MOLECULAR DIAGNOSTICS IN VIROLOGY

To boldly go where no assay has gone before...
“It’s tough to make predictions, especially about the future！”

Yogi Berra, catcher New York Yankees, 1946
1. Growing importance of MDx in the clinical virology laboratory
Molecular Diagnostics analyses
University Hospitals Leuven 1999-2011

Beuselinck K., Lagrou K., Van Eldere J. 2011
Viral culture analyses
University Hospitals Leuven 2004-2011

Beuselinck K., Lagrou K., Van Eldere J. 2011
Molecular Diagnostics & Viral culture analyses
University Hospitals Leuven 2004-2011

Beuselinck K., Lagrou K., Van Eldere J. 2011
Viral culture analyses
University Hospitals Leuven 2001-2011

205,464 analyses

Beuseinck K., Lagrou K., Van Eldere J. 2011
Viral culture analyses
University Hospitals Leuven 2001-2011

205,464 analyses

Beuseinck K., Lagrou K., Van Eldere J. 2011
Molecular Diagnostics analyses
University Hospitals Leuven 2001-2011

280.447 analyses

Van Eldere J. 2011
2. Growing importance of MDx in the *in vitro* diagnostics market
Worldwide IVD market

- Clinical chemistry
- Microbiology
- Hematology
- POC testing
- Immunoassays
- Anatomic pathology
- Molecular Dx
- Other (coagulation etc.)

2007: $37 B
2012: $57 B

9% of growth

Scientia Advisors, 2011
**Worldwide IVD market (2009)**

<table>
<thead>
<tr>
<th>Rank</th>
<th>Company</th>
<th>Revenue (USD)</th>
<th>Growth (%)</th>
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<tr>
<td>1</td>
<td>Roche</td>
<td>9,492,000,000</td>
<td>+ 4.1%</td>
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<tr>
<td>2</td>
<td>Siemens</td>
<td>4,712,000,000</td>
<td>+ 9.5%</td>
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<td>6</td>
<td>BioMerieux</td>
<td>1,651,000,000</td>
<td>+ 10.0%</td>
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</table>

Xavier Nogue, 2011
Global molecular diagnostics market: 4.800.000.000 USD in 2010
Compounded annual growth rate (CAGR): 10%

*MarketResearch.com, 2011*

Global molecular diagnostics market: 15.000.000.000 USD by 2014
Compounded annual growth rate (CAGR): 19%
Dominant and fastest growing segment: infectious diseases

*Research and Markets, 2010*
Worldwide MDx market

Scientia Advisors, 2011
Fastest growing segment:

MDx of infectious diseases in the critical care setting

- MRSA
- Clostridium difficile
- Acute respiratory infections
- Meningitis/encephalitis
Gross margins of MDx players

- **Myriad Genetics**: 87%
- **Ipsogen**: 77%
- **Genomic Health**: 75%
- **Gen-Probe**: 70%
- **Qiagen**: 67%
- **Cepheid**: 44% (2012 Estimate, 60%)

*Scientia Advisors, 2011*
3. Citius, Altius, Fortius
“Citius, Altius, Fortius”

Pierre de Coubertin, 1894
“Everything that can be invented has been invented”

Charles H. Duell, Commission US Office of Patents, 1899
Smaller, faster, easier, cheaper, ubiquitous...

- Short-term trend: MDx will expand from core laboratories to (all) small and mid-sized (hospital) laboratories
**Short-term trend:**
Migration of MDx assays from core laboratories to mid-sized and small hospitals

<table>
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<tr>
<th>Requirements</th>
<th>Traditional MDx systems</th>
<th>New random access, fast TaT MDx systems</th>
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<tr>
<td>Usability w/o training</td>
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<td>✓</td>
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<tr>
<td>Accessibility to small and mid-size hospitals</td>
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<tr>
<td>Random access*</td>
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<td>Bench top (small footprint)</td>
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<tr>
<td>Fast turnaround time (TaT)**</td>
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<tr>
<td>Ease of use</td>
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<tr>
<td>Ability to batch/high throughput</td>
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</tr>
<tr>
<td>Accuracy (sensitivity &amp; specificity)</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
The Verigene® System

Discrete results for 7 respiratory viruses and subtypes in a single test cartridge:

- Influenza A
- Influenza B
- RSV A
- RSV B
- Influenza A Subtypes:
  - Influenza A - 2009 H1N1
  - Influenza A - H1
  - Influenza A - H3
Smaller, faster, easier, cheaper, ubiquitous...

