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Article

TP53 Arg72Pro Polymorphism and Pancreatic Cancer Susceptibility: Assessing rs1042522 as a Risk Factor for Pancreatic Cancer

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Abstract: Background: Pancreatic cancer (PaC) is expected to become the 2nd leading cause of death by 2030 death in Western countries. There is a need to pinpoint high-risk populations since extensive screening would be economically impractical. **Methods:** This study has focused on the polymorphic variants of gene TP53 involved in pancreatic cancerogenesis by next generation sequencing. **Results:** The analysis revealed the TP53 variant rs1042522 in 30 out of 35 patients, with a cytosine (C) replaced by a guanine (G), hence inserting an Arginine in the final protein instead of a Proline. The presence of the rs1042522 variant confers an Odds Ratio of 6,11 for PaC and an OR of 20 for homozygosity G/G when comparing our cohort of PaC patients to a healthy population from the 1000GenomeProject. **Conclusion:** This finding could identify a very high risk population deserving to be screened for PaC, even though a wider validation of rs1042522 as a risk factor is needed. **Impact:** Those preliminary data may open the way for identification of a population more prone to developing pancreatic cancer.

Keywords: pancreatic cancer; TP53; polymorphism; rs1042522; Arg72Pro

1. Background

Pancreatic cancer (PaC) is the 4th cancer-related cause of death in Western countries and is estimated to become the 2nd by 2030 [1]. It has a high mortality rate since only a small percentage of patients are diagnosed in a resectable, hence curable, stage. Approximately 80% of patients are not amenable to resection at diagnosis due to metastatic disease or local infiltration, and another 41.4% of patients candidate to surgery have unresectable tumors found during exploratory laparoscopy/tomy [2]. The economic burden of a hypothetical pancreatic screening restrains from offering a second level imaging to populations with increased odds ratio, from 1.7 to 2.2, for pancreatic cancer. This includes those affected by obesity, pancreatitis and diabetes [3]. Pancreatic cancer screening in asymptomatic patients is impractical. To detect a case, one should screen over 17000 people and to find a resectable, hence curable, patient, over 35000 people should be screened [4]. Finding new genomic susceptibilities to PaC in these high risk populations might narrow the field for early diagnosis.

This study is part of a larger one focusing on the genomic and somatic landscape of the four major driver genes involved in pancreatic cancerogenesis: KRAS, TP53, SMAD4, CDKN2A.

In particular, we focused on the analysis of TP53 polymorphism in a PaC cohort compared to a reference healthy population.

2. Methods

The Institutional Review Board of the University of Sapienza approved the protocol (Prot. 243 SA_2018, 12/12/2018), where every participating center had to subscribe to the IRB. As per the Helsinki Declaration, the protocol was registered at clinicaltrials.gov with the identification number NCT03524677. All consecutive naive patients with non-metastatic PaC who are applicable for upfront pancreatic resection have been enrolled on a multi-center basis from 2017 to 2020. The enrollment took place in 3 institutions: University Hospital Sant’Andrea (Rome), the promoter, University Hospital A. Gemelli (Rome) and Community Hospital G. Salvini (Garbagnate Milanese, MI). After a signed informed consensus, blood samples in EDTA were preoperatively collected and centrifuged twice within 1 hour at 2300 rpm for 10 minutes. Plasma that was obtained was preserved and stored at -80C°. A protected anonymous multi parametric database was filled with relevant information about the patients and disease. Circulating free DNA (cfDNA) was extracted from 1 to 3mL of plasma samples using a Norgen RNA/DNA purification kit as indicated by the manufacturer protocol procedure and sequenced with a Myriapod® NGS 56G Onco panel (Diatech Pharmacogenetics) on the MiniSeq™ Illumina platform for the analysis of hot-spot mutations of 56 genes. The panel allowed us to obtain 263 amplicons, with lengths from 92 to 184 bases, covering the hot-spots and surrounding regions.

A comparison with a reference population was then carried out with data from 1000GenomeProject [5] on healthy subjects from central Italy (Tuscany).

The statistical analysis has been carried out with the online Odds Ratio Calculator [MedCalc Software Ltd. Odds ratio calculator. https://www.medcalc.org/calc/odds_ratio.php (Version 20.115; accessed September 21, 2022)].

