European Journal of Human Genetics

Volume 23 Supplement 1 June 2015 www.nature.com/ejhg

European Human Genetics Conference 2015

June 6 - 9, 2015
Glasgow, Scotland, United Kingdom

Abstracts

nature publishing group npg

EUROPEAN SOCIETY OF HUMAN GENETICS

European Human Genetics Conference

joint with the

British Society of Genetics Medicine

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Considering these observations, we investigated a putative role of EDN3 on ENCC adhesion properties and its functional interaction with β1-integrins during ENS development. We discovered that EDN3 promotes ENCC adhesion in vitro. It stimulates β1-integrin activation and increases the number of ENCCs focal adhesions. Upon EDN3 treatment, ENCCs rapidly exhibited changes in cell shape and membrane dynamics displaying a sustained growth and persistence of lamellipodia. Morever, in vivo double-mutant studies showed that itgb1-/-; Edn3ls/ls mutants displayed an aggravated enteric phenotype and an altered ENS network organization. Ex-vivo live imaging of embryonic guts allowed us to evidence severe migratory defects of double mutant ENCCs that contribute to the enteric defects observed.
Altogether our results reveal that interplays between EDN3 and β1-integrins are crucial for proper ENS ontogenesis.

PS03.21
The analysis of APOB-100, LRPAP1, ABCG5 and ABCG8 genes polymorphisms in gallstone disease patients and healthy donors from Volga-Ural region of Russia

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Gallstone disease (GSD) is a metabolic diseases of the hepatobiliary system, characterized by the formation of gallstones in the gallbladder, common bile duct stones in the liver bile ducts. 10% of the population suffers from gall stones, and the number of patients in the world with each passing decade becomes larger. The aim of this study was to exam the association of polymorphisms of APOB-100 (rs693), LRPAP1 (rs11267919), ABCG5 (rs4131229) and ABCG8 (rs11887534) genes with the risk of gallstone disease. The patient group consisted of 205 patients with with cholelithiasis, the control group included 190 unrelated healthy individuals. Genomic DNA was extracted from peripheral blood leucocytes by standard phenol/chloroform method. Genotyping was performed by PCR followed by restriction digestion. The analysis has revealed that heterozygous genotype X+X- of rs693 of APOB-100 gene is a marker of increased risk of gallstone disease in Russian (p = 0.03; OR = 2,1). For those of Tatar ethnicity shows that rs693*X-allele and C allele of rs4131229 of ABCG5 gene are markers of increased risk of developing the disease (p = 0,002; OR = 2,0 and p = 0,02; OR = 1,7, respectively), while rs693*X +, rs4131229*T alleles and of rs4131229*T/T genotype are a markers of reduced risk of gallstone disease (p = 0,002; OR = 0,5; p = 0,02; OR = 0.6 and p = 0,03; OR = 0,5, respectively). Results of the study shows that the polymorphisms of the APOB-100 and ABCG5 genes are associated with the risk of gallstone disease.

PM03.22
The mutational analysis of the INF2 gene in Czech patients with FSGS and MCD

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We started to screen for mutations in the INF2 gene in 136 Czech patients (49.82 ± 17.91) with FSGS/MCD. The INF2 protein plays a key role in the function of the slit diaphragm in podocytes.
We have so far identified one already known missense mutation with the proven damaging effect on the function of podocytes. The substitution Arg218Gln, which was in the heterozygous state, was found in two brothers (thirty-two and thirty-one years old) with a positive family history who suffered from FSGS. The heterozygous state did not correlate with the rapid progression and early development of the ESRD (twenty-seven and thirty-one years, respectively), while their father developed ESRD at fifty-seven years. For that reason, we suppose the collaboration of more factors, such as other substitutions in the INF2 gene or in other genes connected with FSGS or the influence of environment, in the case of these two brothers.
The other interesting findings were the undescribed heterozygous changes p.Pro208Ser (c.622 C>T) in the anonymous patient and p.Pro1057Leu (c.2640 C>T) in forty-three years old man (FSGS, negative family history). However, according to predictive programs (PolyPhen-2 and PON-P2), this substitution is not probably causal. It was also found the already known substitution p.Arg214His (c.641 G>A) with proven damaging effect in fifty-one years old woman (FSGS, positive family history).
The previous mentioned results are first data from the mutational analysis of the INF2 gene in Czech patients with FSGS/MCD. The similar study focused on the INF2 gene has never been performed in the Czech Republic.
Supported by the grant project PRVOUK- P25/LF1/2.

