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Resúmenes del II Congreso Internacional de Biología Molecular de Cáncer Colorrectal y Mama: Diagnóstico y Tratamiento

14 al 16 de Agosto en Chiclayo, Lambayeque, Perú

Frecuencia de cáncer colon rectal hereditario no polipósico en pacientes atendidos en el Hospital Nacional Almanzor Aguinaga Asenjo 2007 – 2011, Lambayeque – Perú

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El cáncer colonrectal (CCR) es la segunda causa de muerte oncológica a nivel mundial.[1] En el Perú, el cáncer colonrectal según los reportes del INEN se ubica como el más frecuente en varones y el tercero en mujeres.[2] Dentro de esta clasificación se encuentra el cáncer colonrectal hereditario no polipósico (CCRHNP) o también conocido como síndrome de Lynch (SL), siendo la forma más común de CCR hereditario (5-8% de todas las neoplasias de colon), el cual presenta un modo de herencia autosómica dominante, predisponiendo a un desarrollo temprano de otras neoplasias asociadas a este síndrome. Sin embargo para poder identificar a los pacientes con sospecha de CCRHNP es requisito imprescindible que se le realice una correcta historia familiar y el análisis genealógico de por lo menos 3 generaciones del grupo familiar en concordancia con los criterios de Ámsterdam I/II y Bethesda internacionalmente establecidos. **Objetivos:** 1) Determinar la frecuencia de CCRHNP en pacientes diagnosticados con tumor maligno de colon en el Hospital Nacional Almanzor Aguinaga Asenjo durante los años 2007-2011, Lambayeque-Perú. 2) Evaluar la frecuencia de CCRHNP en pacientes diagnosticados con tumor maligno de colon en el Hospital Nacional Almanzor Aguinaga Asenjo durante los años 2007-2011, Lambayeque-Perú. 3) Describir las características clínicas y epidemiológicas de pacientes que cumplan los criterios de Amsterdam I/II y Bethesda, a través de la historia familiar y el análisis genealógico para caracterizarlos como pacientes con cáncer colonrectal hereditario tipo no polipósico en una población diagnosticada con tumor maligno de colon atendido en el Hospital Nacional Almanzor Aguinaga Asenjo, Lambayeque-Perú. **Materiales y métodos:** Diseño del estudio: descriptivo de corte transversal, ambipectivo. Población diana: pacientes con diagnóstico de tumor maligno de colon atendidos en el HNAAA, según reporte de la Oficina de Epidemiología, 2007-2011. Criterios de inclusión: Paciente ≤ 70 con diagnóstico de tumor maligno de colon, resida en el departamento de Lambayeque. Criterios de exclusión: Paciente con diagnóstico de PAF, enfermedad mental grave, analfabeto. Muestra: Pacientes del registro de cancerológica de la Oficina de Epidemiología del HNAAA, 2007-2010. Población elegible: Pacientes que cumplan los criterios de inclusión y exclusión. Variables: Criterios Clínicos CCRHNP, Edad, sexo, presencia de tumor extracolónico, diagnostico histopatológico, comorbilidades, nivel de instrucción. Instrumento de recolección de datos: entrevista: historia familiar y genealogía. Análisis estadístico y procesamiento de datos: Se registrara la información en tablas y gráficos sobre los resultados de la frecuencia. Aspectos éticos: Consentimiento Informado. **Justificación:** Conocer la predisposición familiar al cáncer colonrectal (CCR) es un factor importante en la prevención de la morbilidad y de la mortalidad. La contribución de la herencia en la población peruana sigue siendo desconocida, pero basado en otras poblaciones, se espera que el 20% de los CCR diagnosticados tengan un comportamiento de tipo hereditario. Este nuevo conocimiento podría aportar una base de datos sobre los pacientes y familias donde este tipo de CCR se estaría comportando como un síndrome hereditario y transmitiendo de forma autosomica dominante. En consecuencia haciendo uso del análisis de la historia familiar con la ayuda del árbol genealógico, se tendría la primera caracterización de pacientes y familias con este tipo de neoplasia diagnosticada según los criterios clínicos aprobados internacionalmente, lo cual beneficiaria en el tratamiento y cuidado del paciente y de sus familias, ayudando a predecir la probabilidad de sobrevida en los pacientes que padecen de CCRHNP. **Bibliografía:** 1) Moussa S AB, et al Lynch syndrome in Tunisia: first description of clinical features and germline mutations. Int J Colorectal Dis. 2011;26(4):455-67. 2) Instituto Nacional Enfermedades Neoplasicas [Internet]. Boletín Epidemiológico. Disponible en: http://www.inen.sld.pe/portal/estadisticas/datos-epidemiologicos.html

