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

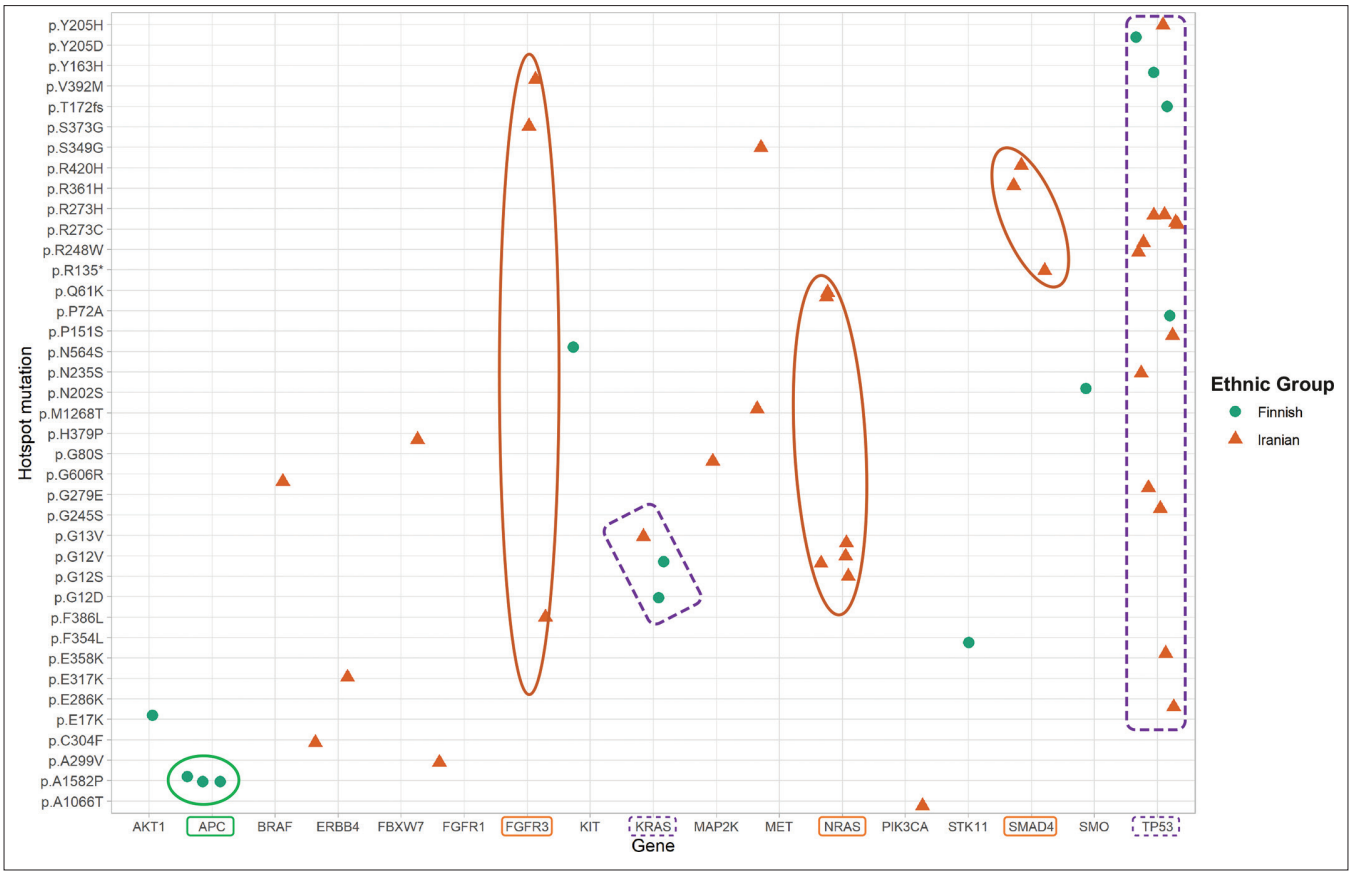

Figure 1: Mutations in stool samples from the Iranian patient cohort were primarily in the MAPK pathway and PI3K-MAPK pathway (circled in orange) with six Iranian patients revealing *NRAS* mutations. The Y-axis illustrates the mutated oncogenic protein. CRC = Colorectal cancer

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.

