EXPERT OPINION

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Germline copy number variation in the *YTHDC2* gene: does it have a role in finding a novel potential molecular target involved in pancreatic adenocarcinoma susceptibility?

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Objective: The vast majority of pancreatic cancers occurs sporadically. The discovery of frequent variations in germline gene copy number can significantly influence the expression levels of genes that predispose to pancreatic adenocarcinoma. We prospectively investigated whether patients with sporadic pancreatic adenocarcinoma share specific gene copy number variations (CNVs) in their germline DNA.
Patients and methods: DNA samples were analyzed from peripheral leukocytes from 72 patients with a diagnosis of sporadic pancreatic adenocarcinoma and from 60 controls using Affymetrix 500K array set. Multiplex ligation-dependent probe amplification (MLPA) assay was performed using a set of self-designed MLPA probes specific for seven target sequences.
Results: We identified a CNV-containing DNA region associated with pancreatic cancer risk. This region shows a deletion of 1 allele in 36 of the 72 analyzed patients but in none of the controls. This region is of particular interest since it contains the *YTHDC2* gene encoding for a putative DNA/RNA helicase, such protein being frequently involved in cancer susceptibility. Interestingly, 82.6% of Sicilian patients showed germline loss of one allele.
Conclusions: Our results suggest that the *YTHDC2* gene could be a potential candidate for pancreatic cancer susceptibility and a useful marker for early detection as well as for the development of possible new therapeutic strategies.

Keywords: copy number variations, germline alteration, pancreatic cancer susceptibility, *YTHDC2* gene

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1. Introduction

Pancreatic cancer accounts for 3% of all new cases of cancer and it is the fourth leading cause of cancer death, with an overall 5-year survival rate of < 5%. This incidence has not changed in nearly 50 years [1]. Pancreatic cancer is one of the most lethal human cancers and its prognosis has not been improved despite advances in diagnostic and therapeutic strategies [2]. Environmental factors such as cigarette smoking [3-5] or diseases such as diabetes [6,7], obesity [8,9] or chronic pancreatitis [10,11] can predispose to pancreatic cancer [12]. Heritable genetic predisposition is also sometimes involved [13,14]. The lifetime risk of pancreatic cancer is

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4.7% for first-degree relatives of patients with pancreatic cancer and the familial risk of pancreatic cancer increases with each affected family member [15,16]. Pancreatic cancer can also be inherited as part of a multi-cancer syndrome such as cancers associated with *BRCA2* mutations [17,18], Peutz-Jeghers syndrome [19], Fanconi anemia syndrome [20] and familial atypical multiple mole-melanoma syndrome [21]. In these cases, the family pedigree reveals the occurrence of other cancers such as breast or intestinal tumors, or melanomas, in addition to pancreatic tumors. However, most pancreatic cancers are sporadic. The molecular mechanism by which they occur involves the same alterations in somatic gene expression as in other cancers, which include *KRAS2*, *BRCA2* and *TP53* mutations, telomere shortening, p21 (WAF1/CIP1) and cyclin D1 upregulations, expression of proliferation antigens, and inactivation of p16^{INK4}/CDKN2A and DPC4/SMAD4 [22]. However, the mechanisms that account for sporadic occurrence remain unknown. The discovery of frequent variations in gene copy number (CN) in germline DNA might further show how the sporadic occurrence of pancreatic cancer occurs [23-26]. Such variations can significantly influence the expression levels of involved genes [27] and it is conceivable that some gene copy number variations (CNVs) lead to peculiar alterations of gene expression that predispose to pancreatic adenocarcinoma [28].

We performed a high-resolution genomic microarray analysis on germline DNA of patients with sporadic pancreatic adenocarcinoma to determine the rate of *de novo* CNV and to identify common DNA regions containing pancreatic cancer-associated CNVs that are not detected in healthy individuals. Specific cancer-associated genes with aberrant CN could be useful biomarkers for the early detection and for the development of possible new therapeutic strategies.

In our study, we identified a CNV-including DNA region that contains the *YTHDC2* gene. This gene maps on chromosome 5 (5q22.2) and encodes for a putative ATP-dependent RNA helicase involved in translation initiation. *YTHDC2* expression was shown in tumor cell lines and TNF-α-treated hepatocytes. However, the function of YTHDC2 in the cancer cells and the mechanism by which the *YTHDC2* gene is transcribed in these cells are largely unknown [29]. In a recent work, Tanabe *et al.* showed that *YTHDC2* plays an important role in cancer cell growth, the activation/recruitment of ATF-2 and c-Jun to the *YTHDC2* promoter region is necessary for the transcription of *YTHDC2*, and that histone deacetylase activity is required for the efficient expression of *YTHDC2* in both hepatocyte and hepatocellular carcinoma cells [29]. Furthermore, rare mutations in the *YTHDC2* gene have been identified in individuals with autism spectrum disorders [30].

2. Patients and methods

2.1 Sample collection

Written informed consent was obtained from all participants. We prospectively collected 72 DNA samples from the peripheral leukocytes of 72 patients with a diagnosis of sporadic pancreatic adenocarcinoma. Patients with a familial pancreatic cancer history were excluded from the study. Diagnosis of adenocarcinoma was confirmed by histologic analysis. All clinical information for each enrolled patient was recorded anonymously and coded. All DNA samples were of sufficient quality to be genotyped. Patients were European (32 from Brussels, 23 from Palermo, 11 from Barcelona and 6 from Leipzig, as self-reported in the presence of the physician). Of these patients, 39 were men and 33 were women, with a mean age of 66 years. Sixty DNA samples from individuals of European origin were used as controls. We used the HapMap database as a reference [23].