Towards personalized immunotherapy in colorectal cancer

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Over the last decade, immunotherapy has entered into the field of colorectal cancer (CRC) treatment with some great successes. Humoral immunotherapy based on the use of anti-EGFR (Epidermal Growth Factor Receptor) antibodies, or targeted therapy, has proven efficacious in metastatic CRCs. However, somatic mutations of KRAS, a key molecule on EGFR signaling pathway, render the tumors resistant to anti-EGFR therapy, and other therapeutic approaches must be developed for these patients. *TP53*, *BRAF*, *PTEN* or *PIK3CA* mutations, among other genetic defaults, could also modify resistance or sensitivity to anti-EGFR therapy. Cellular immunotherapy based on the transfer of autologous anti-tumor specific T lymphocytes, or adoptive cell therapy, is an other very promising approach, which is still, for CRCs, mainly in the field of research. Targeting auto-antigens over-expressed in the tumor could be difficult because of central and peripheral immune tolerance. Targeting neo-antigens due to tumor mutations could be clinically more relevant, with a more limited risk of tolerance. Neo-antigens are particularly numerous in microsatellite unstable CRCs. These CRCs represent approximately 15% of total CRCs and are due to a mismatch repair machinery deficiency that can be of genetic origin (Lynch syndrome in young patients) or not (sporadic microsatellite unstable CRCs in older patients). Mutated KRAS protein could also be a relevant target for anti-EGFR-resistant *KRAS*-mutated tumors found in all forms of CRCs. The main contributions of our laboratory in both humoral and cellular immunotherapy fields for CRCs will be presented and discussed.

Contribution of splicing mutations to the genetic determinism of colorectal cancer and of breast and ovarian cancer

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Lynch Syndrome (LS, often called Hereditary Non Polyposis Colorectal Cancer) and Hereditary Breast and Ovarian Cancer Syndrome (HBOCS) are the most frequent forms of hereditary cancer worldwide. These syndromes are inherited in an autosomal

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dominant pattern. They are essentially due to germline alterations of a gene implicated in DNA repair: *MLH1* or *MSH2* in most LS cases, and *BRCA1* or *BRCA2* in HBOCS. Typical alterations include intragenic rearrangements (large duplications, insertions and deletions) and subtle mutations (small deletions and insertions, nonsense, missense and canonical splice site mutations). The identification of the genetic cause of cancer predisposition is crucial for patient and family follow-up, genetic counseling and, ultimately, cancer prevention. However, many cases remain without a genetic explanation, one of the reasons being the frequent identification of alterations difficult to classify either as neutral or deleterious, such as synonymous mutations, many missense substitutions, and intronic variations. We hypothesized that some of these variants of unknown significance (VUS) could be deleterious by affecting RNA splicing. To test this hypothesis, and because patients' RNA is rarely available, we developed a functional RNA splicing assay based on the analysis of the splicing pattern of representative minigenes transiently transfected into human cells. To date, we analyzed in this assay 222 and 168 VUSs identified in LS and HBOCS patients, respectively. Whenever possible, results were compared with data derived from the analysis of patients' RNA. We found that a large fraction of VUS, 30% in LS and 28% in HBOCS, induce splicing alterations. These results have an important impact on diagnostics. Given their strong effect on RNA splicing, 20% and 15% of the LS and HBOCS VUSs analyzed in our study are now considered deleterious splicing mutations. Variants that induced partial splicing defects (10% of LS and 13% of HBOCS VUSs) remain a challenge for classification.

TBalancing life at increased risk of cancer

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Background: Several studies have shown that most carriers of Lynch syndrome experience increased levels of anxiety, distress and depression after having received a positive test result. In the majority the levels return to normal levels within 12 months. However a subset may be in need of psychosocial support. Identification of these individuals is central for the development of targeted psychosocial interventions. **Materials and methods:** We have applied and compared standardized tools aimed to identify patients with Lynch syndrome who are at increased risk of psychosocial vulnerability from knowledge about hereditary cancer. Self-concept and sense of coherence (SOC) were assessed in the Danish population. Moreover, we explored the lived experiences of hereditary cancer among healthy mutation carriers with Lynch syndrome through interviews with 12 mutation carriers. The data were analysed using a phenomenological approach. **Results:** Evaluation of self-concept showed that the majority of Danish mutation carriers adapt well to the situation though knowledge about risk seems to have a greater impact on women. We also found SOC in scores mutation carriers similar to the general population. In the majority (76%) of the mutation carriers SOC and self-concept were in accordance with adverse scores on both scales in 10%. The findings from the interview study suggest that the increased risk is interpreted and handled in relation to family experiences. The ability to handle thoughts and feelings constitutes an act of balance in which knowledge can confer feelings of security and control. **Conclusion:** These results suggest that tools aimed to reinforce balance related to specific aspects of Lynch syndrome may be relevant to consider in targeted psychological intervention vulnerable subgroups.

TDistinct gene expression profiles in ovarian cancer linked to Lynch syndrome

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Background: Lynch syndrome is a multi-tumor syndrome caused by defective DNA mismatch-repair. Though colorectal and endometrial cancers predominate, female mutation carriers are at increased risk also of ovarian cancer, which typically develops as early-stage tumors at young age. With the aim to identify genetic classifiers of hereditary ovarian cancer, we analyzed gene expression in Lynch syndrome-associated ovarian cancers with comparison to sporadic ovarian cancers. **Materials and methods:** Global gene expression profiling was performed in a histopathologically matched series of 24 Lynch syndrome tumors and 24 sporadic ovarian tumors. Key target genes were validated using immunohistochemical staining. **Results:** The distinct signatures contained 335 genes that were differentially expressed between Lynch syndrome tumors and sporadic tumors. Deregulation affected key targets in the mTOR and MAPK/ERK signaling pathways. **Conclusions:** Upregulation of the mTOR and MAPK/ERK signaling pathways characterize ovarian cancer linked to Lynch syndrome, which implies that targets herein may be relevant for preventive and therapeutic strategies.

