SHORT COMMUNICATION

# The frequency of NRAS mutation in stool samples of Iranian colorectal cancers compared to Finnish patients

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**Background:** Stools from colorectal cancer patients are noninvasive samples that could be used to compare the frequency of hotspot mutations between two different ethnic cohorts. **Materials and Methods:** We collected stool samples from the Iranian cohort (52 patients and 49 controls) and the Finnish cohort (40 patients and 14 controls). Following stool DNA extraction, we used the AmpliSeq Colon and Lung Cancer panel to prepare DNA libraries before sequencing. **Results:** The Iranian cohort exhibited 35 hotspot mutations in the *BRAF*, *ERBB4*, *FBXW7*, *FGFR1*, *FGFR3*, *KRAS*, *MAP2K*, *MET*, *NRAS*, *PIK3C*, *SMAD4*, and *TP53* genes. In the Finnish cohort, 13 hotspot mutations were found in the *AKT1*, *APC*, *KIT*, *KRAS*, *SMO*, *STK11*, and *TP53* genes. Mutations in *NRAS* and *FGFR3* were observed only in the Iranian cohort, while *APC* mutations were exclusive for the Finnish cohort. **Conclusion:** Genes involved in MAPK and PI3K-MAPK pathways showed a higher frequency of mutations in Iranian patients which may have therapeutic implications.

**Key words:** Colorectal, DNA, Finnish, Iranian, MAPK, mutations, NRAS

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## INTRODUCTION

Ethnicity is a sociodemographic factor that affects differences between hotspot mutations frequently encountered in colorectal cancer (CRC).[1,2] Mutations in the RAS pathway (such as *KRAS* G12) and mutations in MAPK pathway (such as *MAP2K1* and *NRAS*) are frequently altered in CRC patients of African heritage.[3] Furthermore, comparisons of mutation frequency between fresh frozen tissues with formalin-fixed paraffin-embedded (FFPE) tissues or with plasma samples usually revealed high concordance.[4,5] As stool samples provide a noninvasive means to study mutations,[6] we demonstrated the feasibility of applying

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next-generation sequencing (NGS) on stool from CRC patients with high coherency between both stool and tissue samples.[7,8] In the current study, we applied our established stool-NGS protocol to explore the frequency of mutations between two CRC ethnic groups.

## MATERIALS AND METHODS

In this pilot prospective study, we collected stool samples from 52 Iranian and 40 Finnish CRC patients and from 49 Iranian and 14 Finnish noncancerous individuals as controls. There were challenges harmonizing the collection of stool from the control individuals. All Finnish controls were healthy asymptomatic individuals from whom we collected stool samples without endoscopy procedures. Finnish diagnostic guidelines do not include endoscopy as a primary procedure but rather at advanced clinical stage. Furthermore, the mean age of Finnish controls was lower than the mean age of Finnish CRC patients (44 and 72 years old, respectively). For Iranian controls, stool samples were collected from individuals with gastrointestinal symptoms assigned for endoscopy who were subsequently shown to be cancer free (no malignant, premalignant, atypic, or dysmorphic lesions). In addition, we followed up Iranian controls with regular endoscopy up to 33 months to ensure that there were no premalignant or malignant changes occurring.

The Ethics Committee of Isfahan University of Medical Science approved the Iranian study (ir.mui.rec. 1394.3.936). The Hospital District of Helsinki and Uusimaa review board approved the Finnish study (ethical permission number 351/13/03/02/2014). Informed written consent was obtained from all recruited participants.

We stored samples at −80°C until DNA extraction. For DNA extraction, we used the QIAamp DNA stool mini kit (Qiagen GmbH, Hilden, Germany) for Iranian stool samples and PSP® Spin Stool DNA Plus Kit (Stratec Biomedical, Birkenfeld, Germany) for Finnish samples, according to the manufacturer's instructions. DNA libraries were prepared from 20 ng of DNA per sample using Ion AmpliSeq Colon and Lung Cancer panel v2 (Life Technologies, California, United States). Coverage analysis was performed using the coverage analysis plug-in (v4.0–r77897) (Thermo Fisher Scientific). Detailed description of the panel composition and sequencing pipeline has been published previously.[7] Both Wilcoxon rank-sum and Pearson's Chi-squared tests were used to calculate statistical differences.

