Southampton University Hospitals NHS Trust

Oxford Radcliffe Hospitals NHS Trust

The Royal Bournemouth & Christchurch Hospitals NHS Foundation Trust

Molecular Analysis of B cell Subsets in Common Variable Immunodeficiency (CVID)

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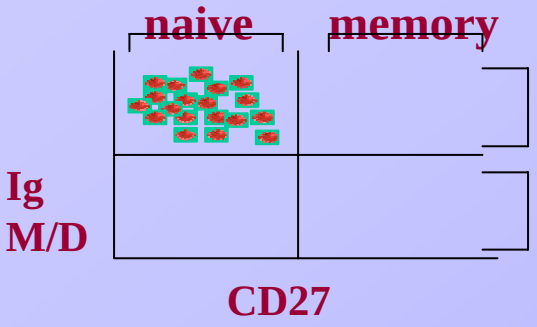
CVID

- 1 in 50,000
- Most common primary antibody deficiency
- Diagnosed between 20 & 40 years
- Heterogeneous syndrome
- Decreased Ig levels
- Recurrent infections
- Other causes of immunodeficiency excluded

Classification of CVID

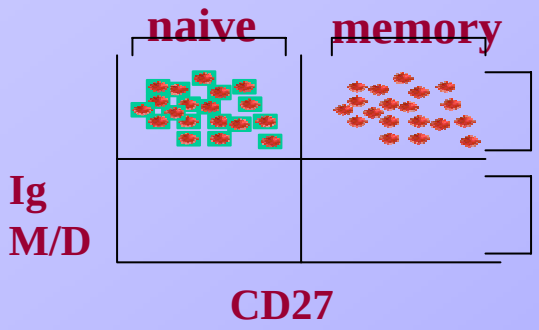


