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Escherichia coli Population-Based Study in Pediatric Crohn's Disease

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Abstract

***Escherichia coli* is assumed to be involved in inflammatory bowel disease (IBD) by many authors. The present Short Report was aimed at analyzing *E. coli* population isolated from ileal samples collected from 4 CD and 3 non-CD (control group) diagnosed pediatric patients. A total of 539 mucosa-associated *E. coli* strains were characterized by: Random Amplified Polymorphic DNA (RAPD), adhesive and virulence factors, and for their phylogenetic groups. A significant separation between RAPD profiles of the two CD and non-CD cohorts ($P < 0.0001$), along with a significant reduction of intra-species genomic variability in *E. coli* populations isolated from CD group ($P < 0.0001$) was found as assessed by Dice index, indicating a different selective pressure in CD intestinal habitat. A predominance of phylogenetic group A was found in control subjects ($P < 0.0001$). Results on adhesive and virulence factors evidenced peculiar genes significantly related to CD *E. coli* populations (K1, IbeA) ($P < 0.0001$) and to total DNA from biopsy specimens K1 ($P < 0.0001$). Results suggest that among *E. coli* population, particular variants may be favorite in the intestinal habitat of CD pediatric patients. These genotype variants could represent the genetic background that, via evolutionary phenomena driven by a persistent inflammatory state, may evolve in Adherent Invasive *Escherichia coli* (AIEC) like strains found in adult CD patients.**

Keywords

***Escherichia coli*, Mucosa-Associated, Crohn's Disease**

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1. Introduction

Increased numbers of mucosa-associated *E. coli* have been observed in adult Crohn's disease (CD) patients [1]-[3], showing enhanced adhesive and invasive capabilities [4]-[7] and conveying to a novel pathovar of *E. coli* called Adherent-Invasive *E. coli* (AIEC) [8]. As defined by Crohn's & Colitis Foundation of America (CCFA), children represent a unique model where the mechanisms involved in CD development are poorly confused/influenced by environmental factors. In pediatric patients it has been reported the presence of an intestinal dysbiosis, along with an increase of mucosa-associated bacterial load of *E. coli* [1] [9] [10], suggesting the presence of particular *E. coli* genotypes [11]. So far, almost all studies aimed at characterizing *E. coli* strains collected from the intestinal habitat, picked up 2/3 strains randomly isolated from samples (feces or biopsies). The intrinsic bias in this kind of study is that such strains are not enough representative of a bacterial population living in the intestinal habitat [12]. Here we report preliminary results of a pilot study aimed at describing the *E. coli* population in a more representative fashion. We characterized 539 mucosa-associated *Escherichia coli* strains (77 from each biopsy) isolated from ileal samples of 4 pediatric naïve CD patients (PCDAI score > 30), and 3 subjects with negative CD diagnosis (controls).

2. Methods

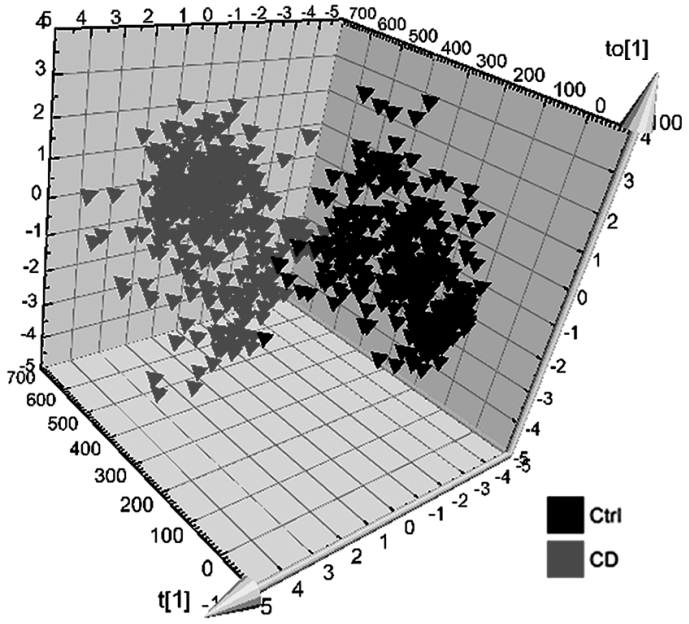
Strains were characterized as follows: 1) genomic typing by Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) with primers 2EC (5'-GTTTCGCTCC-3'), 3EC (5'-GTAGACCCGT-3') and 4EC (5'-AAGAGCCCGT-3'); 2) PCR-phylogrouping as described by Clermont and colleagues [13]; 3) end-point PCR evaluation of 25 specific virulence factors, such as adhesins and toxins, as previously reported [14]. Furthermore, CD discriminant genes found were evaluated for presence/absence directly on total DNA extracted from biopsy samples previously collected from 19 CD patients and 20 controls having similar demographics with the aforementioned 7 subjects. Univariate and multivariate statistics were employed for data analysis. All subjects were enrolled at Department of Pediatrics, Gastroenterology Unit, "Umberto I" Hospital, "Sapienza" University of Rome. None of CD patients had received previous treatment with azathioprine/6-mercaptopurine, antibiotics, or other immunosuppressive and/or biological agents at any time prior to enrolment. All patients underwent ileo-colonoscopy after parental informed written consent according to international protocols. The study was carried out in Rome from 2007 to 2012. *E. coli* AIEC LF82 and *E. coli* MG1655 were used as reference bacterial strains (LF82 strain was kindly provided by Prof. ArletteDarfeuille-Michaud, University of Auvergne, France).