TThe Danish HNPCC-register. A multidisciplinary effort to support individual health care in hereditary colon cancer families

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The Danish HNPCC-register (http://www.hnpcc.dk/) was established in 1991 with the overall aim to improve the prognosis of families with hereditary colon cancer and it is nowadays a national database financed within the National Public Healthcare System. Regardless of residence of the family, epidemiological and genomic data from all families with Hereditary Colorectal Cancer (HNPCC) is included in the register to optimize identifying of family members at risk and initiate and establish screening. All over the country diagnostic data are generated in different departments and laboratories and sent to the register, where these information have been collected over several decades. Until recently paper-based reports were sent to the register and typed into the database. In the EC co-funded – INFOBIOMED network of excellence (www.infobiomed.org), The Danish HNPCC-register

was used as model for electronic exchange of clinical data between diagnosing/treating departments and the HNPCC-register. The aim was to prevent cancer by optimizing organization of screening with digitization of data transport and facilitation of combining genotype-phenotype information. IT-tools, sufficiently usable and generic to be implemented in other countries and for other oncogenetic diseases, were developed. Medical data are very heterogeneous, wherefore the major focuses were on integration, elaboration and dissemination of classification systems and communication standards. In this presentation information on the organization and development of the register for more than 20 years will be presented. What are the challenges and the outcome of a national multidisciplinary effort to prevent cancers in families with increased risk of cancers with the focus on diagnosing the families and establishing the screening procedures. **Biography.** Inge Bernstein is a colorectal surgeon and has completed her Ph.D from Copenhagen University and Master of Health Management from Copenhagen Business School. She is director of The Danish HNPCC-register, a National register including epidemiological and genomic data on all Danish families with Hereditary Non Polyposis Colorectal Cancer. The aim is to improve prognosis by identifying at-risk family members, establish screening and evaluate the outcome. She has published more than 60 papers in reputed journals and is serving as an editorial board member of the Danish Colorectal Cancer Group and the InSiGHT (International Society of Gastrointestinal Hereditary Tumours).

TmiRNAs as non-invasive biomarkers for prostate cancer for diagnostics

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Background: Prostate cancer (PCa) clinical attention is based on early detection using serum prostate-specific antigen test (PSA). Its high sensitivity led to an increase of PCa incidence and detection of early stage of the disease, but also to a risk for overdiagnosis. The drawback of this test is its low specificity, such that benign hyperplasia can also be associated with PSA increase; thus, additional specific biomarkers are needed. A biomarker with great potential for applications in cancer definition and staging are MicroRNAs (miRNA): they are stable small noncoding RNAs that inhibit gene expression of numerous mRNAs through several mechanisms and have been linked to cancer development in different tissue. **Matherials and methods:** We compared the miRNA expression fingerprint from urine and prostate biopsies looking for a correlation in those tissues. The different samples were collected from 50 subjects with PCa and benign hyperplasia. Comparisons of qRT-PCR ratios from ten miRNAs previously shown to be deregulated in PCa were analyzed in order to evaluate the significance of abundance of miRNAs among those tissues. **Results:** We developed a method for extraction of miRNA from urine-exosomes suitable for expression quantification. We also found suitable normalization controls, and optimized multiplex qRT-PCR protocols for each tissue type. So far the, analysis to establish expression correlations in the different samples of the 50 subjects identified two miRNAs with diagnosis potential. **Conclusions:** The results of this project will be the basis for developing a non-invasive PCa diagnostic tool, improving patient's life quality when indicated for PCa testing. **Acknowledgments:** The FP7 Marie Curie Initial Training Network PRO-NEST.

