Migrations et diversité génétique en Méditerranée

Impact du programme MEDIGENE sur le diagnostic moléculaire de résistance à l’insuline et l’état nutritionnel

Florin Grigorescu MD, PhD (CRHC - INSERM)
**International context & Needs for EU research**

**Complex diseases** *(Obesity, Metabolic Syndrome, Type 2 diabetes and cardiovascular complications) – Diabesity*

- impact on morbidity and mortality of human populations
- paralyze HEALTH expenses in 21\textsuperscript{st} century

**Immigrants** - **41 millions for 8.6% of EU population**

**Mediterranean area**: population movements during millennia

- wide diversity of *life styles* (diet, physical activity)
- heterogeneity in *anthropological* population structure

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EPI-Migrant – UK (3M€)

GIFTS - UK (3M€)

MEDIGENE - (3M€)

RODAM - UK (3M€)

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**Genetic and environmental factors of Metabolic Syndrome in Mediterranean populations**
Consortium MEDIGENE: clinical endocrinologists and geneticists, anthropologists, demographists & archaeologists

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Montpellier 2012 (France)

Casablanca, 2014 (Morocco)

Brasov 2013 (Romania)

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Global objectives of MEDIGENE

To study the **insulin resistance (InsRes)** syndrome - **metabolic syndrome** (MetS) - in native and immigrant populations in Europe (**host and home countries**)

**Genetic landscape of Europe:** Balkans, Anatolia, West basin (**Spain, South of France, Italy**) and North Africa (**Morocco, Algeria, Tunisia**)

Solving the **genetic structure** of Mediterranean populations
- Autosomal Single Nucleotide polymorphism (SNP)
- Uniparental mtDNA or Chr Y [*anthropological*] lineages

To discover **new genes** for MetS / insulin resistance
- *Genome wide Association Studies* (GWAS)
- increasing density of SNP up to 1000 Genome Project (1KGP)
- fine-scale haplotytype-mapping

Interaction between culprit genes and nutritional factors (e.g. Mediterranean diet) – new field of *nutrigenomics*

**Finality**: forward-looking policy in the Mediterranean area
- define new genetic markers for Health prevention programs
- ameliorate diagnosis (**CV complications, aging, dependence**)
- Integrate modern demography (**social, economical factors**)

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Why the interest in MetS?

MetS - cluster of obesity, hyperglycemia, dyslipidemia (TG & HDL), high blood pressure (NCEP-ATPIII or IDF definitions)

Questionable candidate for genetic studies (heterogeneous)

• Insulin resistance - unique feature - fundamental biological process – T2D, CV complications & longevity
• Heritability in population: 10 to 30% (higher for each component)
• Epidemiology, MetS per se is correlated with health indices (CV mortality)

High variability in prevalence in ethnic populations (diet/genetic factors)

<table>
<thead>
<tr>
<th>Intuits</th>
<th>Europeans</th>
<th>Immigrants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2%</td>
<td>17-20 %</td>
<td>40 %</td>
</tr>
</tbody>
</table>

More frequent in infertile woman with hyperandrogeny and polycystic ovary syndrome (PCOS) – 3x more obese and more CV complications

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Relationship between migration and Health

Genetic background?

- Home country
- Salmon theory

1st Gen
- Host (Natives)
- Statistically immortal

2nd Gen
- Refugee
- Age of migration
- Life style
- Stress
- Epidemiological paradoxes
- Acculturation

Vulnerable populations

Epi-genetics

Collision with Westernized way of life

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HUMAN MIGRATIONS

Ancestral migrations

Historical migrations

Modern migrations

(Immigrants)
**Populations studies in MEDIGENE**

**Native populations:** Southern France (RA, LR), Spain, Italy, Greece, Romania (*Dacia Mediterranea*), Moldova, Albania and Croatia (continental/island), Lebanon, Lithuania, Basque population