2.2 CNV analysis

DNA was extracted from whole blood using the QIAamp mini kit (Qiagen, Chatsworth, CA, USA), according to the manufacturer's instructions. The DNA yields and purity were determined spectrophotometrically by measuring the absorbance of aliquots at 260 and 280 nm. DNA was prepared for microarray hybridization using the GeneChip Mapping Assay Protocol (Affymetrix, Inc., Santa Clara, CA, USA) as previously described by Pugh *et al.* [31]. The raw images were analyzed using the GeneChip Operating Software (GCOS Ver1.4.1) and GTYPE (Ver4.1) software (Affymetrix). We excluded samples with a genotype call rate < 93% (http://www.biostat.jhsph.edu/~iruczins/teaching/misc/gwas/papers/affymetrix2006.pdf) [32,33]. To assess CN alterations we used CNAT (Ver4.0.1) software.

We set the genomic smoothing at 0.01 Mb and kept default parameters for the other variables. CN estimates were obtained using data from 172 HapMap samples (available online) as a reference. Chromosome X was not analyzed to avoid gender-related complications [34]. Reproducibility of the method was assessed by analyzing six DNAs from patients with pancreatic adenocarcinoma in duplicate, the second analysis confirming > 96% of CNVs obtained in the first analysis.

2.3 Data analysis

Using SAS software and *ad hoc* programs, individual tables generated by CNAT (CNATv4.0.1) were merged and probes were ranked according to their CN value. Two lists containing the probes with CN gains (3 or 4 copies) or loss (1 or 0 copies) were generated. However, to avoid false-positive number variations due to random noise in signal intensity, we retained only the probes that showed the same condition, that is gain or loss, in all 72 patients. Selected probes were merged with their respective gene annotations and physical positions according to the NCBI human genome sequence using the NetAffx web server (Affymetrix).

2.4 Multiplex ligation-dependent probe amplification

Multiplex ligation-dependent probe amplification (MLPA) [35] was performed on genomic DNA isolated from peripheral

Table 1. 'Home-made' MLPA probes and target sequences*.

Probe ID	*YTHDC2* target sequence	Probe oligonucleotide sequences‡		Total probe size (bp) (LPO + RPO)
		LHS	RHS	
1	GTCCAGTCAAAGCAAAAGCGGACTG GAGGTCATGGCAACAGTTTTTTGGGATGC	GCATCCCAAAAAACTGTT GCCATGACC	TCCAGTCCGCTTTTGC TTTGACTGGAC	96
2	CTGGGGCTTTAGCTCTGGTAAGAGATTG GACAGAGTCTTCAGTACATTAAGTTGCTCA	TGAGCAACTTAATGTACT GAAGACTCTGTCC	AATCTCTTACCAGAGC TAAAGCCCCAG	100
3	TCCATTCCCACCAATTCATCACTTCACTGA AACTGTACTTGCCAAGGTCAACTGTGTCCTTC	GAAGGACACAGTTGAC CTTGGCAAGTACAGTT	TCAGTGAAGTGATGA ATTGGTGGGAATGGA	104
4	AGGAACTGGTGACTTTCAAGTTTAGAGCTCT GTCTTAGACCTTTCTGTGAATCCAGCTGCC TTCAGGACA	TGTCCTGAAGGCAGC TGGATTCACAG AAAGGTCTA	AGACAGAGCTCTAAACT TGAAAGTCACCAGTTCCT	112
5	CCCAATGATAGGGGTATACTAATAAACTC CATGATTAAGCTGCTAAATTAACTAACTG CTGATGAACTAAGTTG	CAACTTAGTTCATCA GCAGTTAGTTAA TTTAGCAGCTTAAT	CATGGAGTTTATTAGTA TACCCCTATCATTGGG	116
6	AATCACACTCTGCCACAACTATTGTTTAC TGTCTTTCTTATTAGACTATTTAATTTGATT TTGTGTCTTAGTGCCTAA	TTAGGCACTAAGACA CAAAATCAAATTAAA TAGTCTAATAAG	AAAGACAGTAAACAATAG TTGTGGCAGAGTGTGATT	120
7	CAGGGATTTTCTAGGATGTCTTCTGAGA TTGGAAGGGAAAAGAGTCAGGACTGGGG CTCTGCTGGACTAGGAGGAGTATTTA	TAAATACTCCTCCTA GTCCAGCAGAGCCCC AGTCCTGACTC	TTTTCCCTTCCAATCT CAGAAGACATCCTAG AAAATCCCTG	124

*MLPA probe sizes range from 96 to 124 bp.

‡Sequences do not include the universal primers located at the 5' end of LPO and 3' end of RPO.

LHS: Left hybridizing sequence; LPO: Left probe oligonucleotide; MLPA: Multiplex ligation-dependent probe amplification; RHS: Right hybridizing sequence; RPO: Right probe oligonucleotide.