TMolecular mechanisms in hereditary colorectal cancer

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Colorectal cancer (CRC) is one of the main causes of death in South American countries. The hereditary forms of CRC are, familial adenomatous (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch Syndrome (LS), which is the most common form. CRC develops under influences of a number of molecular cascades to chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP)[1,2,3]. CIN characterizes most sporadic MMR proficient CRC and tumors linked to FAP, *MUTYH*-associated polyposis (MAP), and FCCTX hereditary syndromes. CIN tumors are typically aneuploid, cytogenetically complex and harbor mutations in oncogenes and tumor suppressor genes[1,4,5,6,7]. CRC linked to LS is characterized by MSI, which also characterizes 15% of sporadic CRC with a predilection for the proximal colon[5,8]. Appearance of secondary *BRAF* (p.Val600Glu) mutations may be used to distinguish between somatic and germline *MLH1* inactivation, since these mutations are found in 50% of the somatically *MLH1* methylated cases[8]. Molecular classification of CRC based on MSI and CIMP status is increasingly important, because they reflect global genomic and epigenomic alterations in tumor cells[9,10]. **References:** 1) Lengauer, C., *et al.* (1998) The role of epigenetics in cancer. DNA Methylation, Imprinting and the Epigenetics of Cancer--an American Association for Cancer Research Special Conference. Las Croabas, Puerto Rico, 12-16 1997 December. *Mol Med Today*, 4, 102-3. 2) Jass, J.R. (2007) Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. Histopathology, 50, 113-30. 3) Pritchard, C.C., et al. (2011) Colorectal cancer molecular biology moves into clinical practice. Gut, 60, 116-29. 4) Kim, H., et al. (2004) Different gene expression profiles between microsatellite instability-high and microsatellite stable colorectal carcinomas. Oncogene, 23, 6218-25. 5) Walther, A., et al. (2009) Genetic prognostic and predictive markers in colorectal cancer. Nat Rev Cancer, 9, 489-99. 6) Silver, A., et al. (2012) A distinct DNA methylation profile associated with microsatellite and chromosomal stable sporadic colorectal cancers. Int J Cancer, 130, 1082-92. 7) Woods, M.O., et al. (2010) The genetic basis of colorectal cancer in a population-based incident cohort with a high rate of familial disease. Gut, 59, 1369-77. 8) de la Chapelle, A., et al. (2010) Clinical relevance of microsatellite instability in colorectal cancer. J Clin Oncol, 28, 3380-7. 9) Ogino, S., et al. (2008) LINE-1 hypomethylation is inversely associated with microsatellite instability and CpG island methylator phenotype in colorectal cancer. Int J Cancer, 122, 2767-73. 10) Goel, A., et al. (2011) De novo constitutional MLH1 epimutations confer early-onset colorectal cancer in two new sporadic Lynch syndrome cases, with derivation of the epimutation on the paternal allele in one. Int J Cancer, 128, 869-78.

Colorectal Cancer (CRC), in Colombia: genetic studies sporadic and familial cases

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Background: Colorectal Cancer (CRC), has high rates of incidence and mortality in Colombia. The Genetic factors explain approximately 35% of the variation associated with the risk of CRC. This work attempts to construct a molecular-genetic diagnostic

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panel to distinguish between sporadic and germline forms of 1000 patients with CRC and to establish their genetic variants and clinic-pathologic characteristics. **Patients and methods:** Patterns of expression of DNA repair proteins (MMR) will be established for inmunohistochemistry; the patterns of microsatellite instability (MSI) and the mutations in BRAF, KRAS, APC, TP53 and MUTYH will be evaluated for PCR. The MMR genes will be sequenced after previous characterization of the somatic tumors in patients without IMS and BRAF mutations. The APC and MUTYH genes will be sequenced in patients with adenomatous polyposis along with mutations in KRAS and TP53 to establish a panel of molecular diagnostics. Cases with criteria for genetic syndromes shall be sampled and their relatives will be screened for mutations to do clinico-pathological comparisons between carriers and sporadic cases. The associations derived from the analysis of surveys and pathological reports will be assessed using regression and multivariate analysis. **Results (partial):** Blood DNA from 1161 was extracted and quantified. Analysis of clinic-pathologic information identified 40 families with 24% affected, showing a wide variety of familial syndromes like Lynch Syndrome, P.A.F, hyperplastic Syndrome an Peutz Jegher Syndrome other carcinomas such as CCR (19%) gastric (7%), thyroid (32%) and benign neoplastic type adenoma (32%). IHC are doing. **Acknowledgement:** Financial support CHIBCHA project, EEC, Oxford University, Tolima University. **Conflict of interest:** None declared.

Patient perspectives in hereditary cancer – Influence and psychosocial issues in Lynch syndrome

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Background: Possibilities to undergo predictive genetic testing have largely evolved during recent years. A growing number of healthy individuals thus live with knowledge of an increased risk of disease, but how this knowledge influences life is largely unknown. Though increased levels of anxiety and depression have been demonstrated around the time of genetic counselling, data have been collected on individual level not always taking the psychosocial issues and family into account. Lynch syndrome is characterized by an early age of onset and high life time risks, particularly for colorectal cancer (60-80%), endometrial cancer (40%) and ovarian cancer (10-15%). Surveillance with regular colonoscopies, however, reduces morbility and mortality up to 60%. **Material and methods:** This presentation reviews evidence and knowledge concerning psychosocial impact from Lynch syndrome in the family and in individuals at increased risk. **Result and conclusión:** Current knowledge suggest that most individuals handle life at increased risk well, whereas 5-10% fin this knowledge burdening. Various tools are available for the determination of e.g. self concept and sense of coherence in relation to Lynch syndrome. We review lived experiences and long time perspectives in mutation carriers and strategies related to information and communication about test results with the aim to develop targeted psychological and psychosocial interventions.