IgM Memory ~4%

Class Switched Memory ~1%

MB0
Reduced memory B cells

LPD
Granulomatous
Splenomegaly

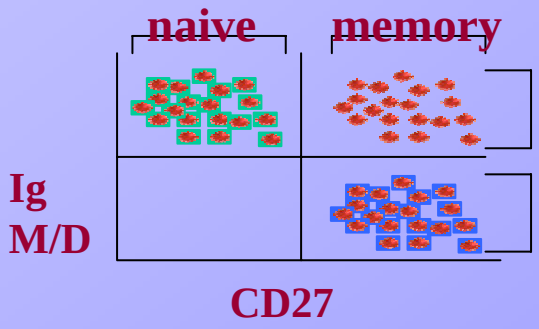


IgM Memory ~20%

Class Switched Memory ~4%

MB1
Reduced class switched memory B cells

Splenomegaly



IgM Memory ~20%

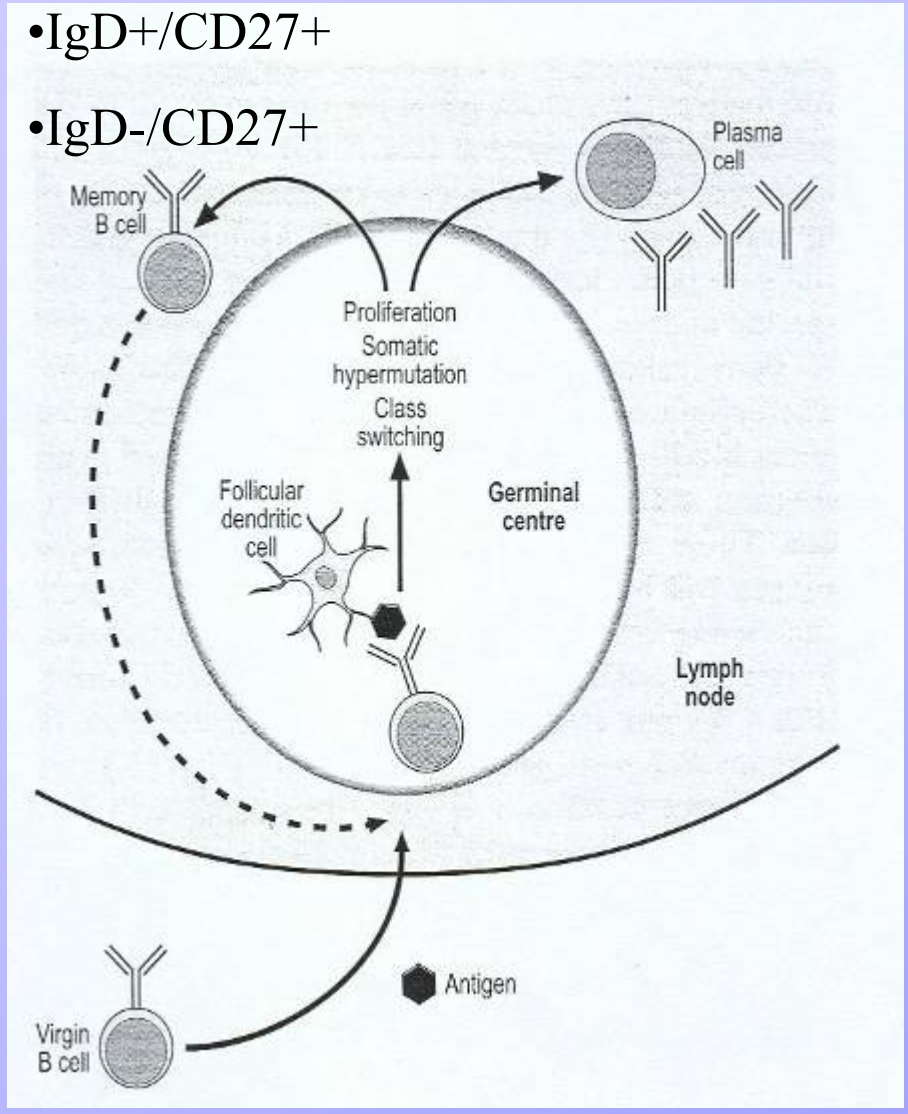
Class Switched Memory ~20%

MB2
Class switched memory B cells

Clinical complications less severe

•IgD+/CD27+

•IgD-/CD27+



IgD+ and CD27-

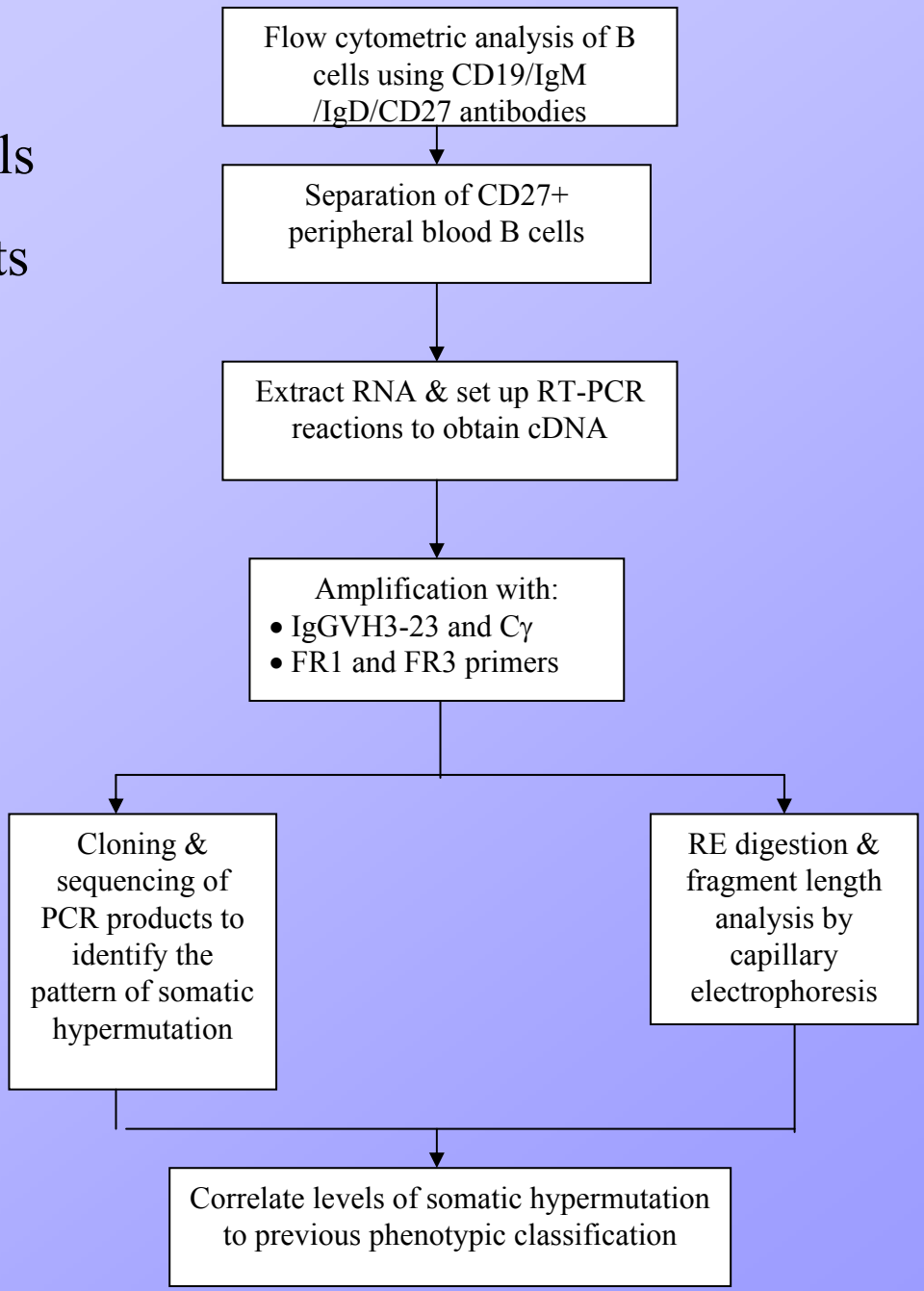
Somatic hypermutation (SHM): nucleotide substitution in V region to increase affinity for antigen