REFERENCES

1. Petrick JL, Barber LE, Warren Andersen S, Florio AA, Palmer JR, Rosenberg L. Racial disparities and sex differences in early- and late-onset colorectal cancer incidence, 2001-2018. Front Oncol 2021;11:734998.
2. Rhead B, Hein D, Pouliot Y, Guinney J, De La Vega FM, Sanford NN. Genetic ancestry differences in tumor mutation in early and average-onset colorectal cancer. J Clin Oncol 2022;40:3620.
3. Myer PA, Lee JK, Madison RW, Pradhan K, Newberg JY, Isasi CR, *et al*. The genomics of colorectal cancer in populations with African

and European ancestry. Cancer Discov 2022;12:1282-93.
4. Gao XH, Li J, Gong HF, Yu GY, Liu P, Hao LQ, *et al*. Comparison of fresh frozen tissue with formalin-fixed paraffin-embedded tissue for mutation analysis using a multi-gene panel in patients with colorectal cancer. Front Oncol 2020;10:310.
5. Min S, Shin S, Chung YJ. Detection of KRAS mutations in plasma cell-free DNA of colorectal cancer patients and comparison with cancer panel data for tissue samples of the same cancers. Genomics Inform 2019;17:e42.
6. Youssef O, Sarhadi VK, Lehtimäki L, Tikkanen M, Kokkola A, Puolakkainen P, *et al*. Mutations by next generation sequencing in stool DNA from colorectal carcinoma patients – A literature review and our experience with this methodology. J Anal Oncol 2016;5:24-32.
7. Youssef O, Sarhadi V, Ehsan H, Böhling T, Carpelan-Holmström M, Koskensalo S, *et al*. Gene mutations in stool from gastric and colorectal neoplasia patients by next-generation sequencing. World J Gastroenterol 2017;23:8291-9.
8. Armengol G, Sarhadi VK, Ghanbari R, Doghaei-Moghaddam M, Ansari R, Sotoudeh M, *et al*. Driver gene mutations in stools of colorectal carcinoma patients detected by targeted next-generation sequencing. J Mol Diagn 2016;18:471-9.
9. Hawkins C, Yu LX. Recent progress in alfalfa (*Medicago sativa* l.) genomics and genomic selection. Crop J 2018;6:565-75.
10. Nevala SM, Knuuttila A, Knuutila S, Sarhadi VK. Concordant results of epidermal growth factor receptor mutation detection by real-time polymerase chain reaction and ion torrent technology in non-small cell lung cancer. J Clin Respir Dis Care 2016;2:1.
11. Jouini R, Ferchichi M, BenBrahim E, Ayari I, Khanchel F, Koubaa W, *et al*. KRAS and NRAS pyrosequencing screening in Tunisian colorectal cancer patients in 2015. Heliyon 2019;5:e01330.
12. Lüchtenborg M, Weijenberg MP, Roemen GM, de Bruïne AP, van den Brandt PA, Lentjes MH, *et al*. APC mutations in sporadic colorectal carcinomas from the Netherlands cohort study. Carcinogenesis 2004;25:1219-26.
13. Christie M, Jorissen RN, Mouradov D, Sakthianandeswaren A, Li S, Day F, *et al*. Different APC genotypes in proximal and distal sporadic colorectal cancers suggest distinct WNT/β-catenin signalling thresholds for tumourigenesis. Oncogene 2013;32:4675-82.
14. Mori M, Yamada R, Kobayashi K, Kawaida R, Yamamoto K. Ethnic differences in allele frequency of autoimmune-disease-associated SNPs. J Hum Genet 2005;50:264-6.
15. Huang T, Shu Y, Cai YD. Genetic differences among ethnic groups. BMC Genomics 2015;16:1093.
16. Gurdasani D, Barroso I, Zeggini E, Sandhu MS. Genomics of disease risk in globally diverse populations. Nat Rev Genet 2019;20:520-35.

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