What about the “Heterozygotes”?

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Definition

How can a heterozygote have the phenotype?

What should one do in the clinical setting?
An autosomal recessive disease

mutations in both alleles
Carrier (“heterozygote?”) frequencies – based on “healthy” individuals

- Non-Askh. Jewish: 1/5  
  Lancet 1998,
- Turkish: 1/5  
  Eur J Hum Genet 2001
- Armenian: 1/3  
  Am J Hum Genet 2000
- Askhenazi Jewish: 1/5-1/7  
  Am J Med Genet 2001
- Arabs: 1/5  
  Eur J Hum Genet 2001

Majority E148Q......
Why was the carrier selected in ancient times?

- MEFV "thrifty" gene?
  Stone Age genes in a Space Age environment

When our ancestors settled between the two rivers they needed the set-up to fight efficiently with microorganisms.
...heterozygotes might have had a survival benefit because of enhanced innate host response enabling a better resistance to microbes

They have certain features in return:

• Higher acute phase proteins
  
  Rheumatology 2006

• They have some complaints: excess febrile episodes and more rheumatic diseases
  
Definition

• A person with mutations on both alleles and the typical “phenotype” and elevated acute phase reactants is a patient.
• A person with one mutation only and without the phenotype is a carrier or “heterozygote”.
• A heterozygote with the typical “phenotype” can be a patient…
Can you have the FMF phenotype with only a mutation in one allele? Or with a mutation + a polymorphism?

- Clinical examples: 20-26%
- Phenotype proven by fulfilling the clinical criteria and appearance of attacks with the cessation of colchicine
- Other AID need to be ruled out
A patient

• An 6 yr old boy presents with an attack of acute abdominal pain and high acute phase reactants. He has had a transient attack of arthritis of the ankle and occasional attacks of (unexplained) fever of 1-2 day duration.

• Has an uncle who died with secondary amyloidosis, a father with ankylosing spondylitis.

• M694V/-
How can one mutation cause the disease in an autosomal recessive condition?*

Speculations?
Any evidence?

*discussions may be extrapolated to the problem with polymorphisms
How can one mutation cause the disease in an autosomal recessive condition?

• Other unidentified mutations – promotor region?
• Another gene - J Med Genet 1997
• Psychological - in a sib of an affected child
• Evidence from digenic inheritance
• Evidence from the modification of phenotype with other factors:
  ➢ Polymorphisms in other relevant genes
  ➢ Environment (severity study)
• Epigenetics
Digenic inheritance examples: Synergistic heterozygosity in independent genes

- Genes affecting different proteins in the glomerular filtration barrier, Type 2 DM and a mitochondrial disease
- At least 3 examples in autoinflammatory diseases:
Digenic inheritance among autoinflammatory diseases

- Coexistent MEFV and CIAS1 mutations (of reduced penetrance mutation) with a FMF-like phenotype and deafness
- MEFV and TRAPS heterozygosity in a patient who develops amyloidosis
- R92Q (low penetrance TRAPS mutation) and a CIAS1 mutation causing overlap features in 2 family members
How can one mutation cause the disease in an autosomal recessive condition?

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Single Gene Diseases

- TRAPS
- CINCA
- MWS
- FCU
- FMF
- HIDS

Complex genetic trait

- JIA (systemic JIA)

Duration of inflammatory attack:
- Hours, days
- Months
Effect of polymorphisms (or else) in genes of the inflammatory pathway

- Complex genetic trait diseases (JIA) are associated with MHC and non-MHC polymorphisms

*MEFV mutations are associated with JIA and severity in RA*


- In FMF—a monogenic disease, polymorphisms in relevant genes affect the phenotype/severity
In FMF, polymorphisms in relevant genes affect the phenotype/severity

Modifying genetic factors increasing the risk of secondary amyloidosis ("severe" disease):

