National and Kapodistrian
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Whole blood next-generation RNA-sequencing in patients with Antiphospholipid syndrome

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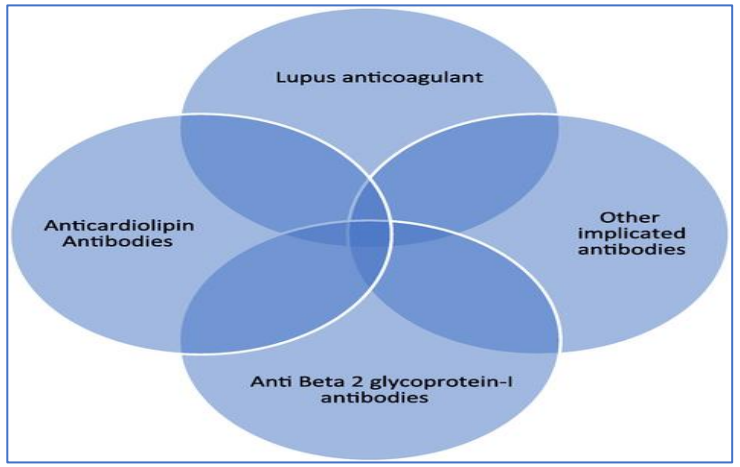
Antiphospholipid syndrome (APS)

Rare systemic autoimmune disorder

- Prevalence: 50 per 100,000 (95% CI: 42-58)



➢Antiphospholipid antibodies

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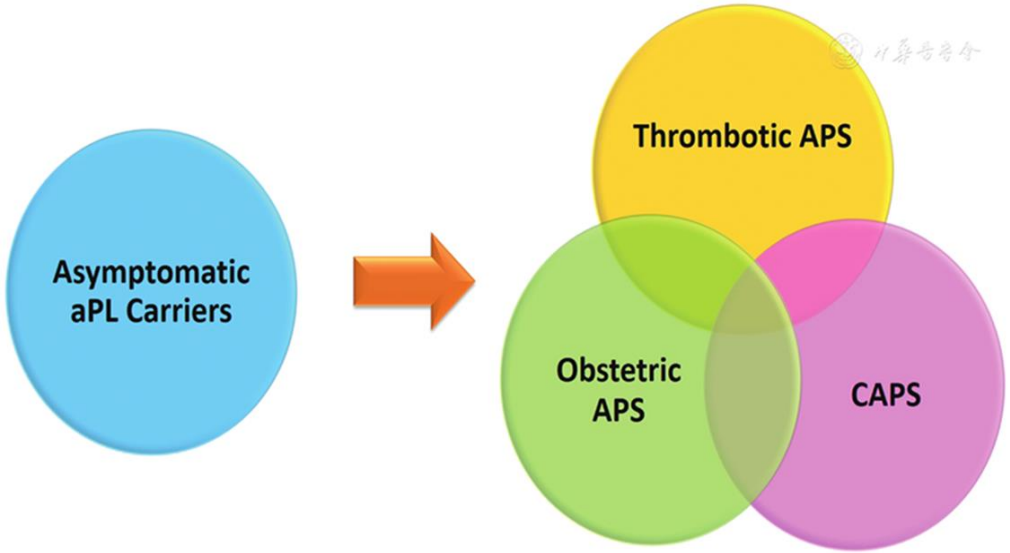
❑**Thrombotic events** (arterial, venous)

and/or

❑**Obstetric events** (miscarriages, fetal loss)



Pathogenesis may differ among different phenotypes

- Obstetric vs thrombotic
- Primary APS vs SLE-related

Thrombotic Primary APS patients (thrAPS) (N=64)

- 21 Arterial thrombosis
- 29 Venous thrombosis
- 31 Triple aPL
- 25 Recurrent thrombosis

Healthy controls (N=32)

"The Greek Research Infrastructure for Personalised Medicine (pMedGR)"

PAXgene RNA stored at –80°C

Pre-processing

- Quality Control (FastQC).
- Counting (GenomicRanges).
- Filtering (>5 reads in >25% of samples).
- Hemoglobin genes (HB) removal.