This study has been reported according to the STrengthening the REporting of Genetic Association studies (STREGA) which is an extension of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement [6].

3. Results

3.1. TP53 Polymorphism

Patients characteristics are listed in Table 1. Next generation sequencing revealed the rs1042522 variant of gene TP53 in 30 out of 35 patients. In 4 patients it yielded a low coverage (<500 reads) with a variant frequency of >99%, therefore making them included in the analysis. In all 30 patients with rs1042522, a cytosine (C) was replaced by a guanine (G), leading to a amino acid change from Proline to Arginine in position 72. The five patients without any call for rs1042522 were classified as homozygous C/C, with a Pro72 phenotype. 19 patients with rs1042522 and a VF >99% were deemed as polymorphic G/G homozygotes, with an Arg72 phenotype. 11 patients in between were presumed to be heterozygous C/G-G/C, with a mixed phenotype.

Table 1. PaC Cohort: patients characteristics.

Clinical Characteristics	
Sex (M/F)	20/15
Age (yr)	68 (range 45-81)
M0/1	29/6

TP53 rs1042522	85,7%
TP53 homozygous C/C	14,3%
Variant Frequency range	49,93-100%

3.2. TP53 Arg72Pro in Local Population

A comparison with the public repository Ensemble [5] from 1000GenomeProject was conducted. The healthy population from Tuscany (central Italy) held in this library, accounts for 6 homozygous G/G, 54 homozygous C/C and 47 heterozygous C/G-G/C [7]. The presence of the rs1042522 variant confers an OR of 6,11 (95%CI 2.20-16.94, p=.0005) towards pancreatic cancer when comparing our cohort of PaC patients to a healthy population coming from the same geographical area of Tuscany-central Italy (Table 2a). Moreover, if we consider only subjects deemed homozygous for G alleles in exon 4, locus 72, they present an OR of 20 (95%CI 6.93-57.61, p<.0001) towards pancreatic cancer (Table 2b).

Table 2. TP53 polymorphism, PaC cohort vs healthy subjects.

a			b		
ODDS RATIO = 6,11	PaC patients	TSI Healthy individuals	ODDS RATIO = 20	PaC patients	TSI Healthy individuals
G/G - C/G - G/C	30	53	HOMOZYGOUS G/G	19	6
HOMOZYGOUS C/C	5	54	C/C - C/G - G/C	16	101

[a] G allele carriers (G/G-G/C-C/G, rs1042522+) vs C/C homozygous (rs1042522 absent); (b) Homozygous G/G vs C allele carriers (C/C-G/C-C/G).

4. Discussion

First discovered in 1986, p53 has two main species: one has an Arginine in position 72 that has a long positive charged side chain and the other, which has a Proline in the same position, has a small nonpolar side chain [8]. Rs1042522 is a single nucleotide variant (SNV) caused by the substitution of the wild type guanine (G) to cytosine (C) as in our findings, or adenosine (A) or thymine (T) [7]. According to the reference SNP (rs) report from the National Library of Medicine [9], G frequency ranges 0.11-0.495. 1000Genomes Project Phase 3 reports a frequency of G allele of 46% worldwide, being 0.276 in a Tuscan population. This European subpopulation accounts for 107 patients from central Italy, including the 6 patients with presented homozygous GG alleles in position 72 of TP53 gene [7].

Assuming that all patients from our cohort with a variant frequency >99% are likely homozygous G/G and that patients without any call for rs1042522 are C/C homozygous, our population had a C allele frequency of 0.3 and a G allele frequency of 0.7.

Regarding the Hardy Weinberg Equilibrium effect on case-control studies [10], according to which disequilibrium in the genetic variation of a population can be charged to different genetic background, inbreeding or genotyping problems, we believe that the multicentric enrollment along with the comparison with a public database overcome its effect on our results.

