protein Kinase Kinase 2, SOK1= Senescence-Related Kinase 1, HSPA9B1= Heat Shock Protein 90 Alpha Family Class B member 1, HSPA9= Heat Shock Protein Family member 9, HSPA1A= Heat Shock Protein Family member 1A, CDC37= Cell Division Cycle 37, HSPA5= Heat Shock Protein Family member 5, PRKACA= Protein Kinase CAMP Activated Catalytic Subunit Alpha.

FunRich tool was used to perform protein–protein interaction network visualization and analysis of BRAF and KRAS. The PPI network showed the selected KRAS and BRAF 47 genes all of which showed interaction with each other (Figure 2). The leading biological pathways (p<0.05) associated with these interacting proteins were found to be Trk receptor signalling mediated by the MAPK pathway, signalling to p38 via Ras-like protein in tissues (RIT) and Ras-like protein in neurons (RIN) GTpases, signalling to ERKs, Frs2-mediated activation, Ankyrin-Rich Membrane Spanning Trk tyrosine receptor kinase (Table 2).
DISCUSSION
Colorectal carcinoma has one of the highest mutational burdens and several somatic mutations have been associated with CRC. Most commonly implicated genes follow CIN pathway and are characterized by chromosome changes that include somatic copy number alterations caused by aneuploidy, deletions, insertions, amplifications, or loss of heterozygosity. The presence of RAS mutations confers a worse prognosis in early-stage CRC, with higher chances of relapse and reduced overall survival. KRAS-driven cancers are considered to be “undruggable” as they mostly resist therapeutic intervention. Our study reveals c.35G>T substitution resulting in p.G12V amino acid mutation as the most frequent KRAS mutation at position 12. The prognostic effect is associated with KRAS codon 12 mutations and left-sided MSS (Microsatellite Stable) tumors while in metastatic tumours, survival is reduced in KRAS-mutated CRC. More important is their negative predictive value in metastatic CRC, with compelling evidence of primary resistance to anti-EGFR mAbs. The codon 12 and 13 KRAS mutations were the first to be causally implicated in primary resistance to anti-EGFR drugs like cetuximab or panitumumab. Mutation in KRAS genes is an important focus while planning cancer therapy as is associated with resistance to anti-EGFR immunotherapy. While our search identified 5 clinical trials based on KRAS mutations in India, there were none revealed in Pakistan. Target therapies which can successfully treat KRAS mutant CRC are unavailable. Even though clinical trials have revealed that inhibitors for the KRAS G12C mutation show anti-tumor activity, KRAS target therapy is yet to establish. Therefore, understanding biological pathways downstream of KRAS and their link to the cancer phenotype needs to be further explored.

KRAS, as a component of epidermal growth factor receptor (EGFR) pathway, leads to constitutive activation of RAF/MEK/ERK pathway, PI3K signaling via MTOR, and the transcription factor NF-kB. A member of RAF protein family, BRAF, may undergo gain of function mutation triggering MAPK pathway. KRAS and BRAF mutations are considered to be mutually exclusive. Missense mutation at valine 600 residue has been documented constitute 90% of all BRAF mutations while they are reported in about 10% of colorectal carcinomas. Our search on COSMIC database revealed similar report with about 57 percent mutations of BRAF mutations to be missense mutations c.1799T>A at 600 position. BRAF/V600E mutations seem to play a crucial role in CRC as they have been documented to be associated with poor treatment response and unique metastatic spread. Literature shows that V600 mutations have worse prognosis as compared to non V600 mutations show poor treatment responses. Since BRAF mutations have been reported to be independent molecular variable that defines poor survival, the clinical management of CRC patients may be directly affected by BRAF mutational status. Furthermore, BRAF mutant cancers have been reported to be unresponsive to anti-EGFR therapy. There is no reported data regarding BRAF mutational status in Pakistani population. Moreover, our search of ClinicalTrials.gov database did not reveal any registered clinical trials based on BRAF mutations in Pakistan (Figure-1b).

There is no defined regime which is proven to treat all CRC patients with same efficacy. However; efforts to add to existing body of information will eventually result in identifying actionable gene targets and novel therapies. Both KRAS and BRAF have been found to be associated with biological pathways that converge at MAPK signalling. It has been reported that ERK activation can result in unchecked proliferation of intestinal cells, while negative feedback to ERK is associated with drug resistance in CRC. The prolonged ERK activation events that are associated with KRAS and BRAF, therefore, facilitate colorectal carcinogenesis. Literature supports our finding that p38 activates MEK cascade signalling via RIT and RIN (p=0.01) and that ARMS mediated activation triggers MAPK cascade (p=0.01) increasing vulnerability to CRC. MAPK pathway activation also results from phosphorylation of Frs2 (p=0.01) and from Trk activation (p=0.047). Literature shows that Trk and MEK inhibition together can regress cancer progression. The identified pathways play a critical role in signal transduction from activated receptors to their downstream effecter proteins and can trigger unchecked proliferation of intestinal cells. Combination therapies targeting KRAS, BRAF and the associated biological pathways can improve patient prognosis. The presence of altered kinases in CRC have clinical implications and show great potential as predictive biomarkers for the efficacy of conventional and targeted treatments, deserving further research.

CONCLUSION
Our study highlights the significant somatic mutations associated with CRC. Targeting several collateral pathways simultaneously may be further explored to improve colorectal cancer therapeutics. Based on lack of comprehensive database of our population and a huge gap in local research, studies focused on molecular profiling of our genetically
distinct population are warranted. With Identification of our population specific mutations, we might be able to stratify subpopulations of CRC to better predict outcome and assign therapies.

AUTHOR CONTRIBUTION
SH, AK and TM conceived the project, designed it and retrieved data for this research. UB contributed to statistical analysis. All authors contributed to manuscript writing and editing.

Data Availability
Following links to online repositories may be used to access the data underlying the findings of our study https://cancer.sanger.ac.uk/cosmic http://www.internationalgenome.org/ https://clinicaltrials.gov/ https://www.ncbi.nlm.nih.gov/clinvar/

Data analyzed using Functional Enrichment tool can be accessed using the following link http://www.funrich.org

Conflicts of Interest:
The authors declare no conflict of interest.

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Supplementary Material:
Supplementary sheet 1 shows the top 20 genes associated with colorectal adenocarcinoma as reported by COSMIC database while Supplementary Sheet 2 shows details of PPI network of selected genes (KRAS and BRAF). Supplementary sheet 3 contains names of suggested reviewers.

REFERENCES


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