Estudio molecular del síndrome de Lynch en Rosario, Argentina

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Introducción: El Síndrome de Lynch es una enfermedad de origen genético de carácter autosómico dominante, de alta penetrancia y caracterizado por el desarrollo temprano de cáncer colorrectal y cánceres extracolónicos (endometrio, ovario, estómago, vías biliares, riñón, vejiga, uréter, piel, cerebro). Los genes afectados codifican para un sistema de reparación del mismatch del ADN (MLH1, MSH2, MSH6, PMS1, PMS2, MLH3). **Materiales y métodos:** Entre setiembre de 2005 y setiembre de 2012 se identificaron 134 casos índice (CI) que cumplían con criterios de Amsterdam. El estudio molecular consistió en un *screening* determinando inestabilidad de microsatélites (MSI) empleando el panel de Bethesda (BAT-25, BAT-26, DS17250, D5S346, D2S123) e inmunohistoquímica de las proteínas MSH2 y MLH1. En la siguiente etapa se secuenciaron MSH2 y MLH1. **Resultados:** De los 134 CI, 54 (40%) adhirieron al estudio. En 8 (15%), el tumor presentó MSI-alta (2 ó más microsatélites inestables) y en 6 (11%) MSI-baja (1 inestable). En 7 de los 8 con MSI-alta se encontraron mutaciones en 4 (3 en MSH2 y 1 en MLH1). En los de baja inestabilidad se secuenciaron 2, de los cuales 1 presentó mutación en MSH2. **Conclusiones:** En este grupo de pacientes predominan las mutaciones del gen MSH2. En nuestro medio el síndrome de Lynch está subdiagnosticado por el desconocimiento de los médicos tratantes, por el bajo nivel de adherencia de los pacientes por razones socioeconómicas y culturales, y por la falta de cobertura de la prestación por parte del estado y las aseguradoras de salud privadas.

Functional Implications of the p.Cys680Arg mutation in the MLH1 mismatch repair protein

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Contract grant sponsors: The European Union (LSHC-CT-2005-018754) and Nordea-fonden.
In clinical genetic diagnostics, it is difficult to predict if genetic mutations causing unknown functional effects on cognate proteins lead to development of disease. Here, we report the clinical identification of c.2038 T>C missense mutation in exon 18 of the human *MLH1* gene and biochemically characterize the p.Cys680Arg mutant MLH1 protein to implicate it in the pathogenicity of the Lynch syndrome. The *MLH1* c.2038 T>C mutation, which causes the amino acid substitution p.Cys680Arg, was identified in an individual with 5 synchronous and metachronous tumors, all of which showed a MSI-high phenotype and loss of MLH1/PSM2 expression. The mutation occurs in a region of the *MLH1* gene that is involved in MLH1-PMS2 interaction. *In silico* analysis unanimously suggested a deleterious effect of this mutation, which was confirmed by functional assays.

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MicroRNA in breast cancer

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MicroRNAs (miRNAs) are a class of ~22 nt small non-coding RNAs that control gene expression through translational repression and regulation of RNA stability. They have been shown to be extensively de-regulated in many different types of cancer and several studies have uncovered their involvement in metastasis promotion or suppression. To comprehensively characterize small non-coding RNAs involved in breast cancer, next-generation sequencing was applied on a total of 15 samples of breast tumors with paired adjacent and normal breast tissue. From the 170 million small RNA reads generated, we identified 361 new human miRNA genes. More than one tenth of the new miRNAs are located in regions that are genomically unstable in cancer. A notable example is miR-4728 encoded within the human epidermal growth factor receptor 2 gene (ERBB2/Her2) an important biomarker and a gene with potent oncogenic capacity that is amplified in many cancer types. To assess the function of this interesting miRNA, we started the functional characterization of miR-4728 in breast cancer cell lines. Our results might have clinical implications since earlier studies indicated miR-4728 to be co-expressed with Her2, which is amplified in approximately 30% of breast cancers.

Next generation sequencing for genetic analysis of at-risk individuals

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Background: Genetic analysis of the BRCA genes is becoming a standard of care for families and individuals with hereditary risk of breast and/or ovarian cancer. The identification of deleterious mutations is essential to direct actions such as early screening to mutation carriers and to adapt treatment where possible. Two factors urge the use of next-generation techniques for mutation detection. First is the greatly increased demand resulting from the expansion of mutation screening criteria to include a progressively larger proportion of the population. Second is the current development of cancer treatments targeting tumors with constitutional mutations, and the rapid turnaround time required for treatment decisions. **Materials and methods:** Amplicons covering the complete coding sequence of the BRCA1 and BRCA2 genes were generated by classic PCR, by Fluidigm 48x48 AccessArray chips, and using the Multiplicom BRCA amplification kit. Sequencing was performed on a Roche/454 GS-Flx or GS-Junior apparatus, and analysed using AVA software with locally developed supports. Variants were confirmed by Sanger sequencing. **Results:** Coverage of coding sequences and splice junctions was more than adequate for all but a handful of amplicons. Using limits of 40x read coverage and 20% variant reads minimized the number of false-positives obtained, without dropping out any variants detected by Sanger sequencing in preliminary experiments. In-house software for the analysis of reads over homopolymers allowed most of these problematic sequences to be correctly genotyped. **Conclusions:** Roche/454 pyrosequencing is a sensitive and specific method for the detection of constitutional mutations. The rapid turnaround and high capacity of the technique makes it ideal for high-throughput analysis of the BRCA genes. We plan to shift all of our BRCA testing to this technology, and to sequence other genes by the same strategy. **Acknowledgements:** Thanks to the GINA sequencing platform and the ERTICa research team at the CJP.