The Arg72 variant induces apoptosis five times better than the Pro72 variant, while the Pro72 represses the G1 phase of cell cycle [11,12]. This higher apoptotic potential is the mechanism thought to weaken the reserves of pancreatic beta cells leading to insulin depletion, causing diabetes. As a matter of fact, rs1042522 has been associated with increased odds of diabetes mellitus, [13] one of the conditions tightly linked to pancreatic cancer, both as a cause and as an early effect. New onset diabetic patients, within 3 years from diagnosis, have a prevalence of PaC of 1%, while long-standing type 2 DM patients have a RR of 1.8-2.1 for developing PaC [14]. If confirmed, the association of GG features to an increased risk of PaC could identify a very high risk population deserving to be screened.

Interestingly rs1042522 gives a susceptibility to gastric and esophageal cancer to Northwestern Chinese populations [15], as well to other cancer types such as gliomas, lung, bladder, prostate, colorectal and breast cancer [16]. On the other hand, a recent metanalysis showed that no association has been found between TP53 Arg72Pro and risk of ovarian cancer [17].

To the best of our knowledge, TP53 Pro72Arg polymorphism has been already described in 3 pancreatic cancer populations from Japan [18,19] and Czech Republic [20]. One study genotyped from FFPE tissues and showed an abnormal high C allele frequency of 0.8 [18]. The second and the latter genotyped from peripheral lymphocytes and have comparable C allele frequency of 0.29 - 0.39 [19,20]. In both cases it was hypothesized a PaC higher risk in C allele carriers.

Despite our results in allele frequency are in line with those of Sonoyama [19] and Naccarati [21], we draw opposite conclusions for our population; in a similar way, p53 exon 4 (Pro) seems to be protective also for head and neck cancer in the Italian population [21].

Limits of this study are the lack of a matched control population as it compares a cohort of patients to a public database, moreover the small sample size along with the great variability of rs1042522 all over Italian population make the results not transposable on a larger scale, hence the need for a prospective large scale validation.

PaC is a major cause of death worldwide due to late diagnosis and the lack of screening programs. This results in a need to pinpoint high risk populations. TP53 is one of the four mountain genes responsible for PaC cancerogenesis and in our PaC population its polymorphism rs1042522 seemed to confer a 6 to 20 fold increased OR for PaC compared to a reference population. Further studies are needed to validate this hypothesis in order to improve patient selection for second level investigations and PaC early diagnosis.

Authors' Contributions: Study concepts and design: Laura Antolino, Stefania Scarpino and Francesco A. D'Angelo. Data acquisition: Laura Antolino, Roberto Santoro, Germana de Nucci, Giuseppe Bianco and Salvatore Agnes. Quality control of data and algorithms: Stefania Scarpino and Gianluca Lopez. Data analysis and interpretation: Laura Antolino, Paolo Aurello, Giuseppe Nigri, Stefano Valabrega, Giovanni Ramacciato, Germana de Nucci. Statistical analysis: Laura Antolino, Niccolò Petrucciani. Manuscript preparation: Laura Antolino and Michaela Segreto. Manuscript review and editing: Stefania Scarpino, Paolo Aurello, Niccolò Petrucciani, Stefano Valabrega, Michaela Segreto, Roberto Santoro, Giuseppe Nigri, Salvatore Agnes, Giovanni Ramacciato and Francesco A. D'Angelo. All authors have read and agreed to the published version of the manuscript.

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Ethical approval and consent to participate: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Sapienza Università di Roma (protocol code 243 SA_2018, 12/12/2018). Patients have subscribed a written informed consent previous to their enrollment.

Patient consent for publication: Informed consent was obtained from all subjects involved in the study for both enrollment and publication of data.

Availability of data and materials: The data obtained from the Pancreatic Cancer cohort of patients presented in this study are freely available at the online public repository OSF at <https://osf.io/b28ne/> and on request from the corresponding author. The data from the 1000GenomeProject is openly available at http://may2021.archive.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=17:7675654-7676654;v=rs1042522;vdb=variation;vf=104315472.