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blood leukocytes using an MRC Holland's EK1 kit, according to the manufacturer's protocol, using self-designed synthetic MLPA probes. The MLPA reaction was performed in five main steps: i) DNA denaturation and hybridization of MLPA probes; ii) ligation reaction; iii) polymerase chain reaction (PCR); iv) separation of amplification products by electrophoresis; and v) data analysis. Following MRC-Holland recommendations, we designed seven sets of synthetic MLPA probes (Invitrogen, Carlsbad, CA, USA) to detect deletions in several regions of *YTHDC2* gene (Table 1). To test the quality of the 'home-made' probes and performed MLPA reactions, as well as facilitate data analysis, we used 'SALSA MLPA kit P200-A1 Reference probemix 1' (MRC-Holland) as a reference internal control (data not shown). Synthetic probe mix was obtained by combining 0.8 µl of each 1 µM oligo (half-probe) solution in a final volume of 200 µl of Tris–EDTA. For each MLPA reaction, we used 1 µl P200 + 0.5 µl synthetic probe mix + 1.5 µl MLPA buffer. Ligation products were amplified by PCR. The PCR conditions were 30 sec at 95°C, 30 sec at 60°C and 1 min at 72°C for 35 cycles; 20 min at 72°C. The resulting amplification products (from 130 to 480 nt in length) were separated and analyzed by capillary electrophoresis using ABI 3100 Genetic Analyzer (Applied Biosystems). GeneMapper® v3.5 software (Applied Biosystems) was used to determine peak heights and areas, and fragment sizes in base pairs (bp). Specific peaks corresponding to each tested region of the *YTHDC2* gene were identified according to their migration in relation to size standards. Peak heights and areas of each fragment were compared to those of 10 non-pancreatic cancer control samples and potential heterozygous deletions were suspected when peak height and area differed by approximately 50%. Furthermore, duplicate assays were performed to check the accuracy of the MLPA analysis data.

3. Results

3.1 Germline DNA analysis reveals specific CNVs in patients with pancreatic adenocarcinoma

In this work, we used the Affymetrix platform to genotype 500,000 unique probes in patients with pancreatic adenocarcinoma to investigate whether patients shared specific CNVs associated with the disease that were not detected in individuals without pancreatic cancer. Several DNA regions that showed different CNV profiles in patients and controls were selected. One of these, where the CNV profile was associated with sporadic pancreatic cancer with high significance (p values from 2.25E-11 to 6.87E-08 depending on the probe set), was found particularly interesting. In this region, one allele was deleted in 36 (50%) of the 72 analyzed patients, but not in the controls. The deleted region is located on chromosome 5 (112,872,760 to 112,962,031) and its size ranges from 85,687 to 41,608 nt (Table 2). This region contains the *YTHDC2* gene (112,877,309 to 112,958,880, total size 81,571 bp). The smallest deletions detected in patients encompassed at least 51% of the *YTHDC2* gene, including several

Table 2. Patients with sporadic pancreatic cancer showing heterozygous deletion of a DNA region containing *YTHDC2**

Chromosome	Start position	End position	Sample identification	Gender	Age (years)	Smoking status	Length (bp)	CNV mean	‡Markers	p value
5	112872760	112958447	210_M_13	F	77	F	85687	1.15	15	1.59E-04
5	112872760	112956131	PBCN-4	F	60	C	83371	0.95	14	4.14E-06
5	112872760	112956131	E4224	M	58	N	83371	1.14	14	1.53E-04
5	112872760	112950168	210_M_16	F	63	F	77408	0.91	13	1.14E-04
5	112872760	112950168	210_M_1A	M	73	C	77408	1.11	13	1.66E-04
5	112872760	112950168	210_M_2A	M	64	C	77408	1.09	13	4.38E-04
5	112872760	112950168	210_M_14	F	96	N	77408	1.03	13	5.93E-03
5	112884180	112956131	210_M_22	F	83	N	71951	1.06	11	1.06E-04
5	112884180	112956131	PBCN6	M	75	F	71951	1.12	11	2.50E-04
5	112884180	112956131	210_M_23	F	74	C	71951	1.19	11	2.98E-04
5	112878566	112950168	Paca6	F	66	F	71602	1.15	11	7.99E-03
5	112888603	112958447	G324	M	58	N	69844	1.13	11	1.96E-04
5	112888603	112958447	G722	M	73	C	69844	1.13	11	1.48E-03
5	112888603	112956131	E4368	M	62	F	67528	1.08	10	1.41E-05
5	112888603	112956131	210_M_10	M	70	N	67528	0.98	10	1.92E-05
5	112888603	112956131	210_M_20	M	89	F	67528	1.00	10	1.96E-05
5	112888603	112956131	210_M_6A	M	61	C	67528	1.06	10	2.86E-05
5	112888603	112956131	210_M_3A	F	54	N	67528	1.06	10	4.06E-05
5	112888603	112956131	210_M_5	F	52	C	67528	0.97	10	1.44E-04
5	112888603	112956131	E4301	F	59	N	67528	1.17	10	1.63E-04
5	112888603	112956131	210_M_8	F	76	N	67528	0.92	10	1.72E-04
5	112888603	112956131	G342	F	66	C	67528	1.09	10	2.05E-04
5	112888603	112956131	210_M_18	M	68	N	67528	1.09	10	2.56E-04
5	112888603	112956131	210_M_21	M	91	F	67528	1.05	10	3.57E-04
5	112888603	112956131	PBCN7	M	73	C	67528	0.99	10	4.10E-04
5	112888603	112956131	210_M_7A	F	67	N	67528	0.97	10	4.84E-04
5	112888603	112956131	210_M_4	F	70	N	67528	1.03	10	9.99E-04
5	112878566	112944715	PBCN-1	M	69	F	66149	1.01	10	9.33E-04
5	112893833	112958447	PBCN-2	F	76	N	64614	0.92	10	1.14E-04
5	112893833	112958447	210_M_11	F	57	C	64614	0.87	10	2.07E-04
5	112893833	112958447	PBCN-3	M	81	N	64614	1.00	10	3.68E-04
5	112893833	112958447	G1809	F	63	F	64614	1.10	10	1.52E-03
5	112907813	112962031	210_M_17	F	80	N	54218	1.03	11	1.49E-03
5	112907813	112962031	G932	M	59	C	54218	1.16	11	6.63E-03
5	112907813	112961060	G426	M	62	F	53247	0.97	10	1.01E-03
5	112920423	112962031	G2619	M	74	N	41608	1.15	10	2.26E-03

