

Autoimmune Polyglandular Syndrome - 1: Clinical and molecular aspects



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and the MEWPE-APS-1 study
group

Czech Republic, Austria, Croatia,
Hungary, Russia and Slovenia

Catherine (born 1982)

- Referred at 4 years as “hepatic failure”
- Jaundice, lethargy, hepatomegaly, elevated liver enzymes
- Liver biopsy: Chronic active hepatitis
- Catherine’s general status markedly improved after the first dose of Prednisolon

- At admission, Catherine had apparently dystrophic nails and onychomycosis
- ECG: Signs of hypocalcaemia; low parathormone; normal serum levels of calcium
- Elevated ACTH even 12 hours following Prednisolon administration → Addison disease

Catherine (born 1982)

Three major components of APS-1:

- Mucocutaneous candidiasis and/or ectodermal dystrophy
- Hypoparathyroidism
- Addison disease

Additional component:

- Chronic active hepatitis

Michaela (born 1987)

- At 6 years, Michaela developed unconsciousness and seizures - during a febrile episode with vomiting;
- At ICU: glycaemia 1.0 mmol/L (18 mg/dL), cortisol 6 nmol/L (normal morning values: 140-700)
- → Addison disease

- Michaela had a history of transient total alopecia at 2 yrs, AITD starting at 4 years, nail dysplasia and frequent infections (tens of episodes of otitis media)

- Following the entire hypoglycaemic episode, she remained in vigil coma and died 9 years thereafter.

Adriana (born 1992)

- Referred at 3 years as “refractory epilepsy”.
- Adriana suddenly had started to suffer from attacks of collapses, unconsciousness, with no seizures, up to 20 times daily. She had “weak legs” and could not walk. Dysarthria followed the attacks;
- EEG: Specific graphoelements;
- However, antiepileptic therapy without any effect;
- Transient total alopecia.

Adriana (born 1992)

- At admission: serum calcium 0.89 mmol/L, phosphate 3.34 mmol/L, parathormone < 0.1 pmol/L (n. 1.5-7.5)
- Following Ca and Calcitriol, attacks resolved.

Five years later, Adriana developed overt Addison disease with hyperpigmentation and fatigue - despite repeatedly normal cortisol levels:

- ACTH 511 pg/mL (n: 10-60)

- ACTH testing
- | | | | |
|----------|-----------|------------|------------|
| | <u>0'</u> | <u>30'</u> | <u>60'</u> |
| cortisol | 288 | 236 | 215 nmol/L |

APS-1 is probably the most troublesome of all paediatric endocrine disorders.

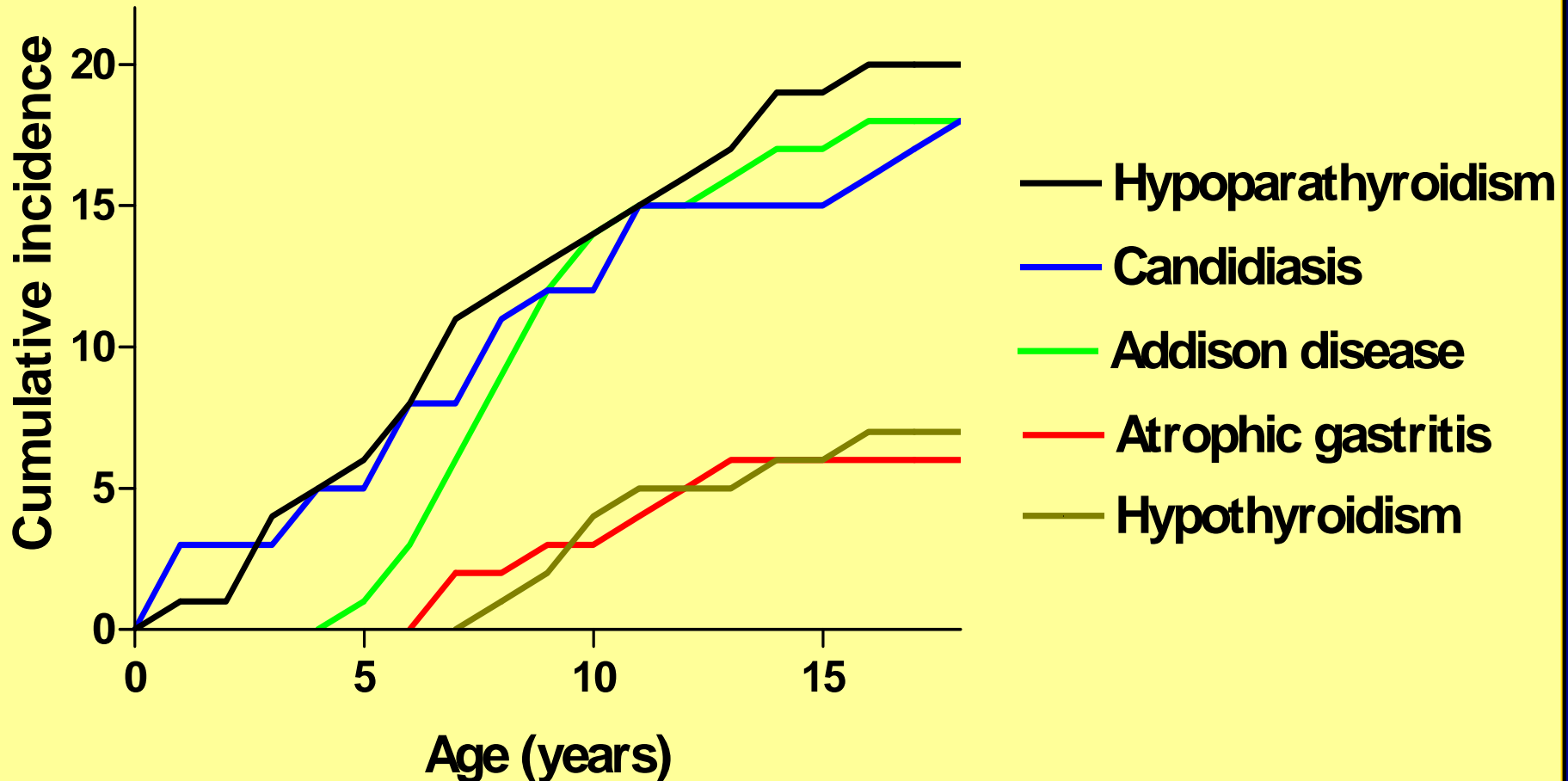
The parents are asking:

- What else will happen to our child ?
- Why our child had to be affected ?

APS-1: Disease components

Mucocutaneous candidiasis	73-100 %
Hypoparathyroidism	76-93 %
Addison disease	72-100 %
Hypogonadism	17-50 %
Alopecia	29-37 %
Chronic active hepatitis	12-20 %
Atrophic gastritis	13-15 %
Pernicious anaemia	13-15 %
Vitiligo	8-15 %
Malabsorption	15-22 %
Sjögren syndrome	0-12 %
AITD	2-11 %
Keratoconjunctivitis	0-35 %
Type 1 diabetes mellitus	2-12 %

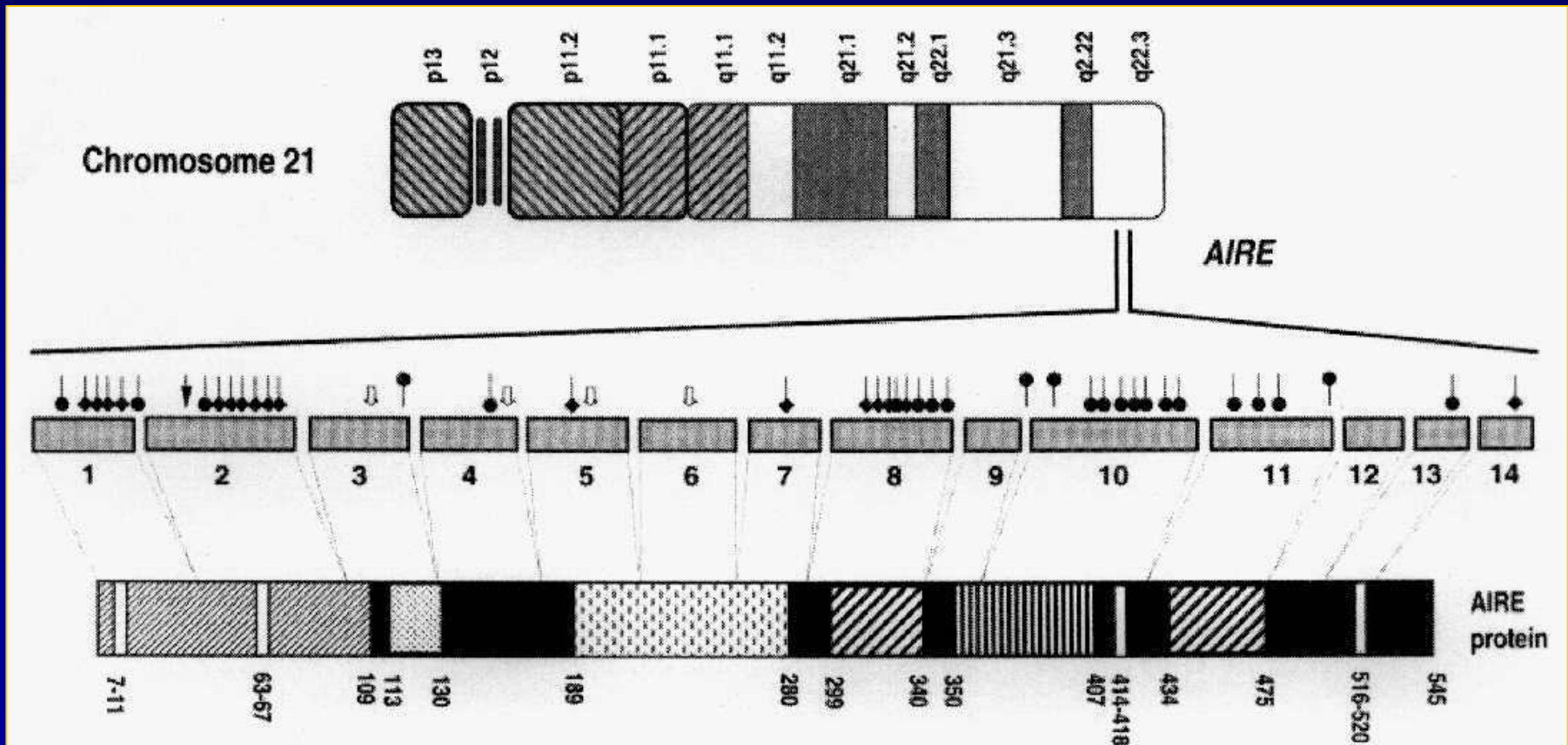
APS-1: Clinical course



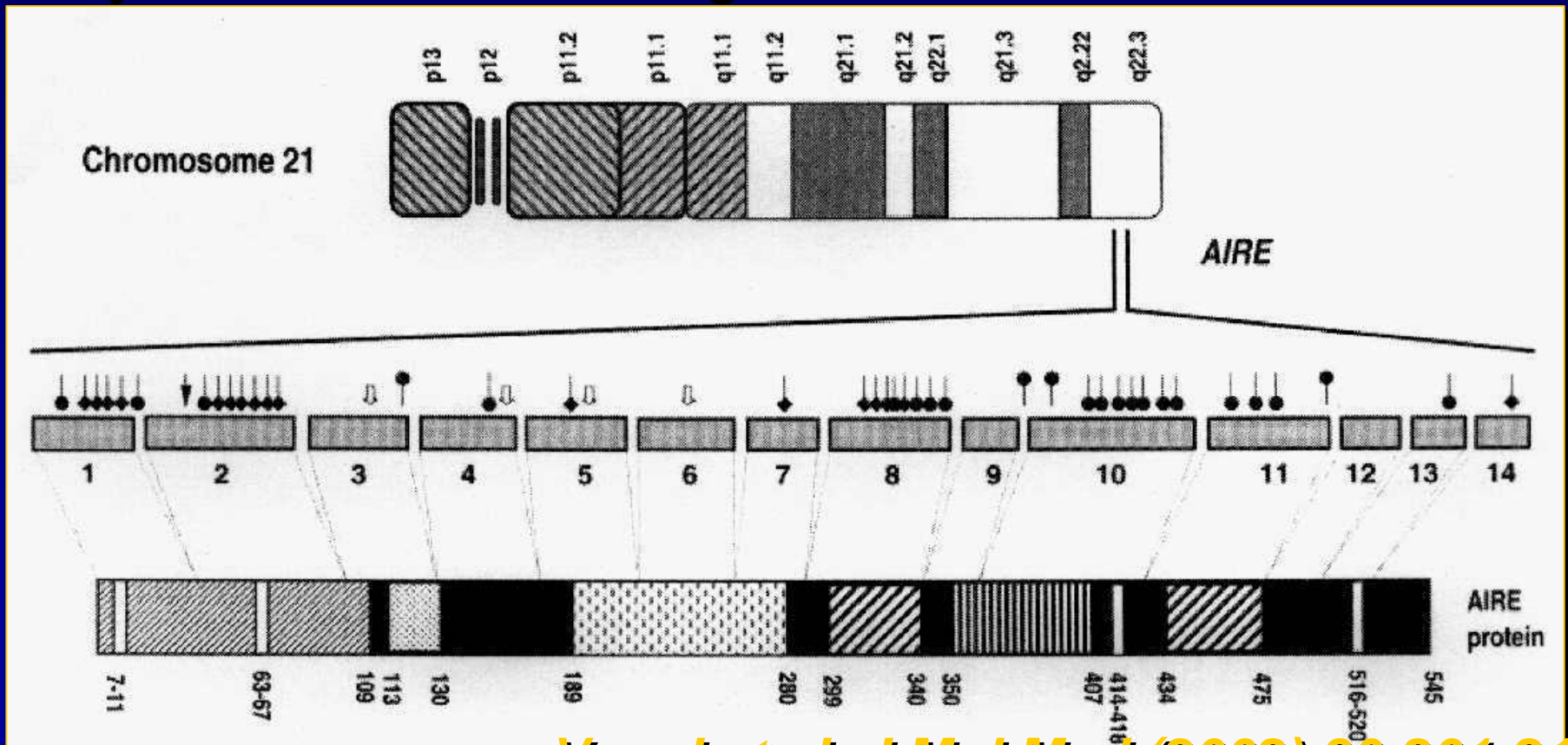
APS-1: Genetic background

- Autosomal recessive inheritance
(incomplete forms in some heterozygotes reported recently)
- No HLA association
- Male/female ratio: 0.8-1.5
- Population-specific incidence:
 - USA, Italy - about 1 : 100.000
 - Finland - 1 : 25.000
 - Sardinia - 1 : 14.000
 - Iran Jews - 1 : 6.500 - 1 : 9.000

- 1997: Identification of the AIRE gene (Autoimmune Regulator)
- Chromosome 21q segment 22.3
- over 40 mutations identified so far



- Encodes the AIRE protein
- Contains two Zinc fingers, prolin-rich regions and LXXLL motifs → transcription regulator
- Expressed in thymus and lymphatic nodes → key role in conserving of immune autotolerance



MEWPE APS-1 study

- Genomic DNA and serum samples of 27 patients (M/F: 10/17) available from Austria (4), Croatia (3), Czechia (5), Hungary (4), Russia (6), Slovenia (5).
- Age: 4-22 years (median 16)
- Age at diagnosis of the first disease component: 0.3-16 years (median 6)
