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The gut-brain axis: interactions between *Helicobacter pylori* and enteric and central nervous systems

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Carabotti *et al* [1] suggested that gut microbiota has an important role in bidirectional interactions between the gut and the central nervous system (CNS). In this regard, we considered gastrointestinal immune system and brain dialogue, particularly implicated in neuroinflammation, as a common feature of the neurodegenerative and neuroinflammatory diseases [2].

Neuroinflammation might initiate from gastrointestinal track (GIT), a vulnerable area through which pathogens influence the brain. A proposed mechanism from GIT (i.e., "little brain") infections including *Helicobacter pylori* (*Hp*) current infection to the CNS (i.e., "big brain") neuroinflammation, is that the pathogen may access the brain through the blood, the oral-nasal olfactory and the faster GIT-associated retrograde axonal transport pathways [2,3]. Specifically, *Hp* induces relative mechanisms and/or mediators such as the synthesis of various cytokines and/or chemokines, which may be detrimental to the CNS inflammation and/or neurodegeneration. *Hp*, by inducing several inflammatory mediators such as tumor necrosis factor-α and interleukin (IL)-6, may contribute to blood-brain barrier (BBB) disruption leading to brain neurodegenerative diseases. In addition, *Hp*-induced vacuolating A cytotoxin exhibits chemotactic activities to the bone marrow-derived mast cells (BMD-MCs) and induces BMD-MCs to produce proinflammatory cytokines leading to BBB dysfunction; MCs can be stimulated by corticotropin releasing hormone, also mentioned by the authors [1], including histamine, IL-8, tryptase and vascular endothelial growth factor which disrupt the BBB. Likewise, human defensins might contribute to *Hp*-related brain pathologies by modulating innate and adaptive immune system responses. Finally, activated monocytes (possibly infected with *Hp* due to defective autophagy resulting in *Hp* replication in autophagic vesicles) might also access the brain due to BBB disruption (Trojan horse theory) contributing to *Hp*-related neurodegeneration [2-5].

Importantly, Hippocrates wisely remarked: "all the diseases begin in the gut" and "death sits in the bowel", thereby creating the hypothesis that gut is responsible for many disorders including neurodegenerative diseases.

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Antiviral therapy leads to histological improvement in HBeAg-negative chronic hepatitis B patients

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We read with great interest the study by Papachrysos *et al* showing that antiviral therapy leads to histological improvement in HBeAg-negative chronic hepatitis B patients [1]. We agree with the authors that long-term antiviral treatment with nucleos(t)ide analogs suppresses hepatitis B virus (HBV) replication, delays disease progression and contributes to resolution of fibrosis [2,3].

In patients with chronic hepatitis B, persistent viral replication is associated with progression of liver disease and treatment is aimed at maximal viral suppression. A number of potential baseline predictors have been suggested to have an impact on the antiviral treatment outcome: demographic (patient age, gender, body weight, duration of infection, alcohol, and/or drug abuse); histological (grading of necroinflammatory activity, staging of liver fibrosis, presence of liver steatosis); virologic (baseline HBV DNA levels, HBeAg status, HBV genotype, genetic polymorphisms); and biochemical parameters (baseline aminotransferase

levels) [4]. The authors showed that hepatic activity index and fibrosis score have significantly declined during the course of treatment. However, information is not clearly available concerning the effect of age, gender, HBV DNA level, genetic subtype, and duration of infection on the histological response to antiviral therapy. Future studies are warranted to determine the predictors associated with the efficacy of antiviral treatment on the histological improvement.

