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Paris, France

Abstracts

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EUROPEAN SOCIETY OF HUMAN GENETICS

European Human Genetics Conference

June 8 - 11, 2013
Palais des Congrès, Paris, France

Abstracts

European Society of Human Genetics

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European Human Genetics Conference 2015
Glasgow, United Kingdom
June 6 – 9, 2015

P15.12

Fast detection of de novo copy number variants from case-parent trio SNP arrays.

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To infer de novo copy number variants from SNP arrays for case-parent trios, we exploit the trio design and define a statistic called „minimum distance" to capture differences in copy numbers between offspring and parents using genome wide SNP array data. We show that this approach reduces technical variation from probe effects and genomic waves, which is the major source of false positive identifications in copy number analyses. Following segmentation of the minimum distance by circular binary segmentation, final inference regarding de novo copy number events is based on a posterior calling step. We apply both the „MinimumDistance" approach and the joint HMM implemented in PennCNV to a study of oral clefts, validate several detected de novo regions by qRT-PCR, and assess the overall concordance of these two algorithms. Our analysis of the oral cleft trios reveals that genomic waves represent a substantial source of false positive identifications in the joint HMM, despite a wave-correction implementation in PennCNV. The minimum distance is an effective statistic for reducing technical variation contributing to such false de novo discoveries. Computationally, MinimumDistance provides a nearly 8-fold increase in speed relative to the joint HMM. Using trios not selected for phenotype as controls, we identify a region on chromosome 7p14.1 with a (genome-wide) significantly higher number of de-novo deletions in the case-parent trios ascertained through an oral cleft.

I. Ruczinski: None.

P15.13

Comparison of copy number variation (CNV) calling performance in large numbers of technical replicate SNP array data using three different, widely-used CNV calling algorithms

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Copy number variants (CNVs) have been shown to explain part of the heritability in various multifactorial diseases. Many of these findings are derived from SNP-array data generated in the course of large genome-wide association studies (GWAS). This is not without challenges, however: SNP-arrays contain an ever increasing density of probes which results in a decreased signal-to-noise ratio. The latter causes problems for automated CNV calling algorithms and is a major cause for the unambiguous calling of smaller (<350kbp) and/or low frequency CNVs. Recent studies have therefore focused on the much more reliable calling of larger CNVs (number of consecutive marker or length) and often considering the easier to detect deletion events only.
This study compares the performance and differences in CNV calling using three widely-used CNV calling algorithms: CNVPartition, QuantiSNP2 (v2.2), and PennCNV. As SNP array data a large number of technical replicates (n>500) all genotyped at the University of Bonn on Illumina's HumanOmni-Express- and HumanOmni1M-arrays were used.
We observed an unexpectedly high fluctuation in the prediction of cnv events throughout the three algorithms. All gave comparable findings for larger findings (> 1Mbp) but suffered to give consensus results for smaller variants. Since the replicates were typed on the same array type, this allowed to evaluate effects of the chemistry or the operator in the lab. Results will be shown and based on that parameters will be presented that allow for a better evaluation of the quality of CNV callings from SNP array data.

S. Herms: None. **A. Hofmann:** None. **L. Priebe:** None. **F.D. Degenhardt:** None. **T.W. Mühleisen:** None. **M.M. Nöthen:** None. **S. Cichon:** None. **P. Hoffmann:** None.

P15.14

CNVs and de novo deletion identified in GSTM1 gene in Rheumatoid Arthritis

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Introduction: At least 12% of the human genome has been identified as copy number variable (CNV). We analysed CNVs of Glutathione S-Transferase Mu 1 class gene (GSTM1), a candidate gene in Rheumatoid Arthritis (RA). Three methods for identifying CNVs were compared in trio families with RA.
Patients and Methods: Nine trio families were genotyped using a multiplex standard PCR (mPCR), which lead to identify three genotypes (-/-, -/+, and +/+) of the gene. Two methods of CNVs quantification were used: a quantitative PCR (qPCR, Life Technologies) for a relative quantification and Droplet Digital PCR (DDPCR, BioRad) for an absolute quantification using up to 20,000 reactions for one sample.
Results: mPCR identified genotypes -/- and -/+. qPCR and DDPCR revealed zero to two copies of the gene. All samples with two copies were heterozygous for GSTM1 deletion (-/++). A de novo deletion of a GSTM1 copy was observed in a RA patient. qPCR analysis using a calibrator with a known CN or the Most Frequent CN (MFC) could have generated heterogeneous results. DDPCR did an absolute quantification of CNVs with a high number of replicates and no calibrator or MFC was needed.
Conclusion: Comparison of technologies identifying CNVs of this candidate gene lead us to conclude that DDPCR is the best method to use in this field of investigation. We highlighted then rare events such as de novo deletion in families of RA patients. Further analyses on larger samples are required to better understand the impact of GSTM1 CNVs in RA.