- Number disease components per patient: 2-12 (median 5)

Mutation analysis

- R257X mutation analysed by Taq1 restriction digestion
- 967-979del13bp detected by fragment length analysis
- The 14 exons of the *AIRE* PCR amplified from genomic DNA and purified by Bandprep Kit
- Dye-terminator sequencing used according to standard protocols for the ABI310 automated sequencer

Results (I)

- 4 different mutations were identified, 2 of them were novel
- No mutation could be identified in 5 patients (2 of them did not match full clinical diagnostic criteria for APS-1)
- In 1 patient, a mutation was found in only one allele
- Thus, in 43 of 54 chromosomes a mutation was established

- R257X: 61 % of alleles - most prevalent mutation in Central and Eastern Europe
- 967-979del13bp: 7 % of alleles
- Novel mutations:
 - W78R in exon 2 - in one allele from a Czech patient
 - 30-52dup23bp - in an allele from a Hungarian and from an Austrian patient

Catherine (“hepatic failure”):

R257X / R257X

Adriana (“refractory epilepsy”):

R257X / W78R

Michaela (unconsciousness and seizures):

wild / wild

Detection of autoantibodies

Autoantibodies against P450c17,
P450c21 and P450scc
steroidogenic enzymes were
tested by immunoblotting

Results (II)

13/17 patients (76%) had autoantibodies against at least one P450 antigen.

Antibodies against P450scc... 7/17 pts (41%)

Antibodies against P450c21... 5/17 pts (29%)

Antibodies against P450c17... 11/17 pts (65%)