- Short-term trend: MDx will expand from core laboratories to (all) small and mid-sized (hospital) laboratories

- Mid/long-term trend: Migration of MDx assays to handheld POC devices
Assays available:
- Anthrax pX01 and Anthrax pX02
- Tularemia
- Plague
- Orthopox
Palm PCR is powered by a Li+ polymer battery that enables more than 4 hours of continuous operation on a single charge. It can be also operated with AC power using an AC/DC adapter. It is designed to conform to the standard 9 mm-spacing well format to use with a disposable plastic sample tube, specially developed for the Palm PCR system. Nearly all kinds of DNA samples, including human genomic DNA of ≤ 0.1 ng, can be amplified within 25 or 30 minutes for up to 1 kbp size. The dynamic range can be extended up to 2 kbp using flow protocols.
Palm PCR is powered by a Li+ polymer battery that enables more than 4 hours of continuous operation on a single charge. It can be also operated with AC power using an AC/DC adapter. It is designed to conform to the standard 9 mm-spacing well format to use with a disposable plastic sample tube, specially developed for the Palm PCR system. Nearly all kinds of DNA samples, including human genomic DNA of ≤ 0.1 ng, can be amplified within 25 or 30 minutes for up to 1 kbp size. The dynamic range can be extended up to 2 kbp using slow protocols.
4. Theranostics: more than just a fashion
PharmaNetics president and CEO John Funkhouser: A business model in developing diagnostic tests directly linked to the application of a specific therapy (new generation POC coagulation tests supporting coagulation therapies).

1998: FDA approves Genentech’s Herceptin (MoAb against Her2) for treatment of stage IV breast cancer and Dako’s HercepTest for diagnosis of Her2 overexpression.

“Theranostics”
John Funkhouser, CEO PharmaNetics
ENOX test to measure the effect of enoxaparin (Aventis) clotting time.
Therapy + Diagnostics = Theranostics

- Pharmacodiagnostic diagnostics
- Companion diagnostics
- Dx/Rx partnering
- Personalized medicine
Therapy + Diagnostics = Theranostics
Mixed Population of Responders and Non-Responders

Theranostics

Non-Responders

Responders
HercepTest negative: no Her2 receptor overexpression

HercepTest positive: 3+ Her2 receptor overexpression

Both Herceptin and HercepTest were FDA approved in 1998
Molecular theranostics in virology

HIV CCR5 tropism and maraviroc
A CCR5 antagonist, like maraviroc, can help block R5 virus.
Viral Entry

- CCR5 cell receptor
- CXCR4 cell receptor

CD4+ target cells

CCR5 cell line

CXCR4 cell line

Cell line expression
R5 HIV
Uses the CCR5 co-receptor only

X4 HIV
Uses the CXCR4 co-receptor only

Dual HIV
Can use either co-receptor

Mixed HIV
Can use either co-receptor

Maraviroc was FDA approved in 2007
Molecular theranostics in virology

HIV CCR5 tropism and maraviroc

ILI: Influenza and neuraminidase inhibitors
Many new viruses begin to form at the cell membrane. Here only one is shown for clarity.
In vitro activity of Neuraminidase inhibitors

MDCK cells + influenza A
In vitro activity of Neuraminidase inhibitors

MDCK cells + influenza A
In vitro activity of Neuraminidase inhibitors

MDCK cells + influenza A

MDCK cells + influenza A + ZANAMIVIR
In vitro activity of Neuraminidase inhibitors

MDCK cells + influenza A

MDCK cells + influenza A + ZANAMIVIR
Molecular theranostics in virology

HIV CCR5 tropism and maraviroc

ILI: Influenza and neuraminidase inhibitors

HCV genotype and antiviral treatment
Response to 48 Weeks of Therapy

RAPID AND SUSTAINED ACHIEVEMENT OF UNDETECTABLE HCV RNA DURING TREATMENT WITH RITONAVIR-BOOSTED DANOPREVIR/PEG-IFNα-2A/RBV IN HCV GENOTYPE 1 OR 4

Danoprevir: protease inhibitor Roche; Reported by Jules Levin
EASL 2012 Apr 18-22 Barcelona Spain
5. Closing the diagnostic gap
xTAG Respiratory Viral Panel
# xTAG Respiratory Viral Panel