PS03.23
Abernathy malformation: a rare association with Goldenhar syndrome

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Abernathy malformation is a rare congenital malformation characterised by absence or hypoplasia of the hepatic portal vein, resulting in a congenital portosystemic shunt. Consequences of the malformation include focal nodular hyperplasia of the liver, hepatoblastoma and hepatic encephalopathy.
Our patient is a 27 year old woman who was referred to the genetics clinic for preconception counselling due to a diagnosis of Goldenhar syndrome. She was diagnosed with focal nodular hyperplasia of the liver aged 15. Review of historic imaging and magnetic resonance cholangiopancreatogram revealed a type 1a Abernathy malformation, likely to be the cause of the focal nodular hyperplasia.
Congenital absence of the portal vein has been previously described in one individual with Goldenhar syndrome. In this case focal nodular hyperplasia developed into hepatoblastoma requiring liver transplantation. Abernathy malformation is a rarely reported association of Goldenhar syndrome that can cause serious hepatic complications.

PM03.24
Metallothioneins are downregulated in ileal mucosa of Familial GUCY2C diarrhea syndrome patients susceptible to Crohn´s disease

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Background: Familial *GUCY2C* diarrhea syndrome (FGDS), first described in a Norwegian family (n=38), is caused by an activating mutation in the gene encoding guanylate cyclase C. Patients with FGDS have early onset mild diarrhea but are also susceptible to ileal Crohn's disease (CD) (7 patients). The aim of the present study was to compare global gene expression in ileal biopsies (non-inflamed mucosa) from FGDS patients (n=11), unrelated CD patients (n=6) and healthy controls (n=16). We also assessed whether CD genetic risk variants segregate with CD in the FGDS patients.
Methods: Global gene expression was examined using Illumina Human HT-12 v4 BeadChip. 140 CD risk variants were genotyped (Immunochip array) and the *NOD2* gene was sequenced in 23 adult FGDS-patients (7 with CD).
Results: Nine metallothioneins were significantly downregulated (1.5-3 fold) in FGDS patients, but not in unrelated CD patients, compared to controls. The polygenic risk score did not differ significantly between FGDS patients with and without CD. However 6 of the 7 FGDS patients with CD carried *NOD2* risk variants, and the two most severely affected patients were homozygous for the rs5743289 risk allele . Three of 16 FGDS patients without CD were heterozygous for *NOD2* risk variants
Conclusion: Metallothioneins were significantly downregulated in non-inflamed terminal ileum of FGDS patients, but not in unrelated CD patients compared to controls. Lower levels of these zinc-binding proteins may cause inflammation due to interference with NOD2-stimulated bacterial clearance and autophagy. Further studies are warranted to investigate guanylate cyclase C-related susceptibility to Crohn´s disease.

PS03.25
Molecular analysis of KAL-1 and GnRHR genes in patients with idiopathic hypogonadotropic hypogonadism

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Introduction: Idiopathic hypogonadotropic hypogonadism (IHH) comprises delayed/absent puberty, infertility and low serum gonadotropins in the context of normal anterior pituitary anatomy and function. Is due to partial/complete absence of gonadotropin-releasing hormone release/action or gonadotropin secretion and its incidence is low (1/10.000-1/86.000) with a nearly 4:1 male-to-female ratio. Approximately two-thirds of individuals with IHH have anosmia or hyposmia (Kallmann syndrome-KS) and one-third