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Register history and characteristics: In 1970, headed by Fernando A. Bonadeo M.D., a colorectal section was organized in the Service of General Surgery. Currently this section is integrated by 5 surgeons and keeps a prospective database of over 4000 colorectal cases, which represents the largest one in Argentina and one of the largest in Latin America. In 1996, an institutional registry on hereditary colorectal cancer (Programa de Cancer Hereditario, Pro.Can.He.), was founded by C. Vaccaro becoming the first one in Argentina focused on HNPCC. One year latter, its experience with 9 families was published as the first report from a registry in Argentina. Currently, the registry has data from 81 families fulfilling Amsterdam Criteria, 40 fulfilling modified Amsterdam Criteria, 435 familial cancers, 26 families with familial adenomatous polyposis, 14 patients with Peutz-Jeghers and 4 with juvenile polyposis. Regardless limitations already stated, the Registry has grown steadily mainly for personal efforts and the collaboration of several foreign centers. A crucial moment for the development of the registry was the meeting with Henry T. Lynch during his visit to Uruguay in 1998 to counsel an Uruguayan family (Fig. 1). By then, a collaborative study was initiated with his help and that of Paivi Peltomaki MD. Since no center had experience with genetic counseling on HNPCC in Argentina, the visits to Registries at Cleveland Clinic Foundation (with James Church and Ellen McGannon) and MD Anderson (with Patrick Lynch) allowed our registry to gain knowledge and carry out counseling in an appropriate manner. As a consequence of this collaboration, as well as the continued help and support received from Terry Berk, several Argentine families could undergo genetic testing and have been counseled. Additionally, several research studies could be accomplished and published.[19-21] **The first 10 years of experience:** Our experience with the first Argentine on epidemiological, molecular and genetic counselling data was published in the *Disease of Colon and Rectum*. Here we present an update over 57 families registered from 1996 to 2007 fulfilling Amsterdam criteria. (an update of the 81 families will be presented at the meeting). These cases were identified from our historical database (upon physician suspicion or early onset of cancer) and more recently among people referred or self-referred to the ProCanHe. Pedigrees were constructed upon family background obtained by personal interview with the proband. Confirmation by pathologic tissues or pathology reports was made whenever possible. Those families which were eligible for genetic testing were counseled according to the Creighton University's recommendations in three stages. The pre-test stage was performed to provide information about all aspects of natural history and current surveillance strategies as well as to discuss advantages, potential disadvantages and limitations of genetic testing. According to the family preference this first stage was done in an individual or familial information session (Fig. 2). For one family, the counseling session was done as a field visit at one member's home (Fig. 3). For the second stage (pre-result stage) the family members were contacted to personally and confidentially receive the results in an individual session during which all potential implications of genetic testing were discussed again with the assistance of a psychiatrist (Fig. 4). The last stage (follow-up) was performed to update information about the family background and to determine degree of accomplishment of surveillance recommendations. At all the stages, participants were asked to complete a survey form including data related to knowledge about