<table>
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<tr>
<th>Viral Family and Subtype</th>
<th>RVP</th>
<th>RVP-FAST</th>
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<tbody>
<tr>
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<td>US-JVD</td>
<td>Health Canada IVD</td>
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<tr>
<td><strong>Respiratory Syncytial Virus (RSV)</strong></td>
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<tr>
<td>RSV A</td>
<td>•</td>
<td>•</td>
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<tr>
<td>RSV B</td>
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<td>•</td>
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<tr>
<td><strong>Influenza A</strong></td>
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<td></td>
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<tr>
<td>Non-specific influenza A</td>
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<tr>
<td>H1 subtype</td>
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<td>•</td>
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<tr>
<td>H3 subtype</td>
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<td>•</td>
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<tr>
<td>H5 subtype</td>
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<td><strong>Influenza B</strong></td>
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<td>Parainfluenza 1</td>
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<tr>
<td>Parainfluenza 3</td>
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<td>•</td>
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<tr>
<td>Parainfluenza 4</td>
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<td>•</td>
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<tr>
<td>Metapneumovirus (hMPV)</td>
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<td>Adenovirus</td>
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<td>Entero-Rhino virus</td>
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<td>Corona HKU1</td>
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<td>Corona 229E</td>
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<td>Corona OC43</td>
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<tr>
<td>Corona SARS</td>
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<tr>
<td>Bocavirus</td>
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</table>
Molecular DNA-based diagnostics are increasingly being used for diagnosis of viral infections. For enteric viruses, PCR assays have also been developed. The aims of this study were to compile and evaluate a comprehensive panel of PCR assays for diagnosis of viruses causing diarrheal disease and to evaluate its use in a largely pediatric population in a 750-bed university medical center. The PCR panel was designed to include assays for detection of adenovirus, astrovirus, enterovirus, norovirus, parechovirus, rotavirus, and sapovirus. The results of the PCR panel were evaluated in relation to conventional viral diagnostics consisting of viral culture and/or rotavirus and adenovirus rapid antigen tests on samples that were taken for routine diagnostics. Comparing conventional with PCR-based testing, the number of viruses detected increased dramatically from 25 to 106 when PCR assays were used. This increase was due mainly to detection of previously undetected viruses, i.e., astrovirus, norovirus, and sapovirus. In 24% of the samples, norovirus was detected. Also, the lower detection limit of PCR-based adenovirus, enterovirus, parechovirus, and rotavirus diagnostics further increased the detection rate. By focusing on samples from patients with complaints of gastroenteritis, detection of a causative agent was increased from 49% by conventional tests to 97% by molecular diagnostics. However, many samples containing low viral loads were found in patients with complaints other than intestinal complaints. In conclusion, the proposed comprehensive PCR panel with appropriate cutoff values can be used for sensitive, rapid, and clinically relevant diagnosis of gastrointestinal viruses.
6. Genomics and next-generation sequencing: a revolution in virology
Next-generation sequencing

Roche: 454

GenomeAnalyzer: Illumina

Life Technologies: SOLiD

Helicos Biosciences: Heliscope
Distribution of next-generation sequencing instruments

March 2010
Next-generation sequencing

- Cost-effective sequencing of PCR products
Human papillomavirus genotyping by 454 next generation sequencing technology

Luisa Barzon, Valentina Militello, Enrico Lavezzo, Elisa Franchin, Elektra Peta, Laura Squarzon, Marta Trevisan, Silvana Pagni, Federico Dal Bello, Stefano Toppo, Giorgio Palù

Study design: Development of an HPV typing method based on 454 next generation sequencing of HPV L1 amplicons generated with MY09/11-based primers. Evaluation of the NGS method in control samples and in a panel of cervical cytological samples. Comparison of the NGS typing method with cycle sequencing and with the reverse hybridization-based INNO-LiPA HPV Genotyping Extra assay (LiPA).

Results: In control samples carrying mixtures of HPV16 and HPV18 DNA, the NGS method could reliably detect genotype sequences occurring at a frequency of 1% in multiple infections with a sensitivity of 100 genome equivalents/μL. In cervical cytology samples, comparison with cycle sequencing demonstrated accuracy of HPV typing by NGS. The NGS method had however lower sensitivity for some HPV types than LiPA, conceivably due to the poor sensitivity of the MY09/11-based primers. At variance, LiPA could not detect HPV types which were present in low proportion in multiple infections (<10% of HPV reads obtained by NGS). In addition, NGS allowed identifying the presence of different variants of the same HPV type in a specimen.