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Authors' reply

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We appreciate the comments of Dr. Basyigit *et al* on our recent study showing that antiviral therapy leads to histological improvement in HBeAg-negative chronic hepatitis B patients. We agree with the comment that there are numerous factors that may have an impact on the degree of fibrosis resolution. Unfortunately, in our data we did not find any correlation between the disease staging and the age of the patients (95%CI -6.4 – 5.7, P=0.91), or the viral load in the first biopsy (95%CI -4.7x10^8 – 1.4x10^8, P=0.27). Male patients had a trend to have a greater change in fibrosis stage (-0.66 vs. -0.42), however the difference was not statistical significant (95%CI -0.18 – 0.38, P=0.23). In the current literature there is data that correlates the female gender and the younger age of the patients with better outcomes in HBeAg negative chronic hepatitis B [1,2]. Our study was an observational study of a small number of patients followed up in our department and unfortunately we did not have enough power to show statistical significant differences between our groups (N=50). Finally, we have no data in our group about the genetic subtype of the virus.

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Familial inflammatory bowel diseases in Northwest Greece

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We read with interest the articles by Ben-Horin *et al* [1] and Arias-Loste [2]. Indeed, inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), have been recognized for many years now to include a hereditary component. Familial occurrence in IBD is probably expected to be less frequent in Greece compared to Northern Europe and America [3]. According to retrospective studies, first-degree familial clustering of IBD in the Mediterranean area, including Greece, seems to not exceed 7% in adult [4,5] and 8.5% in pediatric cases [6].

We herein present the results of our study aiming to record all IBD cases with familial clustering in our area. Medical records of all IBD patients followed up at the Division of Gastroenterology at the Ioannina University Hospital and the Northwest Greece IBD Study Group, were retrospectively assessed and studied concerning the existence of all other family members with a firm diagnosis of IBD.

A total of 557 patients with IBD were analyzed and 32 families with at least 2 IBD affected members (first- and second-degree

relatives) were recorded. Among these 557 patients, 331 were male (59.4%) and 226 female (40.6%). Among male patients, 211 (63.7%) had UC, 75 (22.7%) had CD and 45 (13.6%) had undetermined colitis (IC). Among females, 145 (64.2%) had UC, 64 (28.3%) CD, and 17 (7.5%) IC. Thirty-two families with at least 2 IBD affected members (first- and second-degree relatives) were recorded, totaling 69 patients. Among these families, there were 26 (81.3%) with concordant diagnoses and 6 (18.7%) with disconcordant diagnoses (Table 1).

Table 1 Intrafamilial inflammatory bowel diseases (IBD) in Northwest Greece

Family	Concordance	1st Family member with IBD	2nd Family member with IBD
1st	C	Father UC (2000) left sided	Son UC (2008) left sided
2nd	C	Son CD (2001) left sided	Uncle CD (2000) left sided
3rd	C	Brother CD (2002) ileocolitis	Sister CD (2007) ileitis (twins) Sister CD (2008) ileitis
4th	C	Sister UC (2003) pancolitis	Sister UC (2000) left sided
5th	C	Daughter UC (2009) left sided	Grandfather UC (1984) pancolitis
6th	C	Father CD (1990) left sided	Son CD (1993) left sided
7th	C	Brother UC (1987) left sided	Brother UC (1992) left sided
8th	C	Mother UC (1980) left sided	Son UC (1989) left sided
9th	C	Brother UC (1980) left sided	Sister UC (1982) pancolitis
10th	C	Son UC (1999) left sided	Cousin UC (1990) left sided
11th	D	Brother CD (1999) left sided	Sister UC (2002) left sided
12th	C	Daughter UC (1998) left sided	Grandmother UC (1990) left sided
13th	C	Mother UC (2000) left sided	Daughter UC (2002) left sided
14th	D	Son CD (1999) left sided	Son UC (1987) left sided
15th	C	Father UC (1980) left sided	Uncle UC (1989) left sided
16th	C	Son UC (1980) left sided	Cousin UC (1975) left sided
17th	C	Brother UC (1992) left sided	Sister UC (2000) left sided
18th	D	Daughter UC (1987) left sided	Uncle CD (1980) left sided
19th	D	Brother UC (2010) left sided	Sister CD (2000) left sided
20th	C	Son UC (2009) left sided	Grandfather UC (2000) left sided
21st	C	Brother UC (1994) left sided	Brother UC (1990) left sided
22nd	C	Father CD (2005) ileitis	Son CD (1992) left sided
23rd	C	Brother UC (1986) left sided	Sister UC (2004) pancolitis
24th	C	Brother UC (1993) left sided	Brother UC (1990) left sided
25th	D	Son CD (1997) left sided	Grandmother UC (1990) left sided
26th	C	Son UC (1988) left sided	Aunt UC (1987) left sided
27th	C	Daughter UC (1986) left sided	Cousin UC (1986) left sided
28th	C	Mother UC (1971) left sided	Daughter UC (1990) left sided
29th	D	Sister CD (1988) left sided	Sister UC (1980) left sided
30th	C	Brother CD (2005) Ileitis	Sister CD (2000) left sided