M.S. Ben Kilani: None. **L. Michou:** None. **T. Bardin:** None. **F. Cornelis:** None. **V. Chaudru:** None. **E. Petit-Teixeira:** None.

P15.16

Variant discovery in BRCA1 and BRCA2: Comparison of the efficiency of NextGENe software and the Torrent Suite Variant Caller plugin

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Second-generation sequencing technologies are now widely used in a research setting to screen large numbers of samples for known variations, or as primary detection for new ones. Computational methods that rate the discovered variations and separate the sequencing errors from the real SNPs and mutations are an inseparable part of every NGS pipeline.
In our study the BRCA1 and BRCA2 genes were sequenced in 16 patients with breast cancer on a single Ion 316 chip, using the Ion Torrent PGM platform. The native Ion Torrent Variant Caller and the NextGENe software by SoftGenetics were used separately to analyze the results. The calls that appeared in both analyses were forwarded for confirmation with Sanger sequencing.
NextGENe reported a smaller total number of variations over all 16 patients (224) in the sequenced regions than the Variant Caller (312), which was due to the strict settings of the mutation filter. 106 of those variants were common for the two groups. The filtering was tuned down in two steps, yielding two more NextGENe result sets with 325 (123 common) and 1951 (201 common) variants, respectively. The 201 common variants had a higher frequency than the calls made by Variant Caller alone, and higher scores than those made by NextGENe only. This shows that the combined use of the two filters can increase the quality of the final results, even when they are set to register almost all variations in the data, as in the case of de novo variant discovery.

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P15.17

Evaluation of DNA methylation in breast cancer patients

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Epigenetic alterations, in addition to the highly characterized genetic changes, are key contributors to breast carcinogenesis. Altered DNA methylation is commonly observed in the tumorigenesis. The aberrant promoter hypermethylation of cancer-related genes in tumour-derived DNA from plasma samples was presented as a useful tool for detecting and monitoring cancer.
The purpose of our study is to investigate relationship between DNA methylation and breast cancer progression. Quantitative DNA methylation analysis of 11 genes (APC, ADAM23, CXCL12, ESR1, PGR B, CDH1, RASSF1A, SYK, TIMP3, BRMS1 and SOCS1) was performed by pyrosequencing. For determining of methylation levels we analysed DNA isolated from paraffin-

embedded tumour tissues, plasma and blood cells from 37 sporadic breast cancer patients and plasma and blood samples of 50 healthy controls.
We have observed DNA methylation in nearly half of investigated patients in RASSF1A, APC, ADAM23, CXCL12 and SYK genes in tumour samples. Cumulative methylation results showed different methylation levels in tumour and plasma samples where notable higher portion of methylation was found in tumour. Differences between DNA methylation in plasma samples of patients and healthy controls were not significant. This finding cast some doubts on the utility of DNA extracted from plasma samples to identify hypermethylation of specific gene promoters for diagnosis and prognosis of cancer. Preliminary results suggest that DNA methylation in the several studied genes in tumour tissues could be useful biomarkers for the identification of breast cancer with metastasizing potential.
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P15.18
The Association Between TWIST, RARβ2, ESR1 Gene Promotor Hypermethylation and the Histopathologic Type of Breast Cancer in Turkish Population

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Backround: Breast cancer is the most common cancer in women. Histopathology plays an important part in determining the treatment strategy for women with breast cancer.
TWIST expression in breast tumors correlate with increased disease recurrence, metastasis and poor disease-free survival. Steroid receptor gene family members such as RARβ2 and ESR1 genes are methylated and silenced in a fraction of breast cancer.
Method: In this study the promoter methylation levels of TWIST, RARβ2 and ESR1 gene which are associated with breast cancer were investigated by Quantitative Methylation Sensitive High Resolution Melting Analysis (QMS-HRM).We analysed primary tumor core biopsies from 80 high-risk primary breast cancer patients (tumors ≥2 cm and/or lenfatic metastase and/or distant metastases and/or under 40 years) and their histopathologic types were associated with the methylation levels.
Results: In our study the promoter hypermethylation status were observed at different rates; TWIST, RARβ2 and ESR1 methylation frequencies were 25%, 88.75 and 72.5%, respectively. The promoter hypermethylation levels of the genes found to be significant with lymph node positivity, ER positivity and HER2/neu negativity.
Conclusions: Our study is important as being the first study that analyzes the association between histopathologic type of breast cancer and TWIST, RARβ2, ESR1 gene promotor methylation status in Turkish population.
Key words: Breast cancer, Histopathologic Type, TWIST gene, RARβ2 gene, ESR1 gene, Methylation, MS-HRM Analysis

O. Eroglu: None. **B. Durak aras:** None. **S. Artan:** None. **M. Ozdemir:** None. **O. Cilingir:** None.

P15.19
Analysis of Apoptosis regulatory pathway in sporadic breast cancer through Gene Regulatory Network