Conclusions: NGS is a promising method for HPV typing because of its high sensitivity in multiple infection and its potential ability to detect a broad spectrum of HPV types, subtypes, and variants.
Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments

Jelle Matthijnssens¹, Max Ciarlet², Mustafizur Rahman¹,³, Houssam Attoui⁴, Krisztián Bányaí⁵, Mary K. Estes⁶, Jon R. Gentsch⁷, Miren Iturriza-Gómará⁸, Carl Kirkwood⁹, Vito Martella¹⁰, Peter P.C. Mertens⁴, Osamu Nakagomi¹¹, John T. Patton¹², Franco M. Ruggeri¹³, Linda J. Saif¹⁴, Norma Santos¹⁵, Andrej Steyer¹⁶, Koki Taniguchi¹⁷, Ulrich Desselberger¹⁸, and Marc Van Ranst¹

Novel complete genome nomenclature

G1 – P8 – I1 – R1 – C1 – M1 – A1 – N1 – T1 – E1 – H1
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Next-generation sequencing

- Cost-effective sequencing of PCR products
- Unbiased parallel detection of viruses in clinical samples: closing the diagnostic gap
Unbiased parallel detection of viral pathogens in clinical samples by use of a metagenomic approach.


Viral infectious diseases represent a major threat to public health and are among the greatest disease burdens worldwide. Rapid and accurate identification of viral agents is crucial for both outbreak control and estimating regional disease burdens. Recently developed metagenomic methods have proven to be powerful tools for simultaneous pathogen detection. Here, we performed a systematic study of the capability of the short-read-based metagenomic approach in the molecular detection of viral pathogens in nasopharyngeal aspirate samples from patients with acute lower respiratory tract infections (n = 16). Using the high-throughput capacity of ultradeep sequencing and a dedicated data interpretation method, we successfully identified seven species of known respiratory viral agents from 15 samples, a result that was consistent with results of conventional PCR assays. We also detected a coinfected case that was missed by regular PCR testing. Using the metagenomic data, 11 draft genomes of the abundantly detected viruses in the samples were reconstructed with 21.84% to 98.53% coverage. Our results show the power of the short-read-based metagenomic approach for accurate and parallel screening of viral pathogens. Although there are some inherent difficulties in applying this approach to clinical samples, including a lack of controls, limited specimen quantity, and high contamination rate, our work will facilitate further application of this unprecedented high-throughput method to clinical samples.
A Sensitive Assay for Virus Discovery in Respiratory Clinical Samples


In 5–40% of respiratory infections in children, the diagnostics remain negative, suggesting that the patients might be infected with a yet unknown pathogen. Virus discovery cDNA-AFLP (VIDISCA) is a virus discovery method based on recognition of restriction enzyme cleavage sites, ligation of adaptors and subsequent amplification by PCR. However, direct discovery of unknown pathogens in nasopharyngeal swabs is difficult due to the high concentration of ribosomal RNA (rRNA) that acts as competitor. In the current study we optimized VIDISCA by adjusting the reverse transcription enzymes and decreasing rRNA amplification in the reverse transcription, using hexamer oligonucleotides that do not anneal to rRNA. Residual cDNA synthesis on rRNA templates was further reduced with oligonucleotides that anneal to rRNA but can not be extended due to 39-dideoxy-C6-modification. With these modifications .90% reduction of rRNA amplification was established. Further improvement of the VIDISCA sensitivity was obtained by high throughput sequencing (VIDISCA-454). Eighteen nasopharyngeal swabs were analysed, all containing known respiratory viruses. We could identify the proper virus in the majority of samples tested (11/18). The median load in the VIDISCA-454 positive samples was 7.2 E5 viral genome copies/ml (ranging from 1.4 E3–7.7 E6). Our results show that optimization of VIDISCA and subsequent high-throughput-sequencing enhances sensitivity drastically and provides the opportunity to perform virus discovery directly in patient material.
Next-generation sequencing

- Cost-effective sequencing of PCR products
- Unbiased parallel detection of viruses in clinical samples: closing the diagnostic gap
- Virome: viral communities
Viruses in the faecal microbiota of monozygotic twins and their mother