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References

1. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid liver, and pancreas cancer in United States. *Cancer Res.* 2014; 74: 2913-2921
2. Allen VB, et al. Diagnostic accuracy of laparoscopy following computed tomography (CT) scanning for assessing the resectability with curative intent in pancreatic and periampullary cancer. *Cochrane Database Syst Rev.* 2016 Jul 6;7:CD009323
3. Ming-Chu Chang, Jau-Min Wong, Yu-Ting Chang Screening and early detection of pancreatic cancer in high risk population *World J Gastroenterol* 2014 Mar 7;20(9):2358-64. doi: 10.3748/wjg.v20.i9.2358.
4. Kim JE, et al. Clinical usefulness of carbohydrate antigen 19-9 as a screening test for pancreatic cancer in an asymptomatic population. *J Gastroenterol Hepatol.* 2004. PMID: 14731128
5. Kevin L Howe, Premanand Achuthan, James Allen, Jamie Allen, Jorge Alvarez-Jarreta, M Ridwan Amode, Irina M Armean, Andrey G Azov, Ruth Bennett, Jyothish Bhai, Konstantinos Billis, Sanjay Boddu, Mehrnaz Charkhchi, Carla Cummins, Luca Da Rin Fioretto, Claire Davidson, Kamalkumar Dodiya, Bilal El Houdaigui, Reham Fatima, Astrid Gall, Carlos Garcia Giron, Tiago Grego, Cristina Guijarro-Clarke, Leanne Haggerty, Anmol Hemrom, Thibaut Hourlier, Osagie G Izuogu, Thomas Juettemann, Vinay Kaikala, Mike Kay, Ilias Lavidas, Tuan Le, Diana Lemos, Jose Gonzalez Martinez, José Carlos Marugán, Thomas Maurel, Aoife C McMahon, Shamika Mohanan, Benjamin Moore, Matthieu Muffato, Denye N Oheh, Dimitrios Paraschas, Anne Parker, Andrew Parton, Irina Prosovetskaia, Manoj P Sakthivel, Ahamed I Abdul Salam, Bianca M Schmitt, Helen Schuilenburg, Dan Sheppard, Emily Steed, Michal Szpak, Marek Szuba, Kieron Taylor, Anja Thormann, Glen Threadgold, Brandon Walts, Andrea Winterbottom, Marc Chakiachvili, Ameya Chaubal, Nishadi De Silva, Bethany Flint, Adam Frankish, Sarah E Hunt, Garth R Iisley, Nick Langridge, Jane E Loveland, Fergal J Martin, Jonathan M Mudge, Joanela Morales, Emily Perry, Magali Ruffier, John Tate, David Thybert, Stephen J Trevanion, Fiona Cunningham, Andrew D Yates, Daniel R Zerbino, Paul Flicek **Ensembl** 2021. *Nucleic Acids Res.* 2021, vol. 49(1):884–891
6. Erik von Elm, Douglas G Altman, Matthias Egger, Stuart J Pocock, Peter C Gøtzsche, Jan P Vandenbroucke, for the **STROBE** Initiative The Strengthening the Reporting of Observational Studies in Epidemiology (**STROBE**) Statement: Guidelines for Reporting Observational Studies *PLoS Med.* 2007 Oct; 4(10): e296. Published online 2007 Oct 16. doi: 10.1371/journal.pmed.0040296
7. Ensembl! Available online: http://may2021.archive.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=17:7675654-7676654;v=rs1042522;vdb=variation;vf=104315472 (accessed on jan 5, 2022)
8. Harris, N., Brill, E., Shohat, O., Prokocimer, M., Wolf, D., Arai, N., & Rotter, V. Molecular basis for heterogeneity of the human p53 protein. *Molecular and Cellular Biology*, 1986: 6(12), 4650–4656.
9. https://www.ncbi.nlm.nih.gov/snp/rs1042522?horizontal_tab=true#variant_details last access jul 24, 2021
10. Royo JL. Hardy Weinberg Equilibrium Disturbances in Case-Control Studies Lead to Non-Conclusive Results. *Cell J.* 2021 Jan;22(4):572-574. doi: 10.22074/cellj.2021.7195. Epub 2020 Apr 22.