**Romans:** Necropolis of Tarragona (2nd to the 7th century AD) – *ancient* DNA

**More distant Russian:** Karachays, Balkars, Kmyys, Nogays, Chuvashes, *Tatars*, Bashkirs, Yakuts

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## New DNA Collections in MEDIGENE

### Declaration at MESRI: ethical, import/export, CNIL

<table>
<thead>
<tr>
<th>DNA samples</th>
<th>Geographic region concerned for anthropological study</th>
<th>Ethnic Population</th>
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</thead>
<tbody>
<tr>
<td>1-49</td>
<td>Southern France (FR)</td>
<td>Languedoc Roussillon</td>
</tr>
<tr>
<td>1-14</td>
<td>Lebanon (LB) - Aaikar, Chouf, Beirut, South-Lebanon, Nabatieh, Mont-Liban, North-Lebanon</td>
<td>Lebaneese</td>
</tr>
<tr>
<td>1-14</td>
<td>Lithuania (LT) - Kaunas</td>
<td>Lithuanians</td>
</tr>
<tr>
<td>1-58</td>
<td>Romania (RO) D Giurgiu, Galati, Damboviita, Valea, Bucharest, Brăila, Ifig, Calarasi, Maramures, Sucej, Satu Mare, Alba, Bistrita Násaud, Cluj</td>
<td>Romanians</td>
</tr>
<tr>
<td>1-60</td>
<td>North, Center and South Italy (IT) D Emilia Romana, Rome, Calabria</td>
<td>Italians</td>
</tr>
<tr>
<td>1-14</td>
<td>Greece (GR) - Peloponense, Thessaly, Epirus, Attica, Crete, Thrace, Macedonia</td>
<td>Greeks</td>
</tr>
<tr>
<td>1-336</td>
<td>Russia (RU) D Republic of Kabardino-Balkaria, Chuvashia, Moldova, Karachai-Cherkess, Dagestan and Bashkortostan, Kazakhstan, Tatarstan, Uzbekistan, Siberia and West Ukraine region of Russia</td>
<td>Balkars, Bashkins, Yakuts, Chuvashes, Karachais, Kazakhs</td>
</tr>
</tbody>
</table>

**DNA samples**

<table>
<thead>
<tr>
<th>Country</th>
<th>Age (y)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algeria (DZ)</td>
<td>256</td>
<td>57.5±1.0</td>
</tr>
<tr>
<td>152</td>
<td>55.8±1.1</td>
<td>52</td>
</tr>
<tr>
<td>Russia (RU)</td>
<td>183</td>
<td>57.0±0.5</td>
</tr>
<tr>
<td>Turkey (TR)</td>
<td>32</td>
<td>59.5±1.4</td>
</tr>
<tr>
<td>Italy-C (IT)</td>
<td>71</td>
<td>60.2±1.7</td>
</tr>
<tr>
<td>Tunisia (TN)</td>
<td>356</td>
<td>55.6±0.7</td>
</tr>
<tr>
<td>Spain (ES)</td>
<td>300</td>
<td>52.7±0.7</td>
</tr>
<tr>
<td>Romania</td>
<td>136</td>
<td>30- Metabolic Syndrome</td>
</tr>
</tbody>
</table>

### Anthropological Studies

**2019**

**Metabolic Syndrome**

### 1921

PCOS ± MetS in infertile women

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Components of the Metabolic Syndrome

Brain
- Thriftiness
- Eating behavior
- Socio-ecological adaptation
- Reproduction

Visceral (central) Adiposity
- NO production
- Rennin Angiotensin
- Oxidative stress

Insulin resistance
- Beta cell failure
- Glucose toxicity

Physiological Inactivity

Dyslipidemia
- Resistin
- FFA
- PAI-1

Pro-inflammatory state
- Selectin
- Metalloproteinases
- CD-40
- ICAM-1
- VCAM

Monocyte endothelial cells
- TNF-α
- Interleukin-6, 10
- Interleukin 12, 18

Platelet activation
- P-selectin
- CD-40
- PAI-1
- Tromboxan

Cardio-vascular complications
- Miocardial infarction
- Atherosclerosis
- T2 diabetes
- Microvascular disease