Reyes A, Haynes M, Hanson N, Angly FE, Health AC, Rohwer F and Gordon JI

Viral diversity and life cycles are poorly understood in the human gut and other body habitats. Phages and their encoded functions may provide informative signatures of a human microbiota and of microbial community responses to various disturbances, and may indicate whether community health or dysfunction is manifest after apparent recovery from a disease or therapeutic intervention. Here we report sequencing of the viromes (metagenomes) of virus-like particles isolated from faecal samples collected from healthy adult female monozygotic twins and their mothers at three time points over a one-year period. We compared these data sets with data sets of sequenced bacterial 16S ribosomal RNA genes and total-faecal-community DNA. Co-twins and their mothers share a significantly greater degree of similarity in their faecal bacterial communities than do unrelated individuals. In contrast, viromes are unique to individuals regardless of their degree of genetic relatedness. Despite remarkable interpersonal variations in viromes and their encoded functions, intrapersonal diversity is very low, with >95% of virotypes retained over the period surveyed, and with viromes dominated by a few temperate phages that exhibit remarkable genetic stability. These results indicate that a predatory viral-microbial dynamic, manifest in a number of other characterized environmental ecosystems, is notably absent in the very distal intestine.
Immense populations of viruses are present in the human gut and other body sites. Understanding the role of these populations (the human "virome") in health and disease requires a much deeper understanding of their composition and dynamics in the face of environmental perturbation. Here, we investigate viromes from human subjects on a controlled feeding regimen. Longitudinal fecal samples were analyzed by metagenomic sequencing of DNA from virus-like particles (VLP) and total microbial communities. Assembly of 336 Mb of VLP sequence yielded 7175 contigs, many identifiable as complete or partial bacteriophage genomes. Contigs were rich in viral functions required in lytic and lysogenic growth, as well as unexpected functions such as viral CRISPR arrays and genes for antibiotic resistance. The largest source of variance among virome samples was interpersonal variation. Parallel deep-sequencing analysis of bacterial populations showed covariation of the virome with the larger microbiome. The dietary intervention was associated with a change in the virome community to a new state, in which individuals on the same diet converged. Thus these data provide an overview of the composition of the human gut virome and associate virome structure with diet.
Next-generation sequencing

• Cost-effective sequencing of PCR products

• Unbiased parallel detection of viruses in clinical samples: closing the diagnostic gap

• Virome: viral communities

• Human genome sequencing: viral susceptibility mutations
A Homozygous Nonsense Mutation (428G→A) in the Human Secretor (FUT2) Gene Provides Resistance to Symptomatic Norovirus (GGII) Infections

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Noroviruses (formerly Norwalk-like viruses) are a major cause of acute gastroenteritis worldwide and are associated with a significant number of nosocomial and food-borne outbreaks. In this study we show that the human secretor FUT2 gene, which codes for an α(1,2)-fucosyltransferase synthesizing the H-type 1 antigen in saliva and mucosa, is associated with susceptibility to norovirus infections. Allelic polymorphism characterization at nucleotide 428 for symptomatic (n = 53) and asymptomatic (n = 62) individuals associated with nosocomial and sporadic norovirus outbreaks revealed that homozygous nonsense mutation (428G→A) in FUT2 segregated with complete resistance for the disease. Of all symptomatic individuals, 49% were homozygous (SeSe) and 51% heterozygous (Sese) secretors, and none were secretor negative (se se), in contrast to 20% nonsecretors (se se) among Swedish blood donors (n = 104) (P < 0.0002) and 29% for asymptomatic individuals associated with nosocomial outbreaks (P < 0.00001). Furthermore, saliva from secretor-positive and symptomatic patients but not from secretor-negative and asymptomatic individuals bound the norovirus strain responsible for that particular outbreak. This is the first report showing that the FUT2 nonsecretor (se se) genotype is associated with resistance to nosocomial and sporadic outbreaks with norovirus.
IL10 Family Member Genes IL19 and IL20 Are Associated With Recurrent Wheeze After Respiratory Syncytial Virus Bronchiolitis