- SAA1 alpha/alpha genotype (Odds ratio up to 7) - confirmed in both Jewish and Turkish patients
- Major histocompatibility complex related gene A
  Arthr Rheum 2001
- Ala138Gly alteration in the MEFV gene
  Hum Mutat 2003
TLR2 polymorphisms in FMF: affecting the innate immune response to pathogens

- TLR2 polymorphism more common among patients who develop secondary amyloidosis

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<th>n</th>
<th>(%)</th>
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<tbody>
<tr>
<td>Healthy Controls (116)</td>
<td>7</td>
<td>6,0</td>
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<tr>
<td>FMF without amyloidosis (75)</td>
<td>14</td>
<td>18,6</td>
</tr>
<tr>
<td>FMF with amyloidosis (40)</td>
<td>15</td>
<td>37,5</td>
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Effect of polymorphisms (or else) in genes of the inflammatory pathway

• In diseases of complex genetic trait there are associations with relevant genes
• In FMF—a monogenic disease, polymorphisms in relevant genes affect the phenotype/severity
• Can polymorphisms in relevant genes produce an *FMF* phenotype with one mutation only or one polymorphism?
• Could this explain the differences with low-penetrance mutations/polymorphisms of E148Q? R202R?
Environment

The lack of amyloidosis in Armenians in the US
The Meta-FMF Study – Arthr Rheum 2007
The first study comparing the severity in different geographic areas

- The severity of disease in Turkish children living in Turkey compared to those in Germany
- MEFV mutations and age comparable
- Severity assessed both by an adapted Livneh score and Pras score
- More severe in Turkey
- Different milieu of infections: triggering the fragile innate immune pathway through TLRs?
Gene Environment Interaction

• Gene environment interaction has been defined as
  – a different effect of an environmental exposure on disease risk in persons with different genotype or,
  – a different effect of a genotype on disease risk in persons with different environmental exposures.
Can polymorphisms in relevant genes produce an *FMF* phenotype with one mutation only

Anti-inflammatory cytokines

Pro-inflammatory cytokines

Interaction with the environment

Non-cytokine genes

Epigenetics

Other?: the access of caspase-1 to some positions?

The phenotype /Penetrance
How can one mutation cause the disease in an autosomal recessive condition?

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EPIGENETICS

• Waddington–1946: ‘interactions of genes with their environment that bring the phenotype into being’

• DNA methylation, histone modifications (e.g. acetylation and methylation), chromatin modification and control of mRNA expression by non-coding RNAs are epigenetic mechanisms
Expression of the mutant allele – pronounced?

• Examples in certain cancers
• Sophisticated studies are needed to analyse critical regions in the promoters
• May offer explanation for reduced / “exaggerated” penatrance
When does a heterozygote need treatment? - importance of SAA

• Only with high SAA – level of recommendation?
• SAA levels a part of the therapeutic trial

Neth J Med 2007

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<tr>
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<th>Before Rx</th>
<th>After Rx</th>
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<tbody>
<tr>
<td>CRP</td>
<td>1.22mg/dl</td>
<td>0.10mg/dl</td>
</tr>
<tr>
<td>SAA</td>
<td>244ng/ml</td>
<td>35 ng/ml</td>
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J Rheumatol 2004
What would you do with the patient?

- Does he meet classification criteria for FMF
- Check his SAA levels
- Be sure of other AIDs
- Response to colchicine: withdrawal period
- Placebo?
Avoid overdiagnosis

- Differential Diagnosis: Other AIDs, Kawasaki disease, Systemic JIA
- Justification to use colchicine trial in a non-risk ethnic group – criteria need to be validated
- Collaboration for sophisticated clinical and molecular studies are needed
• A Bakkaloglu
• N Besbas
• R Topaloglu
• A Duzova
• F Ozaltin
• N Aktay
• E Aypar
• Y Bilginer
• U Saatci

• E Yilmaz
• B Balci and the Dept. of Medical Biology
• The patients...