Contd...

Table 1 Intrafamilial inflammatory bowel diseases (IBD) in Northwest Greece

Family	Concordance	1st Family member with IBD	2nd Family member with IBD
31st	D	Son UC (1998) left sided	Uncles UC & CD (1990) left sided
32nd	C	Father UC (2000) left sided	Daughter UC (2004) pancolitis

C, concordant; D, disconcordant in IBD diagnosis ulcerative colitis or Crohn's disease; UC, ulcerative colitis; CD, Crohn's disease

This study showed that any type of confirmed inheritance (first- or second-degree relatives) seems to play a role in IBD familial clustering with a crude rate of 12.4% of our IBD cohort. In this IBD cohort, there was a male predominance and a clear increase in familial UC predisposition compared to CD.

The time frame of sequential diagnoses, like a domino effect, in many of these families we described herein, points towards an environmental factor to which all siblings, and probably also their parents, were exposed during the same period of time.

Of interest, we also recorded one familial IBD case of immigrants in our area [7]. Familial IBD in immigrants have rarely been reported and seem to be of exceptional interest towards a better understanding of disease etiopathogenesis and potential risk factors related also to immigration [8]. Immigrant family cases illustrate potential bias in genetically based studies of CD that rely solely on phenotypic expression. Indeed, it seems that many people might have IBD in their genetic background but either they never express it phenotypically or they express it only under special environmental circumstances such as in the immigration land.

It would be of exceptional interest if any other family members could be investigated for any evidence of silent CD at the time of diagnosis of the disease in their siblings [9]. It can be anticipated that systematic familial screening for silent IBD cases following the first family member diagnosis may explain some of the mystery of the familial IBD aggregation.

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Ex vivo tracing of pancreatic neuroendocrine tumors with bio-conjugated fluorescent quantum dots: a paradigm of nanoparticle-based diagnostics

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We read with great interest the article published recently by Kartalis *et al* [1]. Our study group investigated the application of fluorescence with the use of nanoparticles as a novel hybrid imaging method of pancreatic neuroendocrine tumors (pNETs). We demonstrated that a novel biomarker, namely calreticulin (CRT), is present in pNETs and may be targeted with fluorescent gold quantum dots (AuQDs).

CRT is a ubiquitous Ca^{2+}-binding protein with chaperone activity and a rather complex relation to various cancers: its overexpression is positively associated with various solid tumors, usually as an adverse prognostic indicator [2]; however, contrary to its positive correlation to tumorigenesis and poor cancer prognosis, CRT also seems to promote immunogenic cancer cell death.