## RESULTS

By applying the Phred quality score at 15 (quality metric which estimates the probability of a base was called incorrectly, given on a negative log scale)[9] and mutant allele frequency at 3%,[10] we excluded the samples of two patients and one control due to DNA quality issues from the Iranian cohort, resulting in an NGS success rate of 96% and 98% in patients and controls, respectively. In the Finnish cohort, the samples of five patients and one control were excluded due to poor DNA quality, resulting in a success rate of 87.5% and 92.9% for patients and controls, respectively. The mean depth was 1270 in Iranian patients and 1151 in Finnish patients.

Hotspot mutations identified in Iranian patients were in the *BRAF*, *ERBB4*, *FBXW7*, *FGFR1*, *FGFR3*, *KRAS*, *MAP2K*, *MET*, *NRAS*, *PIK3C*, *SMAD4*, and *TP53* genes [Supplementary Table 1]. The most frequently mutated gene was *TP53* followed by *NRAS*, *FGFR3*, and *SMAD4*. The most frequently occurring mutations were codon 273 of *TP53* (four patients) and *NRAS* codon 12 (three patients), codon 61 (two patients), and codon 13 (one patient) mutations. Finnish patients had hotspot mutations in the *AKT1*, *APC*, *KIT*, *KRAS*, *SMO*, *STK11*, and *TP53* genes. *TP53* was the most frequently mutated gene, followed by *APC* and *KRAS*. We reported 35 hotspot mutations in 16/50 Iranian patients (average mutation/patient = 2.19), and 13 mutations in 9/35 Finnish patients (average/patient = 1.44) ($P$ = 0.6, Wilcoxon rank-sum). MAPK pathway genes studied in our pilot were *BRAF, ERBB4, FGFR3, KRAS, MAP2K, MET, NRAS,* and *PIK3CA*. The frequency of mutations in these genes was higher ($P$ = 0.1, Pearson's Chi-squared) in the Iranian patients compared to the Finnish cohort [Figure 1].

The Iranian controls showed nine hotspot mutations in the *ALK*, *BRAF*, *DDR2*, *EGFR*, *PIK3CA*, *PTEN*, and *TP53* genes in addition to 22 novel mutations in the *DDR2*, *EGFR*, *ERBB2*, *ERBB4*, *FBXW7*, *FGFR2*, *FGFR3*, *MET*, *PIK3CA*, *PTEN*, *SMAD4*, and *STK11* genes. The Finnish controls showed only two novel mutations in *ALK* and *STK11* genes.[7]

## DISCUSSION AND CONCLUSIONS

As we were unable to collect adequately harmonized control samples due to differences in sample collection and endoscopy guidelines between the Iranian and Finnish cohorts, a comparison of results between these cohorts should be done with caution. On the other hand, our patient cohort data were selected with uniform criteria that permit comparison between the Iranian and the Finnish cohort cancer samples.

We observed a clear difference in the frequency of mutations in the MAPK and PI3K-MAPK pathways between both cohorts, and these findings are consistent with previous observations.[8] Similarly, Myer *et al*. reported frequent