Kidney disease
- Hypertension
- Hyperglycemia

Adipocyte
- Leptin
- Adiponectin

TG
HDL
LDL
Bioinformatics structure in Mediterranean area

MEDIGENE PORTAL

MAGDB

MEDIPAD

INTACTILE design

Partners

IDIBAPS
Barcelona
Spain

Genotyping

Medical Practitioners

MAGDB

Principal server XY

Calculating server XX (REDHAT)

Medico team

Archiving

Backup

NAS Synology

Genotyping team

BC platform

SNPmax

File transfer

Coordination team

Backup

IBGU, Ufa, Russia

Russian team

Soft installed

GOLDENHELIX
EIGHENSOFT
STRUCTURE
ADMiXTURE
BEAGLE

IBGU, Ufa, Russia

Analysis

Calculating server XX

Genotyping

IDIBAPS, Barcelona, Spain

QC Affymetrix

IURC, Montpellier, France

Genotyping

(integrated into the analysis)

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backup NAS Synology

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IBGU, Ufa, Russia

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Genotyping
Genome wide association studies (GWAS)

Screening of the whole genome with SNP

Manhattan Plot

Discovery of new loci for complex disorders
Fine-scale haplotype mapping

Ancestral haplotype

Linkage disequilibrium

Unphased DNA

Phased DNA

Recombination

Evolution

Natural selection

Genetic drift

Admixture

Rare SNP

Haplotypes

Classical GWAS ("frequentist")

Phylogeny
Customized DNA chip Affymetrix Axiom_MEDISCOPE

758 000 SNP/patient
Methods of MEDIGENE

**Next Generation Sequencing**

- GS Junior (Montpellier)
- FLX 454

**Genome Wide Association studies**

- AXIOM Chip
- GeneTitan (Barcelona)
- Primary analysis (Montpellier)

**Bioinformatics & statistics**

- Mirrored
- Barcelona
- BC-platforms
- Montpellier

**Replication**

- Roche
- NimbleGen

**Data integration**

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Main results of MEDIGENE

**GWAS of MetS in multiethnic Mediterranean populations**

- Discovery of the role of **AVPRA1** in hypertension
- Discovery of **KLB gene** in insulin resistance & FGF21
- Identify influential genes in **BCAA metabolism**
- Influential role of genes in **Zn metabolism**

**GWAS of PCOS (French, Italian, Romanian and Greeks)**

- Fine-scale mapping of several candidates - DUSP9, FTO
- Describes genes in DHEAS secretion in PCOS
- FSHb and LHCGR genes in FR versus TN
- Anthropological markers for **baldness** (men) in PCOS

**Anthropological studies**

- Autosomal **SNP markers in Mediterranean** area
- Ancient DNA in antique **Romans** in **Tarragona** Necropolis
- **Neanderthal ancestry** in humans – genes of lipid catabolism
Nouvelle hypothèse dans la pathogénie de la résistance à l’insuline – Rôle des BCAA

**Apport nutritionnel**

**Génome humain**

**Gènes de susceptibilité**

**BCAA**

**Métabolisme des BCAA**

**Métabolisme énergétique**

**SNC**

**Système de Récompense Cérébral**

**FGF-21**

**Fibroblast Growth Factors**

**Insuline**

**Résistance à l’insuline**

**Syndrome Métabolique (SMet)**

**Complications cardiovasculaires**

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Architecture générale du MEDIPAD

CIQUAL 2012
1440 aliments
Plats grecques (n = 141)
Plats turcs (n = 72)
Plats roumains (n = 22)
Plats marocains (n = 32)
Programme SIDI (n = 430)

Calcul des recettes par SIDI

Rappel de 24h (3 jours)