Differential Gene Expression

- DESeq, edgeR, NOISeq, limma, NBPSeq.
- Meta-analysis with PANDORA through metaseqR2.

Machine Learning (ML)

- Stratified nested (n=3) cross-validation (k=10).
- Tuning 3 methods (K nearest neighbours, support vector machine, Random Forests) => training >1000 models.
- Model selection according to accuracy

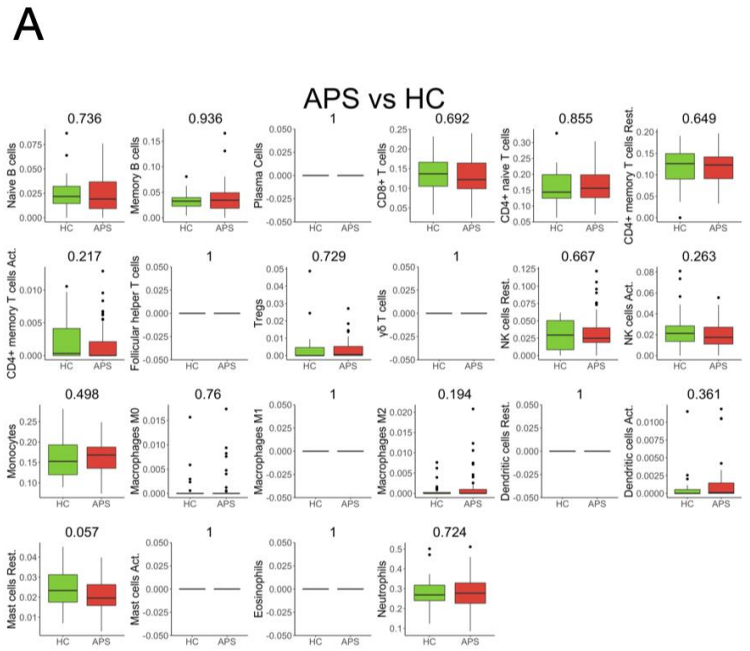
ThrPAPS vs HCs

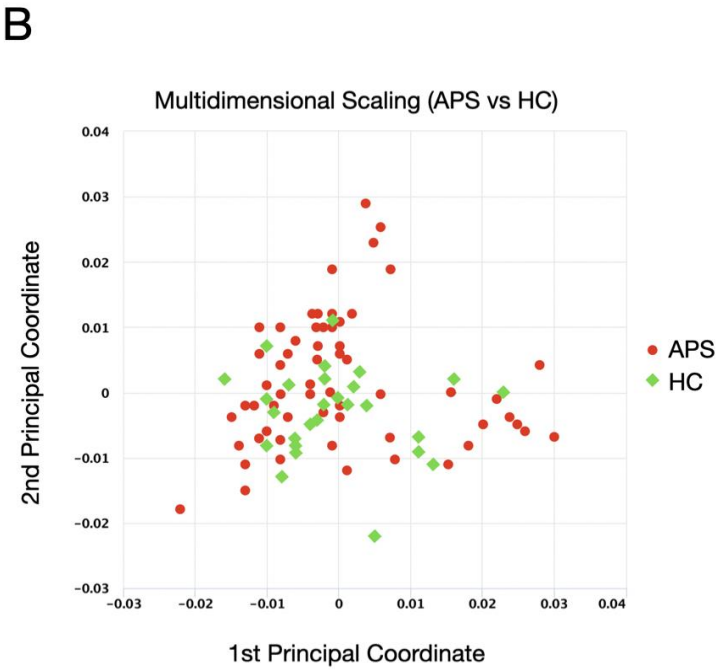
- Differential gene expression analysis (DEGA) of 12.306 genes --34 deregulated genes
- 33 were upregulated by at least 2-fold