***Chromosome** = Chromosome where the segment is located; **Start position** = Base pair position on the chromosome at which the first marker in the segment begins (going from top of the p-arm to the bottom of the q-arm of the chromosome); **End position** = Base pair position on the chromosome at which the last marker in the segment begins; **Sample identification** = Sample name; **Gender** = Male (M) or Female (F); **Smoking status =** Current (C), Former (F), Never (N); **Length (bp)** = Size of the segment of copy number change; **CNV mean** = The mean value of CNV; ‡**Markers** = Number of SNPs + CNV markers within the segment; **p-value** = p-value of CNV region.
bp: Base pairs; CNV: Copy number variation.

exons (**Figure 1**). Interestingly, 19 of 36 patients showing heterozygous deletion in the DNA region containing *YTHDC2* are Sicilian (52.8%). Therefore, 82.6% (19 of 23) of patients from Palermo had a germline loss of one allele in that gene.

Furthermore, very similar results were found when segmentation analysis was performed in parallel, using the Partek Genomics Suite (Partek GS) (data not shown).

3.2 *YTHDC2* may be a potential susceptibility gene for sporadic pancreatic adenocarcinoma

The *YTHDC2* (YTH domain containing 2) gene encodes for a 1430-amino acid protein with a theoretical molecular weight of 160,248 Da. The YTHDC2 protein contains seven well-conserved domains, including an R3H domain (which binds to ssDNA or ssRNA in a sequence-specific manner), a DEAD-like helicase superfamily domain (with helicase activity), an ANK-repeat domain (which mediates protein–protein interactions), another DEAD-like helicase superfamily domain with helicase activity, a HA2 domain found in various helicases, potentially involved in nucleic acid binding, a DUF1605 domain systematically found toward the C-terminus of the DEAD box-containing helicases and a YTH domain, which likely modulates alternative splice site selection in a concentration-dependent manner. Although its function

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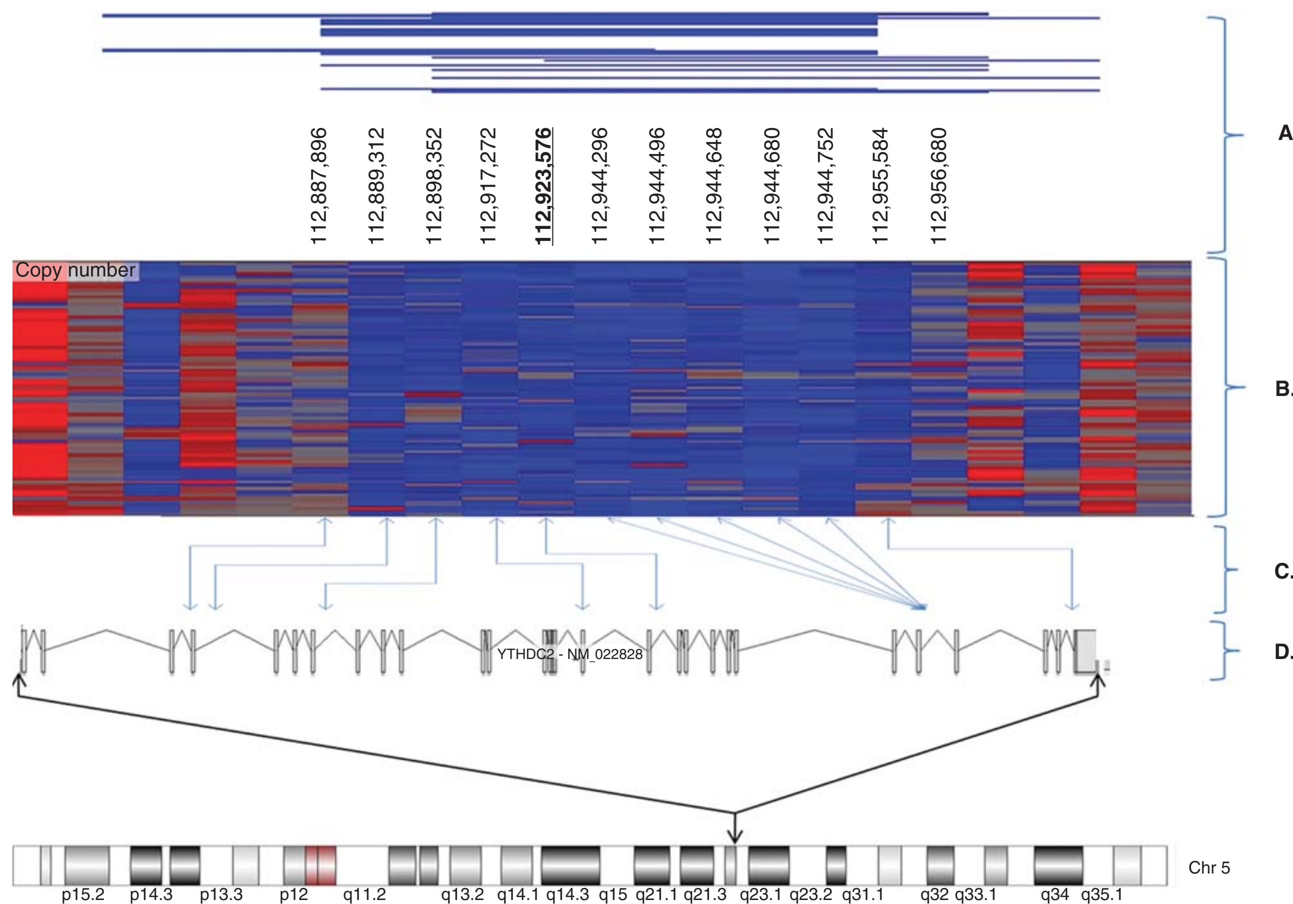