QDs are semiconductor nanocrystals with unique optical properties, such as highly tuneable fluorescence and high photochemical stability; their relatively large surface-to-mass ratio enables them to perform as antibody or drug-carriers, attributes that provide QDs with an almost unlimited potential in cancer theranostics [3]. Our group have synthesized AuQDs with functionalized groups and bio-conjugated to anti-calreticulin polyclonal antibodies (AuQD-antiCRT). These AuQDs were manufactured to emit at 800 nm on excitation in the near infrared (NIR). It can be shown that conjugated AuQDs can be targeted specifically to *in vitro* pancreatic adenocarcinoma cell lines and *ex vivo* human pancreatic lesions. With Human Research Authority approval (REC Reference: 04/q0504/1) and appropriate patient consenting, paired tissue samples from resected pancreatic specimens post-pancreatectomy were obtained and stored in liquid nitrogen. Sections were then cut on a cryostat-microtome at approximately -25°C and mounted on Vectabond coated slides for observation under laser scanning confocal fluorescence microscopy (LSCM, Nikon Eclipse TE 300). Once the fixed tissues were characterized under the LSCM, selected paired slides were incubated with AuQD-antiCRT and observed under the LSCM. The resected pancreatic specimens were further processed and evaluated by a senior histopathologist.

Among the subjects was a 61-year-old female who underwent a Whipple's procedure for what proved to be a completely resected, well differentiated neuroendocrine neoplasm, TNM stage pT3N1pMx. The photomicrographs of the pathological tissues under laser excitation before and after incubation to AuQD-antiCRT are depicted in Fig. 1. Illustrations (A) and (B) were captured by LSCM after 543 nm green laser beam excitation via a 650 nanometres/Long Pass (nm/LP) fluorescence barrier, while (C) and (D) after 488 nm blue and 650 nm/LP filtering. There was minimal signal emission after 543 nm excitation due to lower energy confirming NIR emission (A). Post-incubation with AuQD-antiCRT *(B),* there was NIR emission (pseudocolor) in 650/LP filter cut-off and therefore CRT expression. Images (C) and (D) also demonstrate NIR fluorescence after AuQD-antiCRT incubation, more intense upon He-Ne (488 nm). These two latter photographs also demonstrate some of the architectural appearances typical of pNETs, such as the cell nests (*single arrows*), trabeculi (*double arrows*) and gyriform patterns (arrowheads).

To the authors' best knowledge, this is the first pictorial demonstration of CRT surface expression on pancreatic neuroendocrine malignancy with the use of fluorescent quantum nanotechnology and tracing of pNETs with bio-stable, non-degradable fluorophores, such as the AuQDs. These findings may facilitate further *in vivo* investigation on CRT expression on pNETs and on the application of QD–based theranostics on the detection and treatment of these conditions [4]. Finally, these findings should trigger the design of *in vivo* animal studies on the fluorescence-guided laparoscopic surgery (FGLS) after QD-labeling of CRT-rich solid pancreatic lesions [5]. The advantages of FGLS have already been described by Metildi *et al* [6]; the theranostic advantage of QD-fluorophores as both diagnostic and therapeutic tracers and carriers of immunochemical tumoricidal agents and their safety for *in vivo* applications in the treatment of pNETs are yet to be developed and proven.

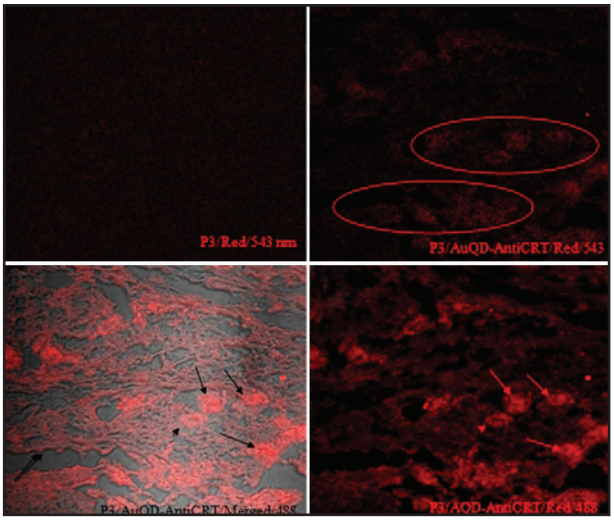

Figure 1 Laser scanning confocal fluorescence microscopy photomicrographs illustrating a pancreatic neuroendocrine tumor before and after exposure to fluorescent gold quantum dots bio-conjugated to anti-calreticulin polyclonal antibodies

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