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the disease and its psychological impact. **Results:** A total of 57 families fulfilled the Amsterdam Criteria (45 AC I and 12 AC II). Eighteen (31%) presented as Lynch syndrome I, 36 (63%) as Lynch syndrome II and 3 (6%) as Muir-Torre syndrome. Table 1 depicts their clinical characteristics. Data from 839 relatives (53% females) of a mean of 46.4 (range: 2 - 94) could be obtained. The median number of individuals per family was 14 (range: 1 to 41) with no statistical difference between AC I families and AC II families (12 [IC95%: 9-24] vs. 8 [IC95% 4-28], respectively, p=0.10). Families characterized as Lynch syndrome I presented a trend to have less relatives compared with families characterized as Lynch syndrome II (8 [IC95: 5-22] and 17 [IC95%: 12-25], respectively, p=0.09). A total of 343 patients (52% females) with cancer were identified among the 839 relatives (40.8%). The median of affected members per family was 6 (IC95% 5-8, range 3-24). In 210 cases (61.2%) CRC was the first diagnosed tumor and 42 (12.2%) developed more than one tumor (33 patients presented 2 cancers, 5 patients 3 cancers, and 4 patients 4 cancers). A paradigmatic case was a woman who developed an adenocarcinoma in the cecum at age 39. By this time her family background, although included many affected members, did not fulfill Amsterdam criteria and genetic testing was not available. She was advised to undergo a prophylactic hysterectomy which refused regardless her complete risk comprehension for being oncologist. One year later she developed an adenocarcinoma of the endometrium. At age 44 she was operated on for a mucinous adenocarcinoma duodenal and at age 49 for a breast adenocarcinoma. This patient was found to carry a novel mutation (hMSH2: exon 12, del C en nt 1910, codon 637 and her family background showed a strong aggregation of breast cancer (Figures 5 and 6). A lack of inmunohistochemical hMSH2 expression was found in all the tumors tissues. A total of 213 cases of colorectal cancer were identified with a median of 3 (IC95% 3-5, range 1-21) cases per family. The mean age at diagnosis was 52.1 (range 21 to 90) with 53% of the cases diagnosed before 50 year old (highest prevalence between 41 and 50 years: 34.8%). This age of onset is similar to that reported from Europe[22] and slightly higher than those reported in Brazil,[23-25] in Uruguay[26,27] and in North America.[28,29] Age of onset was the same in cases identified by AC I and AC II (50 years) and was related to the generation number (Table 2). This anticipation of developing cancer could be explained by a secular time trend in cancer occurrence and or improvement in screening and surveillance strategies. Patients with more than one tumor developed colorectal cancer at an earlier age: 45 (range 21-87) vs. 51 (range: 22-90), p=0.001. One-hundred ninety-six out of the 343 (57.1%) affected members presented colorectal cancer only, 26 (7.6%) associated with extracolonic tumors and 121 (35.3%) presented with extracolonic cancer alone. These relatives proportions are similar to those reported in USA. Regarding extra colonic tumors, breast cancer and gastric cancer were the most common tumors in females and males respectively. **Genetic counseling:** In our previous analysis only 25 (43%) out of the 57 families fulfilling Amsterdam criteria could be counseled by the register. This was mainly due to the inability of the registry to offer adequate support because of resource limitations. In the data regarding the 84 counselled relatives (57% females; mean age 44.7 years old [range: 18-81]), several figures point out the lack of knowledge of people at risk and the need of an educational register program: 1) Seventy-nine percent of the individuals were aware of their risk when a close relative died from (34.5%) or were diagnosed with (44.5%) CRC. Only 1.2% was warned by a physician. 2) Although 71% of the interviewed referred that their personal medical doctors knew about their family background, only 62% had shared information with them. 3) Eighty-one percent of them had received surveillance recommendations. However, this occurred at a mean age of 29 years. Furthermore, this information was provided by a physician in only 32% of the cases. 4) Before counseling, up to 73% of participants had heard little or nothing about genetic testing for cancers. This rate is higher than the 64% found within the American population by a NIH study.[30] 5) Sixty-seven percent referred having new relatives showing interest in being counseled. 6) Ninety-seven percent of the relatives estimated that they would accomplish the surveillance recommendations provided by the registry. 7) After becoming aware of the oncological risk, 61% of the individuals expressed concern and 11%, fear. These figures are similar to those reported from Europe. Regardless of the lack of experience in giving genetic counselling and the fact that the session was led by a surgeon (CAV), all counselled members considered the session adequately implemented, very useful and recommendable for their relatives at risk. Sixty-six percent referred to feel better than before the session and 93% stated to trust in the confidentiality of the data. These results are as satisfactory as those reported by well organized registers. All the people eligible for molecular testing accepted to pursue testing. This is in accordance with a cohort study conducted at the NIH where 97% of the individuals stated this intention.[30] In our series this high level of acceptance could be explained not only for the adequate implementation of the counselling but also by the fact that no fee was charged. An additional cause may include the lack of fear to discrimination by health insurance companies or by employers, which is the most frequent reason (up to 39%) to refuse testing in United States. Among individuals who had no children, 46% identified their own health concern as the most important reason to consider testing. On the other hand, those with children identified learning about their children's risks as the most important motivation. Again, the figures above are similar to those reported among Americans. Data from the United Kingdom show that most people still want screening if at low risk and would make more plans for the future if they were at high risk. In our series 52% of the individuals referred that the genetic testing results would modify their life style or future plans. Although only 50% considered informing their physicians, as much as 87% of the people referred no concern or difficulty sharing the information with their relatives or friends (which is similar to other series). Furthermore, all individuals expressed feeling emotionally supported by their families or friends. Among the people contacted for follow-up, 86% were willing to receive an additional counseling session and 38% would like to receive psychological support. Seventy-six percent of the people interviewed had talked about their cancer risk in the last year, mostly with their family members (71%) and/or with their family doctor (29%). Lack of interest and fear were the most common causes referred by those who had not recently discussed their risk. Ten out of twelve individuals who had children over 18 years old had shared the information with them. Regarding implementation of surveillance recommendations, seventy-six underwent a videocolonoscopy last year.[37,38] Among women, thirty-six underwent surveillance (transvaginal unltrasound and ecography and serum CA125) last year. As worldwide reported, lack of interest and fear were the main reasons not to pursue surveillance. **Final considerations:** Argentina is a developing country with unevenly distributed health resources and no professionals trained to perform genetic counseling for hereditary cancer. Experience in counseling for hereditary cancer is limited to the only 3 Argentine registries which are concentrated in urban centers and have a restricted scope, determined largely by the interest of individual clinicians and researchers and by resource limitations. International collaboration allowed the implementation of genetic testing with a high degree of satisfaction. However, support for continued surveillance and counseling is still limited, which makes it difficult to appropriately run the registry. Although genetic testing for people at risk has been proven to be cost effective even in developing countries, registries do not receive financial support making these programs lack permanent financing and skilled personnel. Regional collaboration is beginning and promising.

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Oncogenetics for breast cancer in medical practice. The French Experience

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Amongst multiple risk factors, hereditary predisposition to cancers is the risk with the far highest PPV (positive predictive value) and NPV (negative predictive value) allowing personalized predictive medicine. It therefore, permits specific medical cares for patients and families. More than 72 known genes are related to hereditary monogenic predisposition to cancers (BRCA1, BRCA2, MLH1, MSH2, MSH6, CDH1, PTEN, APC, TP53…); many others are related to multigenic hereditary susceptibility. The first ones are belonging to the oncogenetics medical practice in about 5% of all cancers, others are mainly in the research field in 25-50% of cancers. Oncogenetics is defined as the medical care and diagnosis of patients and their family with an hereditary or a familial high risk of cancer. Oncogenetics was pioneered in France at Clermont-Ferrand in 1988, then spread in a national network since 1991. Moreover we shared our experience with 24 countries through partnerships or international grants. It is already demonstrated that cancer prognosis is better when an oncogeneticist is within an oncological multidisciplinary team than when not. Actually knowing the hereditary risk to cancer allows specific practices like early diagnosis (and reduces cancer mortality), cancer prevention (and reduces cancer incidence), adapted cancer treatments (allows personalized targeted therapy), genetic counselling … Oncogenetics saves lives of patients and saves money for the country. We develop the "oncogenetics chain" concept as follow: Multidisciplinary care: From the patient (family) to the patient (family) through molecular genetic test