Figure 1. Potentially deleted *YTHDC2* gene region. A. Deletions, with at least 10 markers, detected in chromosome 5. Blue lines are representative of the deleted region detected in each patient. The physical position of the SNPs is represented in base pairs (bp) along chromosome 5. The underlined position, close to the exon 18, was tested using the AB kit. **B.** Dendrogram of signals from cancer samples. Each probe is represented by an equal and non-proportional trait. Each row represents a single patient tested. Each column summarizes genotyping data for the chromosomal region containing the *YTHDC2* gene. Red lines are representative of amplified segments. **C.** Proportional distribution of probes along chromosome 5. **D.** Introns and exons of the *YTHDC2* gene.

has not yet been studied, its primary structure strongly suggests that it is an ssRNA- or ssDNA-binding helicase. The fact that in Drosophila the *maleless* gene product, which has a similar organization (**Figure 2**), is a well-established helicase supports this hypothesis [36,37]. Therefore, YTHDC2 protein is expected to have several molecular functions such as ATP binding, ATP-dependent helicase activity, hydrolase activity, nucleic acid binding and nucleotide binding. **Figure 3** shows some putative interactions of YTHDC2.

3.3 MLPA analysis

Because half of the patients with pancreatic adenocarcinoma showed a germline heterozygous deletion in a region of chromosome 5 containing *YTHDC2*, seven gene regions were selected for MLPA validation to confirm data previously obtained by genome-wide CNV analysis. An MLPA assay was performed in 36 patients showing loss of one allele, using a set of self-designed synthetic MLPA probes specific for seven target sequences of *YTHDC2* (**Table 1**). We normalized the samples by using peak height and area values and comparing the patients with 10 healthy controls that had no CNVs (deletions or amplifications) in *YTHDC2*, as confirmed by a previous analysis (data not shown). Heterozygous deletions were detected when peak height and area of each fragment were reduced by approximately 50% with respect to controls (**Figure 4**). MLPA analysis indicated that *YTHDC2* target sequences showed a putative germline heterozygous deletion only in 10 of 36 (27.8%) patients with pancreatic adenocarcinoma, partially confirming the previously obtained CNV analysis data (Table S1). Interestingly, among these, six patients are from Palermo, in part confirming the relatively higher percentage of patients belonging to this geographical area showing germline loss of one allele of *YTHDC2*.

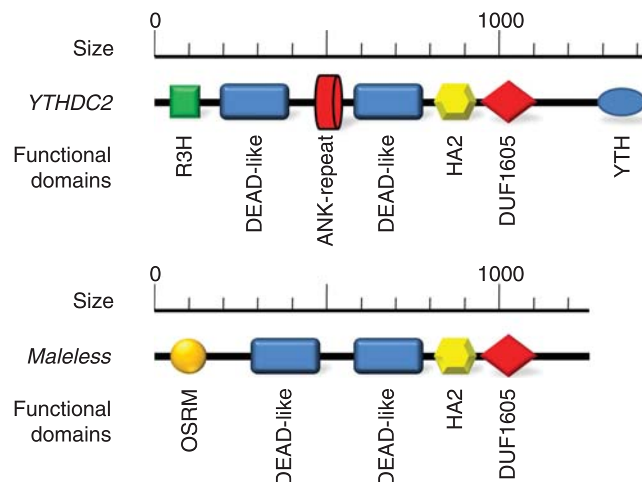

Figure 2. *YTHDC2* is a probable ATP-dependent helicase. Comparison between the structural organization of YTHDC2 and maleless proteins.

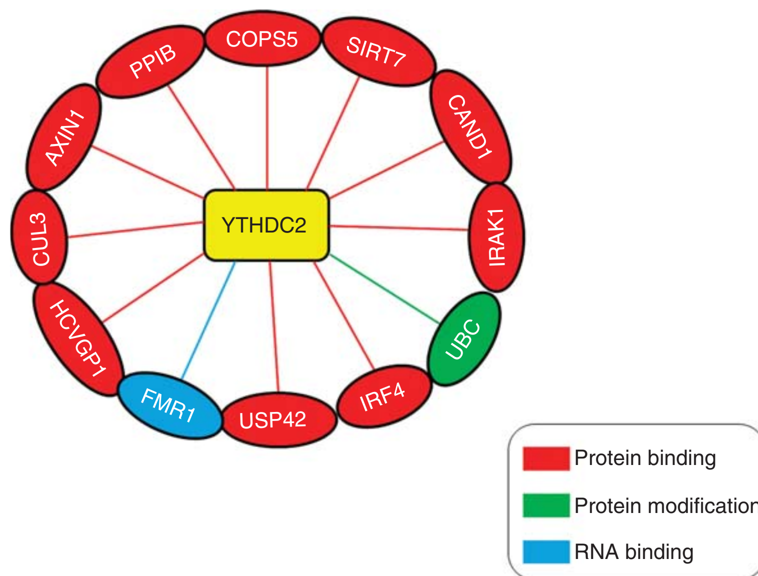

Figure 3. *YTHDC2*: putative interaction network.