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Mechanisms underlying the increased risk of recurrent wheeze after respiratory syncytial virus lower respiratory tract infection (RSV LRTI) are unclear. Specifically, information about genetic determinants of recurrent wheeze after RSV LRTI is limited. We performed a candidate gene association study to identify genetic determinants of recurrent wheeze after RSV LRTI. We investigated 346 single nucleotide polymorphisms (SNPs) in 220 candidate genes in 166 Dutch infants hospitalized for RSV LRTI. Logistic regression analysis was used to study associations between genotypes and haplotypes and recurrent wheeze after RSV LRTI. We found associations with recurrent wheeze for SNPs in IL19, IL20, MUC5AC, TNFRSF1B, C3, CTLA4, CXCL9, IL4R, and IL7 genes. Haplotype analysis of the combined IL19/IL20 genotyped polymorphisms demonstrated an inverse association between the TGG haplotype and recurrent wheeze after RSV LRTI. IL19 and IL20 genes were notably associated with recurrent wheeze in infants without asthmatic parents. The association of IL20 SNP rs2981573 with recurrent wheeze was confirmed in a healthy birth cohort. We concluded that genetic variation in adaptive immunity genes and particularly in IL19/IL20 genes associates with the development of recurrent wheeze after RSV LRTI, suggesting a role for these IL10 family members in the etiology of airway disease during infancy.
Genomic risk of hepatitis C-related hepatocellular carcinoma

Hoshida Y, Fuchs BC, Tanabe KK

To identify the genetic susceptibility factor(s) for hepatitis C virus-induced hepatocellular carcinoma (HCV-induced HCC), we conducted a genome-wide association study using 432,703 autosomal SNPs in 721 individuals with HCV-Induced HCC (cases) and 2,890 HCV-negative controls of Japanese origin. Eight SNPs that showed possible association ($P < 1 \times 10^{-5}$) in the genome-wide association study were further genotyped in 673 cases and 2,596 controls. We found a previously unidentified locus in the 5' flanking region of MICA on 6p21.33 (rs2596542, $P$ (combined) = $4.21 \times 10^{-13}$, odds ratio = 1.39) to be strongly associated with HCV-induced HCC. Subsequent analyses using individuals with chronic hepatitis C (CHC) indicated that this SNP is not associated with CHC susceptibility ($P = 0.61$) but is significantly associated with progression from CHC to HCC ($P = 3.13 \times 10^{-8}$). We also found that the risk allele of rs2596542 was associated with lower soluble MICA protein levels in individuals with HCV-induced HCC ($P = 1.38 \times 10^{-13}$).
Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus.


RESULTS:
In Caucasians, the CC IL-28B type was associated with improved early viral kinetics and greater likelihood of RVR (28% vs 5% and 5%; P < .0001), complete early virologic response (87% vs 38% and 28%; P < .0001), and SVR (69% vs 33% and 27%; P < .0001) compared with CT and TT. A similar association occurred within African Americans and Hispanics. In a multivariable regression model, CC IL-28B type was the strongest pretreatment predictor of SVR (odds ratio, 5.2; 95% confidence interval, 4.1-6.7). RVR was a strong predictor of SVR regardless of IL-28B type. In non-RVR patients, the CC IL-28B type was associated with a higher rate of SVR (Caucasians, 66% vs 31% and 24%; P < .0001).

CONCLUSIONS:
In treatment-naive HCV-1 patients treated with pegylated interferon and ribavirin, a polymorphism upstream of IL-28B is associated with increased on-treatment and sustained virologic response and effectively predicts treatment outcome.
Genetic variation in *IL28B* predicts hepatitis C treatment-induced viral clearance

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Association of IL28B SNP With Progression of Egyptian HCV Genotype 4 Patients to End Stage Liver Disease

Mostafa K. El-Awady 1, Lotiaf Mostafa 2, Ashraf A. Tabll 1, Tawfeek H. Abdelhafez 1, Noha G Bader El Din 1, Naglaa Zayed 3, Reem El Shenawy 1, Yasmin El Abd 1, Reham M. Hasan 1, Hosam Zaghlol 4, Hesham El Khayat 3, Ashraf O. Abdel Aziz 3
RAPID AND SUSTAINED ACHIEVEMENT OF UNDETECTABLE HCV RNA DURING TREATMENT WITH RITONAVIR-BOOSTED DANOPREVIR/PEG-IFNα-2A/RBV IN HCV GENOTYPE 1 OR 4

Reported by Jules Levin
EASL 2012 Apr 18-22 Barcelona Spain
If you aren’t confused, you don’t know what’s going on!

Jack Welch, former CEO General Electric
May you live in interesting times

Sir Hughe Knatchbull-Hugessen, ambassador to China, 1936

Old Chinese curse