Organization: linked by a dedicated bio-informatics "Gataca" software. From one highly specialized multidiscipinary oncogenetics clinic. To a tightly linked high-quality molecular diagnosis laboratory to a network of physicians for Personalized Oncogenetics Follow-up Programme (POF-UP). **When to address a patient to an oncogenetics consultation?** Because germ-line mutations are rare in the general population (<1%) family history is the best tool for identifying mutation carriers in cancer hereditary predisposition genes. There are two main situations: 1) The most frequent situation is linked to an aggregation of at least 3 histological confirmed breast cancers in a family branch. In small families 2 cases may indicate oncogenetics consultations especially when cancers were diagnosed at an uncommon early age in patients (<35 yrs –old) and/or when patients developed multiple cancers in their life (breast and ovarian). 2) A familial clinical scoring is commonly used in France for BRCA genes testing. Excellent indication of oncogenetics consultation is considered if scoring is ³ 5, possible indication if scoring is at 3 or 4. Score is for each case of BC (breast cancer) or OC (ovarian cancer) in a family branch: Known BRCA germ-line mutation: 5; BC woman < 30 yrs: 4; BC woman 30 - 40 yrs: 3; BC woman 40 - 50 yrs: 2; BC woman 50 - 70 yrs: 1; BC man: 4; OC: 3. If a women has successive breast or ovarian cancer, her scoring is additive. More oncogenetics consultation indications (and BRCA tests) are now recommended, without knowing the familial context when: male breast cancer, ovarian cancer <60 yrs-old, triple-negative or basal-like breast cancers. The rarer situation is linked to hereditary diseases in the evolution of which, cancer risk is high: Cowden disease, Li-Fraumeni syndrome, Peutz-Jeghers disease … In 2011 in France oncogenetics is organized in 48 main consultations, making 36.000 consultations per year, about half of them for breast cancer families. In Clermont-Ferrand consultation, we have more than 5.000 families and 150.000 people with their pedigrees in our data base. With the clinical suspicion, **gene test** is required in families to make the diagnosis, or confirm it, or weaken it, or to look for one or many predisposition genes, or help to adapt medical care. 2 independant blood (or jaw smears) samplings after the signature of an informed consent sheet are required for gene tests. When a deleterious germ-line mutation is found the high risk of cancer is described to patients in a new consultation and predictive test is available for all relatives of the family branch. For instance, cumulative cancer risks over life, linked to BRCA mutations are: breast in women: 80-85%; breast in men: 5-7% for BRCA2; Invasive ovarian: 40 - 50 % for BRCA1, 10 - 20 % for BRCA2; controlateral breast: 30 - 45% (3%/yr); Fallopian tuve: 3% (RR: 50-120); peritoneal cavity: 1-2% (RR: 44.6); prostate <65 yrs: RR: 2.6 - 23 (BRCA2); biliary ducts, gallbladder, pancreas melanoma: RR 3-5 for BRCA2. The cumulative risk may be modulated by other acquired factors. For instance, breast cancer risk is higher in nulliparous with natural menopause at 50 yrs-old women who smoked from age 25. Therefore **early diagnosis** of main cancer risks is recommended to all mutation carriers and discarded for non carriers (Personalized Oncogenetics Follow-up Programme). In BRCA families it consists of: a) Breast for women and men (when appropriate) : clinical exam every 6 months from 20 yrs-old and annual mammography/MRI/U.S. from 25 yrs-old. b) Ovaries from 35 yrs-old : clinical exam & plasmatic CA 125 every 6 months + U.S. yearly. Whenever possible **prevention** is the goal to reach for those families in order to avoid cancer treatments and burdens. Prevention is mainly by surgery and removal of the target tissues of cancer development in BRCA positive people (always after multidisciplinary discussions of each case): bilateral annexectomy in BRCA women after completion of child project (and >40yrs-old), total mastectomy. Medical recommandations are always explained : encouragements for breast feeding and physical activity, avoid alcohol, overweight and weight gain, toxic exposuse (radiations, xeno-oestogens …), long-term explosition to oestrogens treatments, multiple ovarian stimulations. **In conclusion**, nobody can anymore ignore oncogenetics in its medical practice. Henceforth, good medical practices must address patients and their families to highly specialized multidisciplinary oncogenetics clinics when an hereditary predisposition to cancer is suspected. Oncogenetics is a paradigm for personalized predictive medicine through identification of the genetic « soil » of cancer in healthy people. But many people still ignore medical oncogenetics in 2013 in too many countries: there are urgent needs for international network & oncogenetics schools collaborations widely opened.