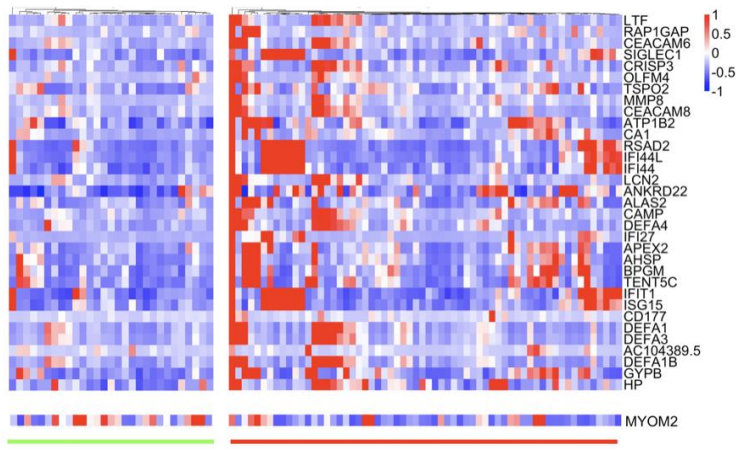
>40% were type I and II interferon-regulated genes (IRGs)

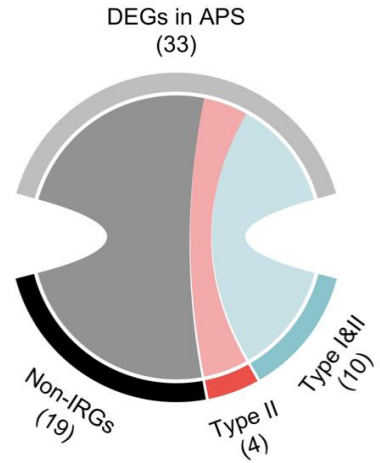
Machine learning:

- 79% accuracy to discriminate thrPAPS from HCs
- 86% when only IRGs were analyzed