4. Discussion

Pancreatic cancer is one of the most common fatal malignant tumors worldwide with poor prognosis and frequent resistance to conventional therapies such as radiotherapy or chemotherapy [38-40]. CNV has recently gained considerable interest as a source of genetic variation as it seems to play a role in phenotypic diversity and evolution [25]. Associations between CN changes and complex diseases were discovered in whole-genome association studies. Furthermore, genome analysis provided a powerful approach to test for evidence of genetic variations within and between geographical regions and local populations [41,42]. In a recent work, Chen *et al.* reported that whereas the overall CN variant frequencies are similar between populations, their distribution is highly specific to the population of origin [43]. DNA samples used in this study include those from groups of individuals from different geographical areas so as to facilitate detection of possible population-specific common variants. However, this approach involves that variants that are rare and population-restricted could be not detected because the number of individuals examined in each population is small. The germline CNV identified in this study was found in 36 of the 72 - analyzed patients with sporadic pancreatic adenocarcinoma, but in none of the 60 controls, indicating that this DNA anomaly is frequent in patients and rare in non-affected

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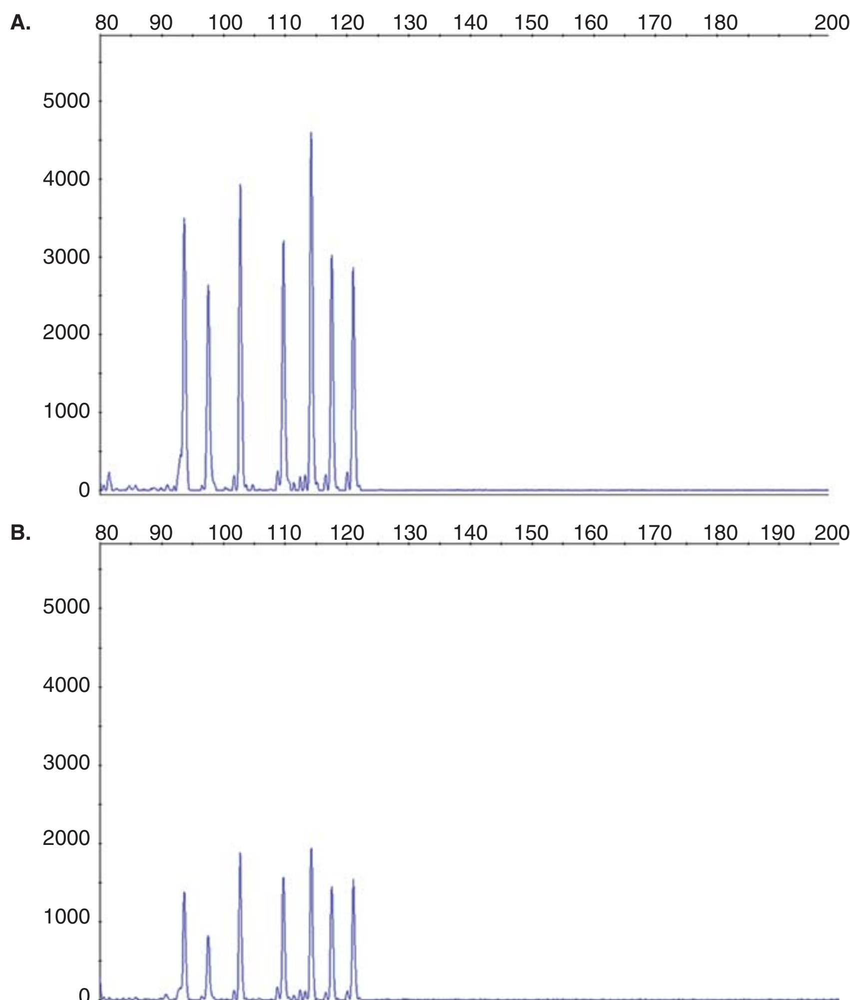

Figure 4. Heterozygous deletion of *YTHDC2* gene. MLPA-derived amplification products were separated by electrophoresis and peak patterns were generated from a control DNA sample (**A**) and a patient sample (**B**). Relative amounts of probe amplification products, compared to a control DNA sample, reflect the relative copy number of seven target sequences. The comparison between the peak heights showed a reduction by approximately 50% in patient sample with respect to control.
MLPA: Multiplex ligation-dependent probe amplification.

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people. If this alteration generates a functional defect involved in the occurrence of sporadic pancreatic cancer, its absence in half of the patients should be explained. At least two possibilities can be considered. The first is that other functional defects, other than a defect in *YTHDC2* gene, can trigger pancreatic adenocarcinoma. Indeed, we found other CNVs associated with pancreatic cancer with a lower frequency that might account for such defects. The second possible explanation is technical. We cannot exclude the possibility that short DNA losses or other DNA anomalies involving this region were undetectable with our approach. These include inversions, insertions and more complex rearrangements, which are found in other patients with pancreatic cancer.

Interestingly, our genome microarray analysis detected that 19 of 23 (82.6%) patients from Palermo (Italy) showed germline heterozygous deletion in the DNA region containing *YTHDC2*. MLPA analysis further confirmed that most of the patients showing loss of one allele in *YTHDC2* (27.8%) belongs to geographical area of Palermo (Italy). Because CNVs may differ greatly among different populations, we hypothesize a putative population-specific CNV in this region. However, further investigations are needed to confirm this hypothesis. Therefore, we suggest that *YTHDC2* could be a potential candidate for susceptibility to pancreatic cancer. To our knowledge, this is the first report that associates a particular CNV with susceptibility to a sporadic cancer. Interestingly, results from a microarray analysis on familial pancreatic cancer [44] showed that patients with a familial history of pancreatic cancer had a total of 56 unique germline genomic regions with CNVs that were not present in the controls, including 31 amplifications and 25 deletions. These patients did not show DNA

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anomalies in the *YTHDC2* gene region, suggesting that familial and sporadic pancreatic cancers develop through different pathways. In addition, a truncating germline mutation in the *PALB2* gene was found in several patients with familial pancreatic cancer. PALB2 protein is a binding partner for BRCA2. PALB2 mutations have previously been reported in patients with familial breast cancer, and *PALB2* is now considered to be a susceptibility gene for pancreatic cancer [45]. An attractive hypothesis is that genomic alterations associated with inherited pancreatic cancer have sufficient penetrance to trigger the disease, whereas alterations associated with sporadic pancreatic cancer require the additional influence of environmental factors [22]. In 2009, Amundadottir *et al.* [46] identified an SNP that maps to the first intron of the ABO blood group gene, which is significantly associated with pancreatic cancer, suggesting that people with blood group O may have a lower risk than those with groups A or B. However, the involvement of this SNP in susceptibility to pancreatic cancer remains to be established.