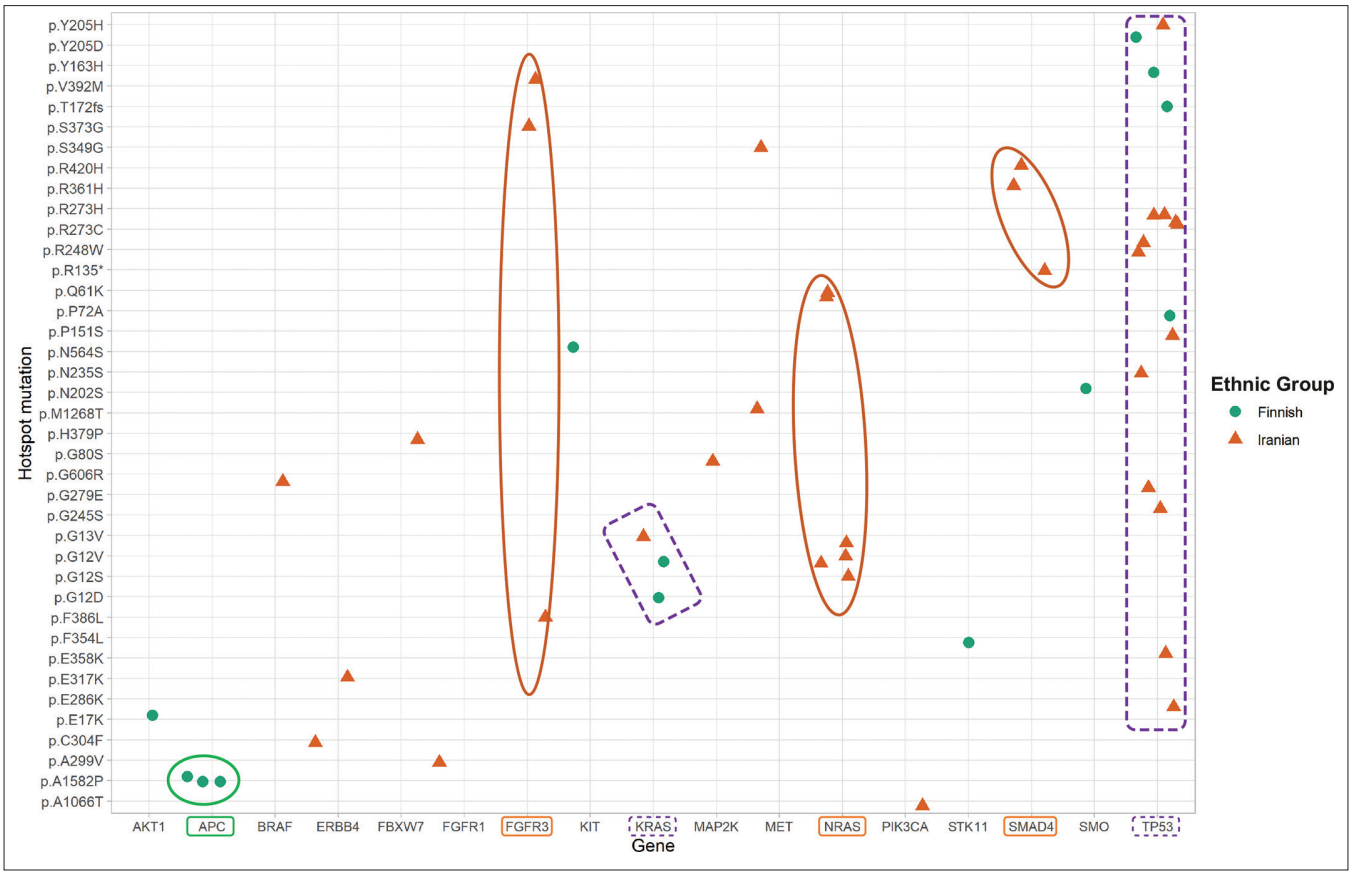


**Figure 1:** Mutations in stool samples from the Iranian patient cohort were primarily in the MAPK pathway and PI3K-MAPK pathway (circled in orange) with six Iranian patients revealing *NRAS* mutations. The Y-axis illustrates the mutated oncogenic protein. CRC = Colorectal cancer

alterations in MAPK pathway genes in CRC patients of African ancestry, with fewer *BRAF* mutations compared to those of European ancestry.[3] In addition, *NRAS* mutations were found in 6.9% of FFPE samples from Tunisian CRC patients.[11] In our series, *SMAD4* gene mutations (wnt/β-catenin pathway) were also exclusive to the Iranian cohort [Figure 1].