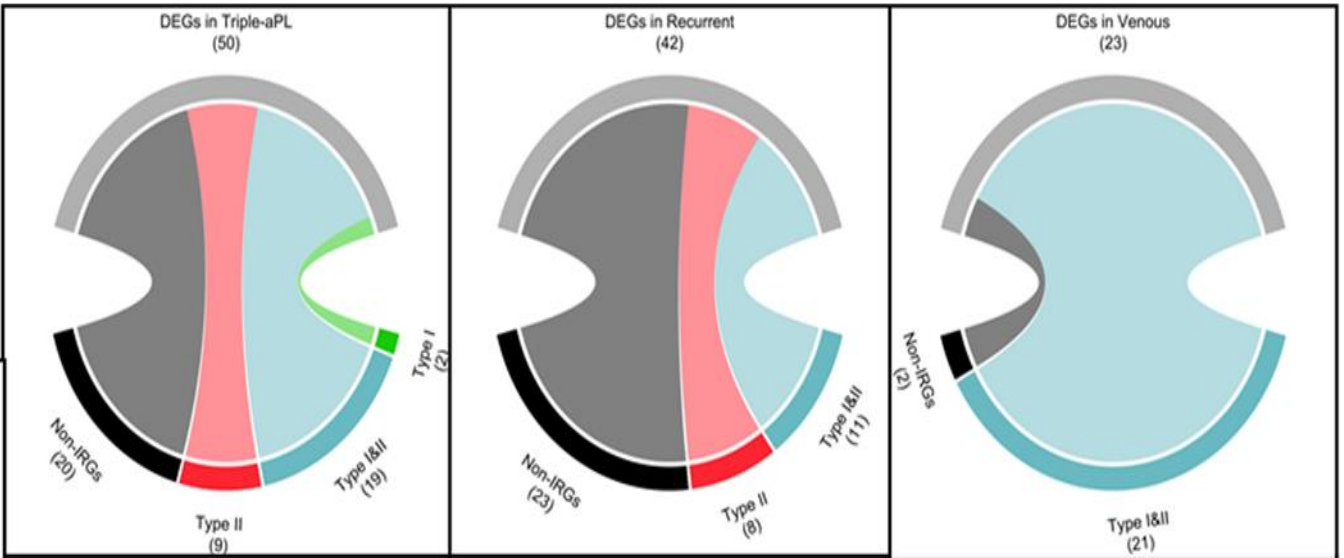






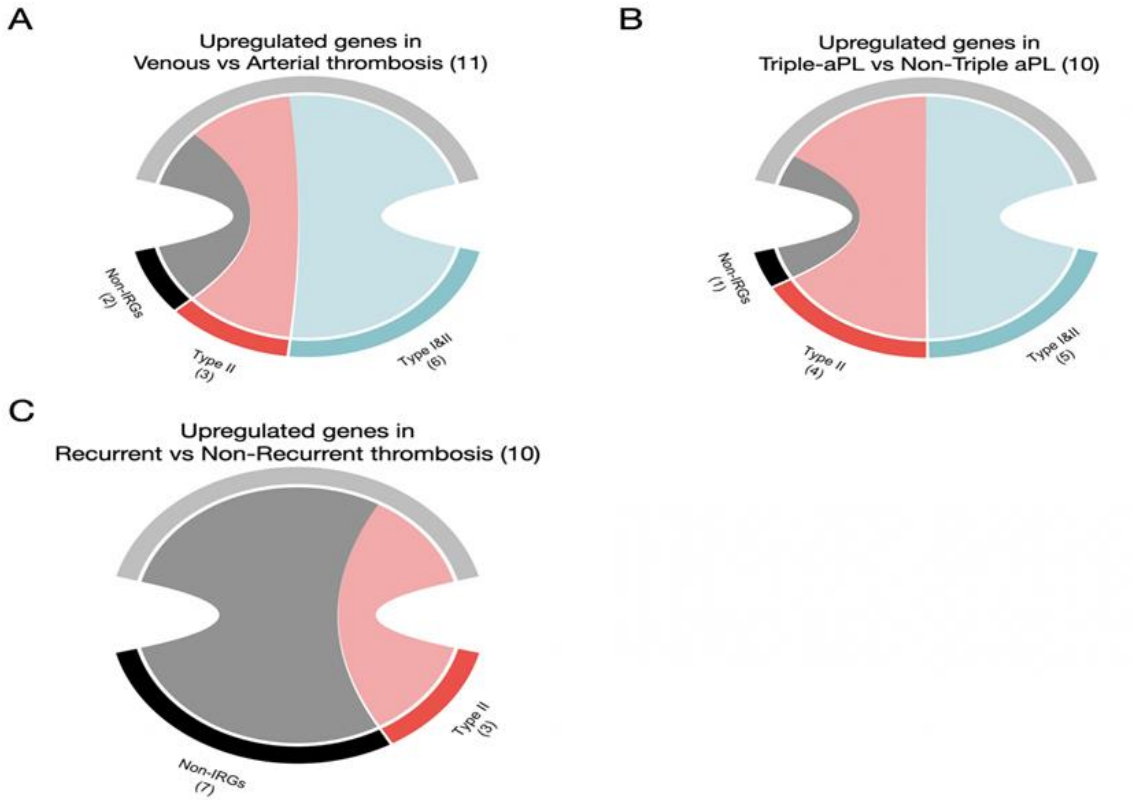
ThrPAPS subgroups vs HCs: majority of upregulated genes are IRGs

- Venous thrombosis: 21/23, **91%**
- Triple aPL: 30/50, **60%**
- Recurrent thrombosis: 19/42, **45%**



DEGA **among thrPAPS subgroups**:

- Venous vs arterial thrombosis: 9/11 (**82%**)
- Triple-aPL vs non-triple aPL: 9/10 (**90%),**
- Recurrent vs non-recurrent thrombosis: 3/10 (**30%**)



Conclusion:
IRGs are key regulators in thrPAPS and high-risk subgroups