- Three of patients with positive antibodies had no clinically apparent Addison disease at testing (all had 2 positive antibodies);
- However, in one of them (in Adriana) Addison disease manifested within the subsequent 6 months.

- What is wrong with the immune system of APS-1 patients ?
- How does AIRE protein influence immune reactions ?
- Will this knowledge contribute to our general understanding of autoimmunity - or even to the development of new treatment options ?

“Immunological substudy”

Four APS-1 patients and their family members;

- $\text{INF}\gamma$ production decreased in APS-1 subjects compared to controls (455 ± 191 vs. 910 ± 406 pg/mL; $p=0.055$);
- IgM, CD3+CD4+ lymphocyte count increased;
- Interestingly, all fathers of APS-1 subjects had substantially elevated IgA and activated T lymphocytes

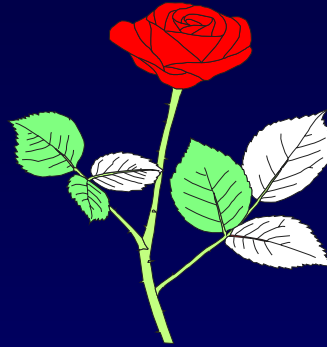
Conclusions (I)

- R257X was found to be the most common *AIRE* mutation in Central and Eastern Europe
- Two novel *AIRE* mutations were identified
- Some other genes may be involved in APS-1 patients with normal findings in *AIRE* (e.g., in Michaela)
- P450 autoantibodies may help to detect patients with high risk of developing Addison disease

Conclusions (II)

- APS-1 patients tend to produce less INF_γ
- Heterozygotes may have subclinical activation of the immune system
- However, the mechanism of action of the AIRE protein remains unclear

Thanks...



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