We cannot exclude the possibility that a specific CNV is also associated with other forms of cancer. Evaluating pancreatic specificity by repeating the analysis with a series of DNA samples from patients with other cancers was beyond the scope of this study. However, if cancer is the consequence of a given series of inherited genetic imbalances, the corresponding CNV should be found in a population of individuals chosen at random, in a proportion corresponding to cancer occurrence in humans (American Cancer Society 2007). We did not find the same proportion of CNV (~20%) in our control population, suggesting that there is no clear genetic predisposition to cancer in general, but that more specific genetic abnormalities predispose individuals to cancers of specific organs. The CNV identified in this study, associated with susceptibility to pancreatic adenocarcinoma, might be an example of such specificity.

The CNV selected in this study comprises at least 51% of the *YTHDC2* gene, including several exons, indicating that one allele is inactivated in patients with sporadic pancreatic cancer. The *YTHDC2* gene encodes for a putative helicase, well conserved in pluricellular eukaryotes, including early organisms. The YTHDC2 function has not yet been established, but several proteins with similar structures are DNA and/or RNA helicases. How the deletion of one *YTHDC2* allele is a potential susceptibility factor for pancreatic adenocarcinoma also remains to be elucidated. However, activity loss of several helicases has been implicated in breast and prostate cancer susceptibility. Helicases are involved in DNA and RNA metabolism, including DNA repair and recombination, chromosomal stability, splicing and removal of proteins from RNA. Some may act as viral receptors, others may be involved in transcription regulation and some are involved in the initial steps of eukaryotic translational mechanism. Many functions are strongly associated with the cancer development.

5. Conclusions

This is a preliminary/pilot study performed on individuals from different geographical areas in order to identify new possible common variants in sporadic pancreatic adenocarcinoma. However, we are looking at expanding our sample size to carry out a research more extended and to further confirm obtained results. The CNV reported in this study may help increase our understanding of the physiopathology of pancreatic cancer because gene deletions generate imbalances in the corresponding mRNA and encoded protein levels. For genes and pathways critically dependent on a fixed amount of a functional product, it seems likely that CNVs could account for individual variations in disease susceptibility [47]. Also, the interaction between germline modifications and somatic mutations could influence the phenotype. For instance, the result of a somatic mutation occurring in a gene whose expression is restricted to a single allele would correspond to a knock-out. In conclusion, these data suggest that the occurrence of pancreatic cancer involves a combination of specific somatic mutations and germline alterations. Knowledge of the genes whose CN is altered in pancreatic cancer could be useful for identifying patients at high risk of developing this disease both for diagnosis and also to possibly reveal new gene targets for preventive or curative strategies.

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D Fanale and JL Iovanna contributed equally to this work.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Bibliography