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Evaluation de l’apport alimentaire en BCAA : enquêtes MEDIGENE par rapport à l’INCA2
Total alcohol consumption in Mediterranean area
Etude des gènes du KEGG pathway des BCAA

RESEARCH ARTICLE

Fine-scale haplotype mapping of MUT, AACS, SLC6A15 and PRKCA genes indicates association with insulin resistance of metabolic syndrome and relationship with branched chain amino acid metabolism or regulation

Sara Haydar¹, Florin Grigorescu¹*, Mădălina Vintilă², Yannick Cogne¹, Corinne Lautier³, Yıldız Tutuncu³, Jean Frederic Brun⁴, Jean Marie Robine⁵, Michel Pugeat⁶, Christophe Normand⁷, Patrick Pouchere⁷, Monica Livia Gheorghiu⁸, Carmen Georgescu⁹, Corin Badiu⁹, Nicoleta Băculescu², Eric Renard⁹, Dorina Ylli¹⁰, Stephanie Badiou⁴, Thibault Sutra⁴, Jean Paul Cristoi⁴, Jacques Mercier⁴, Ramon Gomis¹¹, Josep Maria Macias¹², Serghey Litvinov¹³, Elza Khusnudinova¹³, Catalina Poiana⁴, Renato Pasquali¹⁴, Davide Lauro¹⁵, Giorgio Sesti¹⁶, Sabrina Prudente¹⁷, Vincenzo Trischitta¹⁷, Agathocles Tsatsoulis¹⁸, Sonia Abdelhak¹⁹, Abdelhamid Barakat²⁰, Akila Zenati²¹, Agron Ylli¹⁰, Ilhan Satman³, Timo Kanninen²², Yves Rinato²³, Sasa Missoni²⁴
Genetic makeup of Europe was made by successive waves of colonization (migrations) – demic diffusion

Strata are stable from Mesolithic and recognize remnants (Basque), East colonization (Greeks in Magna Grecia), Indo-European populations (LCT gene), invasion by Barbarian (Germanic) populations.

Population structure may be solved by mDNA & Chr Y lineages and extraordinarily by bio-geographic location of autosomal SNP in the human genome

- increase chances to discover pathogenic allelic variants
- increase density of SNP in GWAS is very useful but not sufficient
- genomic studies should be combined with haplotype mapping/phylogeny

Neanderthal introgression

Summary and conclusions - 2

There is a large “reported prevalence” of MetS in native and immigrant populations (no systematic analysis and standardized methods: ATPIII/IDF, randomized recruitment)

• Good correlation between HOMA (robust) and cumulative criteria for MetS
• differences in lipid profile, East-West gradients

Understanding the pathogenesis of InsRes: energy allocation mechanisms (insulin) are obsolete. New actors in glucose homeostasis in direct interrelation with alimentary behavior, sweet preference, alcohol consumption: BCAA intake and FGF-21 (fibroblast growth factor)

Both BCAA and FGF-21 can act on the brain – involving the rewarding system

• alcohol consumption – diabetes – behavior
• involvement of dopamine in the rewarding system (DR4) make the link between novelty seeking, pathological gambling, financial risk, alcoholism, addiction

• evolutionary explanation: exodus out of Africa, nomadism, male/male competition, agricultural scarcity/famine, climate changes/water
Perspectives and ethical considerations

Forward goals in migration studies

Understanding the way of life of different people in their natural (ancestral) ecological niche, consequences of migration in a broader perspective as a potential factor of global increase in prevalence of diabetes/obesity – measure the impact of deregulation of food supplies in modern times.

High Ethical attitude

Understanding and accepting human genetic diversity, in opposition to biological egalitarism represents a major advance in current thinking and a positive attitude.

Group differences will help to understand how genetic and environmental factors produce biological outcomes. We should bear in mind that genetic diversity is a virtue of mankind not a defect, and a source of evolutionary resilience and adaptation assuring survival and health with potential positive economic consequences on agriculture and environment.