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In multicellular organisms, gene regulatory networks along with transcriptional factors (TF) control the global gene expression and the dynamics of protein output in living cells. An extensive analysis of networks facilitates to analyze coordinated gene expression changes to find altered molecular pathways and genes in cancer development. The aim of the study was to predict the innovative biological networks that describe transcriptional alteration (up or down regulation) in genes/pathways which could contribute to the pathogenesis of cancer and other associated disease. The expression of several candidate genes (MDR1, ATM, BCL2, CASP3, CASP8, CHK2, TR10C, TR10D, TR10B, CFLAR, H2AX, IFNG, IL10, IL4, IL6, P53, MDM2, TGFB1, TNFA, TNF10, BRCA1 and BRCA2), as a part of another study in sporadic breast cancer was used for constructing the biological network which comprised of both transcriptional regulatory relationships and integrated the protein interactome. We also developed a set of TF-gene regulatory relationships, using UCSC genome browser. The human cancer combinatory gene regulatory network was found to be a hierarchical scale-free network with PAX4-transcription factor as the most important regulators. It is known that PAX4 functions as a potent tumor suppressor and plays a critical role in Cancer growth as well as in the functional morbidity causes Diabetes, which also provides a logical link between cancer and diabetes. We believe our work provides a scaffold combinatorial gene regulatory network allowing systematic study of apoptotic gene regulation, and provides a pipeline which could be extended to reveal conditional combinatorial regulatory landscapes correlating with specific cellular contexts.

S. Haider: None. **K. Ponnusamy:** None. **R.N.K. Bamezai:** None.

P15.20
Methylation analysis of NFkB-related genes in celiac gut mucosa

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Celiac disease (CD) is a complex, immune-mediated enteropathy caused by ingested gluten with strong genetic and environmental risk determinants, and is an excellent target for the study of gene methylation. IL6 has been shown to induce nuclear translocation of DMT1 through NFkB and JAK/STAT pathways and to alter CpG island methylation. In this work, we studied the methylation pattern of the promoter regions of several NFkB-related genes.
Using pyrosequencing of bisulfite treated DNA, we analyzed 4-6 CpG islands in 8 NFkB-related gene promoters in 17 celiac biopsy pairs (at diagnosis and after >2 years on gluten free diet) and 13 non-celiac biopsies. We also studied IL6 expression. Additionally, samples from 8 patients at diagnosis and 8 treated patients were incubated for 12 hours with gliadin, with a modulator of NFkB pathway, with both compounds or without any of them.
Compared to controls, RELA presented lower methylation levels at diagnosis while MAP3K7 and TRADD showed the opposite pattern. Treated patients presented intermediate methylation percentages, suggesting a partial reversion of the aberrant methylation induced by active disease. Methylation levels of different genes showed significant linear correlation in patients but not in controls. Methylation percentages did not correlate with IL6 RNA levels, although IL6 was overexpressed in active CD. Preliminary results suggest that incubation of biopsies from treated patients alters methylation in several genes, and NFkB modulation seems to partially reverse gliadin effects. Correlation between different genes was not observed in these samples, pointing to cell subtype selection underlying the observed events.

N. Fernandez-Jimenez: None. **X. Elcoroaristizabal:** None. **L. Plaza-Izurieta:** None. **A. Jauregi-Miguel:** None. **T. Lopez-Euba:** None. **I. Irastorza:** None. **M. M. de Pancorbo:** None. **J. Bilbao:** None.

P15.21
Genome-scale DNA Methylation Analysis of Sporadic Colorectal Cancer by Infinium HumanMethylation 450 BeadChips.

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Research Institute of Physico-Chemical Medicine, Moscow, Russian Federation.

Infinium HumanMethylation 450K BeadChip arrays were used to examine genome-wide DNA methylation profiles in 22 sample pairs of colon cancer (CRC), adjacent tissues and 19 colon tissues samples from cancer-free donors. We show that tumor and both normal methylation profiles can be clearly distinguished from one another and that the main source of methylation variability is associated with disease status. Although we have identified a number of genes potentially involved in the field cancerization their total impact on methylation in normal tissue is rather low.
At CpG sites level we showed that common CRC-specific methylation patterns consist of at least 15,667 CpG sites significantly different from both versions of the norms. 10,342 of them were hypermethylated and 5,325 hypomethylated. Hypermethylated sites were common in maximum number of sample pairs, located mostly in CpG islands and significantly enriched for known cancer-specific differentially methylated regions, while hypomethylated were mostly located in CpG Shores and were generally sample-specific.
Despite of considerable variability in methylation data we selected a panel of 14 highly robust candidate methylation markers located in genes SND1, ADHFE1, OPLAH, TLX2, C1orf70, ZFP64, NR5A2 and COL4A. This set was