3. Results

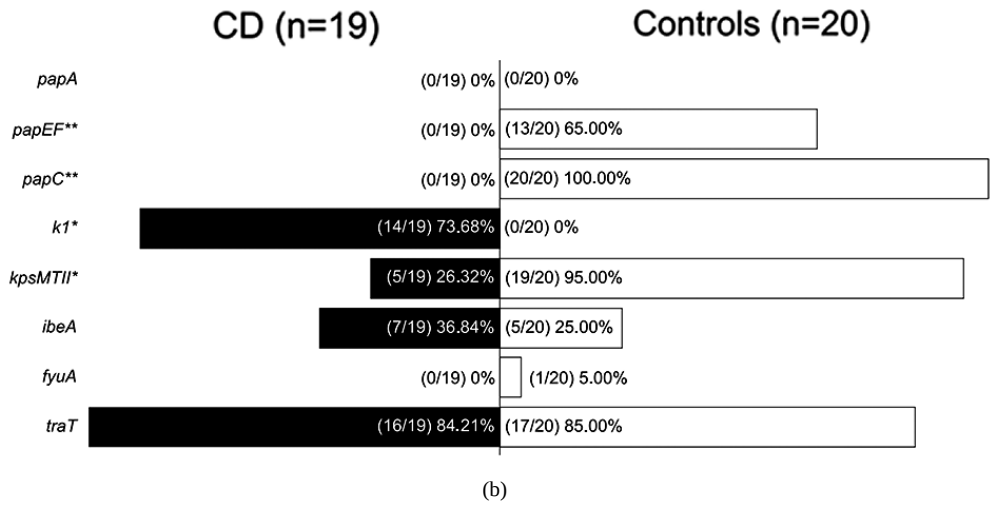
Phylogroup A strains were significantly higher in control subjects, while the abundance of phylogroup B1 strains was significantly higher in isolates from CD patients ($\chi^2 = 30.46$, $P < 0.0001$, $DF = 1$). OPLS-DA analysis of RAPD profiles clearly showed a highly significant separation between CD and control subjects (Fisher's $P < 0.0001$), with a predictability of 96.1% (**Figure 1**, panel (a)). Evaluating the intra-group similarity of all RAPD profiles made by Dice index (D), showed an intra-species variability significantly lower among *E. coli* populations isolated from CD patients compared to those isolated from control subjects (DCD = 51.5% ± 0.5%, DControls = 34.9% ± 0.5%, Mann-Whitney U-test $P < 0.0001$). Among all the virulence factors tested, seven (papA, papEF, PAPC, k1, kpsMTII, ibeA, fyuA) were significantly different for their presence and/or absence in the *E. coli* population. In particular, papA, papEF, PAPC, kpsMTII, and fyuA were significantly found in controls ($P < 0.0001$), while k1 and ibeA were significantly related to CD ($P < 0.0001$). Of the seven genes only four were confirmed to be significantly different in the two groups studied in biopsy samples: papEF, PapC, k1 and kpsMTII. The capsular gene k1 was significantly related to CD, while papEF, PapC and kpsMTII, were significantly related to controls (**Figure 1**, panel (b)).

4. Discussion

In conclusion, we found that among *E. coli* population colonizing the mucosa of CD pediatric patients are already present particular genetic variants that could represent a pathobiont sub-population. Such strains, through evolutionary phenomena driven by a persistent inflammatory state, could evolve in peculiar pathogenic *E. coli* (such as AIEC-like strains), frequently encountered in adult CD patients. Furthermore preliminary results on



(a)



(b)

Figure 1. Panel (a) 3D score plot of RAPD profiles. Panel (b). Prevalence of the selected bacterial virulence factors in ileal samples collected in CD and control patients. Statistical significance. *Virulence factors associated to CD ($P < 0.0001$), **Virulence factors associated to controls ($P < 0.0001$).

invasion ability on Caco 2 cells, and survival ability on macrophage indicate the presence of *E. coli* strains AIEC like in both CD and control group, but significantly more abundant among *E. coli* population of CD pediatric patients (data in progress no shown). Preliminary results in our Short Report need to be improved adding more pediatric subjects, but give an insight on the *E. coli* population concept of particular importance in this kind of study, and on the necessity to individuate such pathobionts population within CD pediatric patients, in order to escape the evolutionary process leading in pathogen forms.

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