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62:10-29
2. Hamada S, Shimosegawa T. Pancreatic cancer stem cell and mesenchymal stem cell. In: Grippo PJ, Munshi HG, editors. Pancreatic cancer and tumor microenvironment. Chapter 6. Transworld Research Network, Trivandrum (India); 2012
3. Mack TM, Yu MC, Hanisch R, et al. Pancreas cancer and smoking, beverage consumption, and past medical history. J Natl Cancer Inst 1986;76:49-60
4. Momi N, Kaur S, Ponnusamy MP, et al. Interplay between smoking-induced genotoxicity and altered signaling in pancreatic carcinogenesis. Carcinogenesis 2012;33:1617-28
5. Bosetti C, Lucenteforte E, Silverman DT, et al. Cigarette smoking and pancreatic cancer: an analysis from the International Pancreatic Cancer Case-Control Consortium (Panc4). Ann Oncol 2012;23:1880-8
6. Everhart J, Wright D. Diabetes mellitus as a risk factor for pancreatic cancer. A meta-analysis. JAMA 1995;273:1605-9
7. Chari ST, Leibson CL, Rabe KG, et al. Pancreatic cancer-associated diabetes mellitus: prevalence and temporal association with diagnosis of cancer. Gastroenterology 2008;134:95-101
8. Lin Y, Kikuchi S, Tamakoshi A, et al. Obesity, physical activity and the risk of pancreatic cancer in a large Japanese cohort. Int J Cancer 2007;120:2665-71
9. Larsson SC, Orsini N, Wolk A. Body mass index and pancreatic cancer risk: a meta-analysis of prospective studies. Int J Cancer 2007;120:1993-8
10. Malka D, Hammel P, Maire F, et al. Risk of pancreatic adenocarcinoma in chronic pancreatitis. Gut 2002;51:849-52
11. Chung SD, Chen KY, Xirasagar S, et al. More than 9-times increased risk for pancreatic cancer among patients with acute pancreatitis in Chinese population. Pancreas 2012;41:142-6
12. Huang L, Teng D, Wang H, et al. Association of copy number variation in the AHI1 gene with risk of obesity in the Chinese population. Eur J Endocrinol 2012;166:727-34
13. Raimondi S, Maisonneuve P, Lowenfels AB. Epidemiology of pancreatic cancer: an overview. Nat Rev Gastroenterol Hepatol 2009;6:699-708
14. Vincent A, Herman J, Schulick R, et al. Pancreatic cancer. Lancet 2011;378:607-20
15. Wang L, Brune KA, Visvanathan K, et al. Elevated cancer mortality in the relatives of patients with pancreatic cancer. Cancer Epidemiol Biomarkers Prev 2009;18:2829-34
16. Shi C, Klein AP, Goggins M, et al. Increased prevalence of precursor lesions in familial pancreatic cancer patients. Clin Cancer Res 2009;15:7737-43
17. Lal G, Liu G, Schmocker B, et al. Inherited predisposition to pancreatic adenocarcinoma: role of family history and germ-line p16, BRCA1, and BRCA2 mutations. Cancer Res 2000;60:409-16
18. Murphy KM, Brune KA, Griffin C, et al. Evaluation of candidate genes MAP2K4, MADH4, ACVR1B, and BRCA2 in familial pancreatic cancer: deleterious BRCA2 mutations in 17%. Cancer Res 2002;62:3789-93
19. Giardiello FM, Brensinger JD, Tersmette AC, et al. Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology 2000;119:1447-53
20. Couch FJ, Johnson MR, Rabe K, et al. Germ line Fanconi anemia complementation group C mutations and pancreatic cancer. Cancer Res 2005;65:383-6
21. Goldstein AM, Fraser MC, Struewing JP, et al. Increased risk of pancreatic cancer in melanoma-prone kindreds with p16INK4 mutations. N Engl J Med 1995;333:970-94
22. Hezel AF, Kimmelman AC, et al. Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev 2006;20:1218-49
23. Redon R, Ishikawa S, Fitch KR, et al. Global variation in copy number in the human genome. Nature 2006;444:444-54
24. Conrad DF, Pinto D, Redon R, et al. Origins and functional impact of copy number variation in the human genome. Nature 2010;464:704-12
25. Zhang F, Gu W, Hurles ME, et al. Copy number variation in human health, disease, and evolution. Annu Rev Genomics Hum Genet 2009;10:451-81
26. Bruder CE, Piotrowski A, Gijsbers AA, et al. Phenotypically concordant and discordant monozygotic twins display different DNA copy-number-variation profiles. Am J Hum Genet 2008;82:763-71
27. Henrichsen CN, Chaignat E, Reymond A. Copy number variants, diseases and gene expression. Hum Mol Genet 2009;18:R1-8
28. Fanale D, Iovanna JL, Calvo EL, et al. Analysis of germline gene copy number variants of patients with sporadic pancreatic adenocarcinoma reveals specific variations. Oncology 2013;85:306-11
29. Tanabe A, Konno J, Tanikawa K, et al. Transcriptional machinery of TNF-alpha-inducible YTH domain containing 2 (YTHDC2) gene. Gene 2014;535:24-32
30. O'Roak BJ, Vives L, Girirajan S, et al. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 2012;485:246-50
31. Pugh TJ, Delaney AD, Farnoud N, et al. Impact of whole genome amplification on analysis of copy number variants. Nucleic Acids Res 2008;36:e80
32. Hong H, Su Z, Ge W, et al. Assessing batch effects of genotype calling algorithm BRLMM for the affymetrix genechip human mapping 500 K array set using 270 hapmap samples. BMC Bioinformatics 2008;9(Suppl 9):S17
33. Fu B, Xu J. A new genotype calling method for affymetrix SNP arrays. J Bioinform Comput Biol 2011;9:715-28
34. Andersen CL, Wiuf C, Kruhoffer M, et al. Frequent occurrence of uniparental disomy in colorectal cancer. Carcinogenesis 2007;28:38-48
35. Schouten JP, McElgunn CJ, Waaijer R, et al. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 2002;30:e57

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36. Izzo A, Regnard C, Morales V, et al. Structure-function analysis of the RNA helicase maleless. Nucleic Acids Res 2008;36:950-62
37. Lee CG, Chang KA, Kuroda MI, et al. The NTPase/helicase activities of Drosophila maleless, an essential factor in dosage compensation. EMBO J 1997;16:2671-81
38. Biankin AV, Waddell N, Kassahn KS, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature 2012;491:399-405
39. Hidalgo M. Pancreatic cancer. N Engl J Med 2010;362:1605-17
40. Hidalgo M. New insights into pancreatic cancer biology. Ann Oncol 2012;23(Suppl 10):x135-8
41. Park H, Kim JI, Ju YS, et al. Discovery of common Asian copy number variants using integrated high-resolution array CGH and massively parallel DNA sequencing. Nat Genet 2010;42:400-U461
42. Huang RS, Chen P, Wisel S, et al. Population-specific GSTM1 copy number variation. Hum Mol Genet 2009;18:366-72
43. Chen W, Hayward C, Wright AF, et al. Copy number variation across European populations. PLoS One 2011;6:e23087
44. Lucito R, Suresh S, Walter K, et al. Copy-number variants in patients with a strong family history of pancreatic cancer. Cancer Biol Ther 2007;6:1592-9
45. Jones S, Hruban RH, Kamiyama et al. Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. Science 2009;324:217
46. Amundadottir L, Kraft P, Stolzenberg-Solomon RZ, et al. Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. Nat Genet 2009;41:986-90
47. Freeman JL, Perry GH, Feuk L, et al. Copy number variation: new insights in genome diversity. Genome Res 2006;16:949-61

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