have normosmic IHH (nIHH). Mutations in *KAL1* gene cause X-linked KS, and in *GnRHR* gene autosomal recessive IHH (almost 2% of nIHH patients).
Material and Methods: 45 patients with IHH (42 males, 3 females), with and without hyposmia/anosmia were studied. Mutation analysis of *KAL1* and/or *GnRHR* was performed by SSCP/DHPLC-PCR or by PCR-direct DNA sequencing.
Results: We found two *KAL1* mutations: c.769C>T (p.Arg257*) in a 15-years-old anosmic male; and a novel one, an extensive deletion encompassing exons 4 to 14 confirmed by MLPA, in a 3-years-old boy (detected also in his mother) with micropenis and maternal family history of HH. Two nIHH male patients were compound heterozygous for *GnRHR*: c.[2T>C];[785G>A], p.[(M1T)];[(R262Q)] (39-years-old, prepubertal testicules, gynecomastia, P1-A0 pilosity) and c.[317A>G];[416G>A], p.[(Q106R);(R139H)] (35-years-old with a brother non-tested with similar phenotype). All four mutations are known to be disease-causative.
Discussion: We were able to find the genetic defects in 4 patients. The low detection rate of mutations (8.8%) is related with the existence of several genes implicated in the IHH' pathogenesis. The NGS analysis in patients with IHH may improve the molecular diagnosis as it allows the screening of different genes simultaneously.

PM03.26

Systematic analysis of chromatin interactions at disease associated loci links novel candidate genes to Inflammatory Bowel Disease

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Introduction: Genome wide association studies (GWASs) have revealed numerous genomic loci that are associated with complex genetic diseases. Subsequently, many candidate genes have been defined, mainly based on the functional relationships between genes found in the vicinity of the identified loci. However, many of these loci can be linked to regulatory DNA sequences and it is now widely appreciated that part of the GWAS associations is due to sequence variation in regulatory elements. Therefore, the genes controlled by these regulatory elements should be considered as possible candidate genes. Since regulatory elements can regulate genes via chromatin-chromatin interactions that comprise up to 1 Mb, these genes cannot be identified based on base-pair distance from the regulatory regions. To address this, we used chromatin conformation capture-sequencing (4C-seq) to systematically determine the genes that are physically interacting with regulatory units that overlap the disease associated SNPs in Inflammatory Bowel Disease (IBD).
Results: We assayed chromatin interactions in monocytes, lymphocytes and in DLD-1 cells – major cell types implicated in IBD pathogenesis. We performed 4C-seq for 92 IBD-associated loci that localize to regulatory elements in all three cell types. Our approach links 815 novel genes, including *IL10RA, SMAD5, SMAD6* and *PIAS1*, to IBD.
Conclusion: We have performed a novel candidate gene approach in which chromatin interaction data on GWAS-susceptibility loci are intersected with the information about DNA regulatory elements and gene expression in relevant cell types. This revealed 815 novel candidate genes, consisting of multiple notable genes like *SMAD6, IL10RA, PIAS1* and *SMAD5,* thereby complementing previously reported candidate gene approaches.

PS03.27

Johanson-Blizzard syndrome in an Omani infant with neural tube defect: a coincidental findings or a consequence of UBR1 mutation ?

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Johanson-Blizzard syndrome (JBS), #243800 is a rare, autosomal recessive multisystem disorder characterized by exocrine pancreatic insufficiency and aplasia/hypoplasia of alae nasi. Additional common features include ectodermal dysplasia, hypothyroidism, growth hormone deficiency, sensorineural hearing loss, urogenital and anorectal anomalies and cognitive dysfunction of variable degrees. Mutations of *UBR1* (MIM #605981) are known to cause JBS. The UBR1 represents one of at least four E3 ubiquitin ligases of the N-end rule pathway, an evolutionary conserved and ubiquitously expressed intracellular proteolytic pathway involved in ubiquitin-mediated degradation of many proteins. JBS has wide and highly variable clinical manifestation with rare malformations observed in some patients with molecularly confirmed JBS. We report a newborn Omani with JBS and a novel truncating mutation in *UBR1*. The clinical features include a beaked nose, hypoplasia of nasal wings, exocrine pancreatic insufficiency presenting with severe failure to thrive and septicemic shock, severe anemia requiring frequent blood transfusion, anal atresia, sparse hair, scalp defect, lumbosacral meningo-myelocele and hydrocephalus.This is a second report of neural tube defect in association with JBS implying that this association is as a result of *UBRI* mutation rather than coincidental. The phenotypic defects in JBS involve several organ systems in addition to pancreas suggesting that UBR1-mediated protein degradation plays a critical role at certain stages of human development, and in specific cell types.