11. Burgdorf KS, Grarup N, Justesen JM, Harder MN, Witte DR, Jørgensen T, Sandbæk A, Lauritzen T, Madsbad S, Hansen T; DIAGRAM Consortium, Pedersen O. Studies of the association of Arg72Pro of tumor suppressor protein p53 with type 2 diabetes in a combined analysis of 55,521 Europeans. *PLoS One*. 2011 Jan 20;6(1):e15813.
12. Thomas M, Kalita A, Labrecque S, Pim D, Banks L, Matlashewski G. Two polymorphic variants of wild-type p53 differ biochemically and biologically. *Mol Cell Biol*. 1999 Feb;19(2):1092-100. doi: 10.1128/MCB.19.2.1092.
13. Gaulton KJ, Willer CJ, Li Y, Scott LJ, Conneely KN, Jackson AU, Duren WL, Chines PS, Narisu N, Bonnycastle LL, Luo J, Tong M, Sprau AG, Pugh EW, Doheny KF, Valle TT, Abecasis GR, Tuomilehto J, Bergman RN, Collins FS, Boehnke M, Mohlke KL. Comprehensive association study of type 2 diabetes and related quantitative traits with 222 candidate genes *Diabetes*. 2008 Nov;57(11):3136-44. doi: 10.2337/db07-1731. Epub 2008 Aug 4.
14. Roy A, Sahoo J, Kamalanathan S, Naik D, Mohan P, Kalayarasan R. Diabetes and pancreatic cancer: Exploring the two-way traffic. *World J Gastroenterol*. 2021 Aug 14;27(30):4939-4962. doi: 10.3748/wjg.v27.i30.4939.
15. Cao J, Chen Z, Tian C, Yu J, Zhang H, Yang J, Yang W. A Shared Susceptibility Locus in the p53 Gene for both Gastric and Esophageal Cancers in a Northwestern Chinese Population. *Genet Test Mol Biomarkers*. 2020 Dec;24(12):804-811. doi: 10.1089/gtmb.2020.0192. Epub 2020 Dec 7.
16. Eiholzer RA, Mehta S, Kazantseva M, Drummond CJ, McKinney C, Young K, Slater D, Morten BC, Avery-Kiejda KA, Lasham A, Fleming N, Morrin HR, Reader K, Royds JA, Landmann M, Petrich S, Reddel R, Huschtscha L, Taha A, Hung NA, Slatter TL, Braithwaite AW. Intronic TP53 Polymorphisms Are Associated with Increased Δ 133TP53 Transcript, Immune Infiltration and Cancer Risk. *Cancers (Basel)*. 2020 Sep 1;12(9):2472. doi: 10.3390/cancers12092472.
17. Zhang A, Shi TY, Zhao Y, Xiang J, Yu D, Liang Z, Xu C, Zhang Q, Hu Y, Wang D, He J, Duan P. No association between TP53 Arg72Pro polymorphism and ovarian cancer risk: evidence from 10113 subjects. *Oncotarget*. 2017 Nov 21;8(68):112761-112769. doi: 10.18632/oncotarget.22603. eCollection 2017 Dec 22.
18. Hori Y, Miyabe K, Yoshida M, Nakazawa T, Hayashi K, Naitoh I, Shimizu S, Kondo H, Nishi Y, Umemura S, Kato A, Ohara H, Inagaki H, Joh T. Impact of TP53 codon 72 and MDM2 SNP 309 polymorphisms in pancreatic ductal adenocarcinoma. *PLoS One*. 2015 Mar 3;10(3):e0118829. doi: 10.1371/journal.pone.0118829
19. Sonoyama T, Sakai A, Mita Y, Yasuda Y, Kawamoto H, Yagi T, et al. TP53 codon 72 polymorphism is associated with pancreatic cancer risk in males, smokers and drinkers. *Mol Med Rep*. 2011; 4: 489–495. doi: 10.3892/mmr.2011.449 PMID: 21468597
20. Naccarati A, Pardini B, Polakova V, Smerhovsky Z, Vodickova L, Soucek P, et al. Genotype and haplo-type analysis of TP53 gene and the risk of pancreatic cancer: an association study in the Czech Republic. *Carcinogenesis*. 2010; 31: 666–670. doi: 10.1093/carcin/bgq032 PMID: 20110284
21. Galli P, Cadoni G, Volante M, De Feo E, Amore R, Giorgio A, Arzani D, Paludetti G, Ricciardi G, Boccia S. A case-control study on the combined effects of p53 and p73 polymorphisms on head and neck cancer risk in an Italian population. *BMC Cancer*. 2009 May 8;9:137. doi: 10.1186/1471-2407-9-137

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