Mutations in the *APC* gene were observed in Finnish cancer samples [Figure 1]. All three reported *APC* mutations were in codon 1582 in the central part of the reading frame and close to the mutation clustering region and Ser-Ala-Met-Pro repeats, in which almost 70% of somatic mutations occur.[12,13] Differences in the allele frequency of the same genetic mutation have been reported also in different diseases.[14] CRC-related risk variables, such as dietary habits, smoking status, and lifestyle, differ remarkably between the Iranian and Finnish ethnic groups. Population demography and ethnic diversity exert genetic drift on the same genes by different levels.[15,16] In addition to genetic differences, it is challenging to identify the drivers behind the difference in mutation frequency status, and more studies with a larger sample size from both ethnic groups are needed. However, these observations may have important therapeutic implications. Our study also shows the usefulness of stool samples for performing mutation profiling in CRC research.

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## Conflicts of interest

There are no conflicts of interest.

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**Supplementary Table 1: Genes and mutations reported for each patient in both Iranian and Finnish cohorts**

| Patient ID | Gene | Mutation | Ethnic group |
|---|---|---|---|
| T1 | *BRAF* | 606R | Iranian |
| | *ERBB4* | E317K | Iranian |
| | *FGFR3* | V392M | Iranian |
| | *TP53* | E358K | Iranian |
| | *TP53* | G279E | Iranian |
| T10 | *TP53* | P151S | Iranian |
| T13 | *NRAS* | G13V | Iranian |
| T15 | *KRAS* | G13V | Iranian |
| T23 | *FGFR3* | F386L | Iranian |
| | *MET* | M1268T | Iranian |
| T27 | *FGFR3* | S373G | Iranian |
| | *MAP2K* | p.G80S | Iranian |
| | *TP53* | Y205H | Iranian |
| | *TP53* | N235S | Iranian |
| T3 | *TP53* | R273C | Iranian |
| T4 | *NRAS* | Q61K | Iranian |
| | *TP53* | R248W | Iranian |
| T40 | *NRAS* | G12V | Iranian |
| | *TP53* | R248W | Iranian |
| T43 | *NRAS* | G12V | Iranian |
| | *SMAD4* | R361H | Iranian |
| | *TP53* | R273H | Iranian |
| T44 | *TP53* | G245S | Iranian |
| T45 | *FBXW7* | H379P | Iranian |
| T5 | *NRAS* | Q61K | Iranian |
| | *TP53* | E286K | Iranian |
| *T52* | *TP53* | R273C | Iranian |
| T6 | *TP53* | R273H | Iranian |
| T9 | *ERBB4* | C304F | Iranian |
| | *FGFR1* | A299V | Iranian |
| | *MET* | S349G | Iranian |
| | *NRAS* | G12S | Iranian |
| | *PIK3CA* | A1066T | Iranian |
| | *SMAD4* | R135* | Iranian |
| | *SMAD4* | R420H | Iranian |
| F12 | *APC* | p.A1582P | Finnish |
| | *TP53* | p.P72A | Finnish |
| F20 | *KIT* | p.N564S | Finnish |
| F21 | *APC* | p.A1582P | Finnish |
| F22 | *SMO* | p.N202S | Finnish |
| | *STK11* | p.F354L | Finnish |
| F23 | *APC* | p.A1582P | Finnish |
| F28 | *AKT1* | p.E17K | Finnish |
| | *KRAS* | p.G12D | Finnish |
| F31 | *TP53* | p.Y205D | Finnish |
| F55 | *KRAS* | p.G12V | Finnish |
| | *TP53* | p.T172fs | Finnish |
| F68 | *TP53* | p.Y163H | Finnish |