PM03.28

Targeted panel sequencing of 399 renal genes reclassifies primary disease diagnoses in young end stage renal disease patients

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Background: About a quarter of patients with end stage renal disease (ESRD) before age 30 do not have a primary renal disease diagnosis. Previous genetic studies have focused on specific clinical diagnoses. We took an innovative approach by sequencing a panel of 399 renal disease genes in 200 cases with ESRD onset before age 30, regardless of their clinical diagnosis. Data for the first 132 cases are presented.
Methods: We designed the "RENome" using SureSelect/Agilent with 399 genes involved in hereditary renal disease. We used SOLiD™ 5500XL for sequencing and an in-house developed bioinformatics pipeline for mapping, variant calling and QC. Variants were annotated using CARTAGENIA software.
Results: On average >95% of in target bases were genotyped, with >99% sensitivity and specificity. Stringent filtering criteria allowed only for coding variants with percentage variant reads of >15%, novel or with allele frequency of <0.005, that were listed as disease-causing in HGMD Pro, had a SIFT score <0.05 and were not predicted to be benign in PolyPhen2. We also selected samples with likely CNVs. Extended analyses, with less stringent filtering criteria and in depth copy number analyses, are presented at the meeting.
Conclusion: This filtering strategy yielded a molecular diagnosis in 15 patients (11.4%), confirming the registered primary disease in 6, and unexpectedly reclassifying it in 9. Considering the stringency of filtering, these numbers underestimate the diagnostic potential of our innovative approach. Adding early RENome sequencing to the diagnostic work-up in all young ESRD patients, improves etiologic classification and genetic counseling.
AMvE is supported by the Dutch Kidney Foundation and Fonds NutsOhra

PS03.29

Significant association of KIR2DL3/HLA-C1 combination with susceptibility to Crohn´s disease

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Introduction: The killer cell immunoglobulin-like receptors (KIRs) form a group of regulatory molecules that specifically recognize HLA class I molecules. The aim of this study was to analyze the possible association of specific KIR genes and KIR/HLA-C genotypes with the susceptibility to Crohn's disease (CD) in a Spanish population.
Materials and Methods: A total of 125 patients with RA and 339 healthy control subjects were selected for this study based on clinical criteria. The commercial KIR-SSO typing kit from Luminex (Tepnel Lifecodes) was used to investigate KIR and HLA-C typing.
Results: The centromeric A/A genotype was more frequent in CD patients ($P<10^{-3}$). When we included HLA-C analysis, we found that the centromeric A/A genotype and HLA-C1 combination was significantly increased in CD patients ($P<10^{-3}$). Moreover, KIR2DL2/2DL3 genotype demonstrated a decreased frequency in CD patients (P<0.0005), whereas the KIR2DL3/2DL3 genotype was significantly increased in CD patients (P<0.0005). Remarkably, we also observed a highly significant increase of the KIR2DL3/KIR2DL3 HLAC1/HLAC1 homozygosis in CD patients (P<0.0005).
Conclusion: Our results confirm the relevance of the KIR2DL2/KIR2DL3 genes and their interaction with HLA-C in CD. The presence of a particular KIR-HLA pair may confer functional competence on NK cells and influences differences in NK cell functional responses among individuals. We show that the contribution of the KIR genes to CD susceptibility extends beyond the association with individual KIRs, with an imbalance between activating and inhibitory KIR genes seeming to influence the susceptibility to CD.