Class switch recombination (CSR): B-cells substitutes expression of IgM and IgD for IgG, IgA or IgE by deleting DNA between switch regions

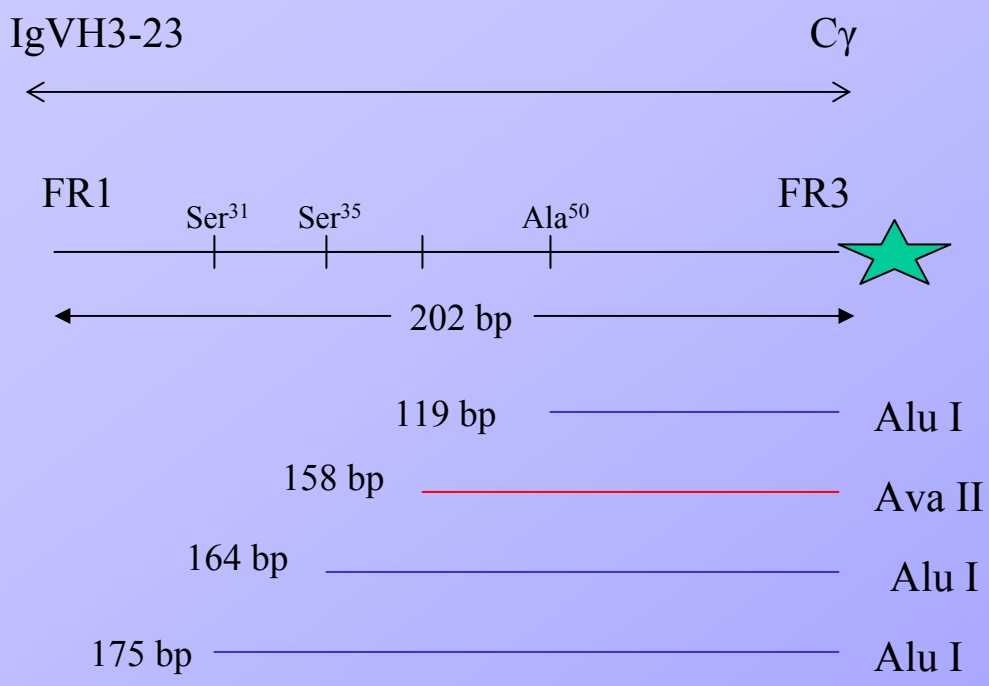
Aim

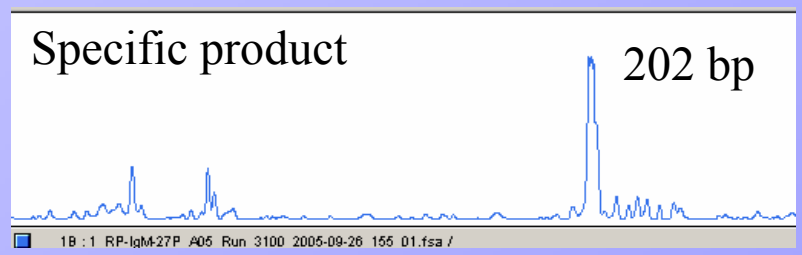
- Molecular methodology to develop CVID classification to a molecular level
- Investigation of the pattern and frequency of somatic hypermutation in B cell subgroups as defined by CD27 expression
- Restriction enzyme-based hot-spot mutation assay (REHMA): screening test to detect the presence of SHM in B cells of CVID patients
- To allow improved characterisation, prognosis and management of CVID patients

- 3 CLL patients
- 9 healthy controls
- 10 CVID patients

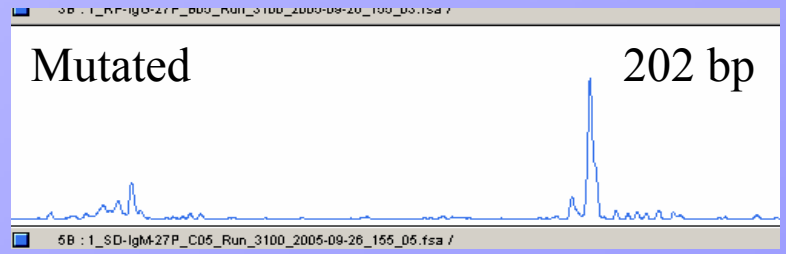


Ig Heavy Chain SHM Analysis

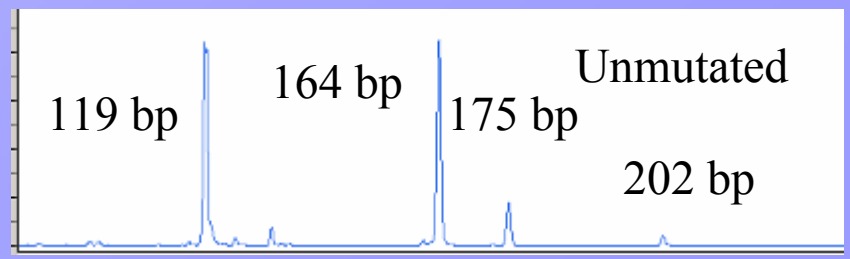




2nd round PCR product



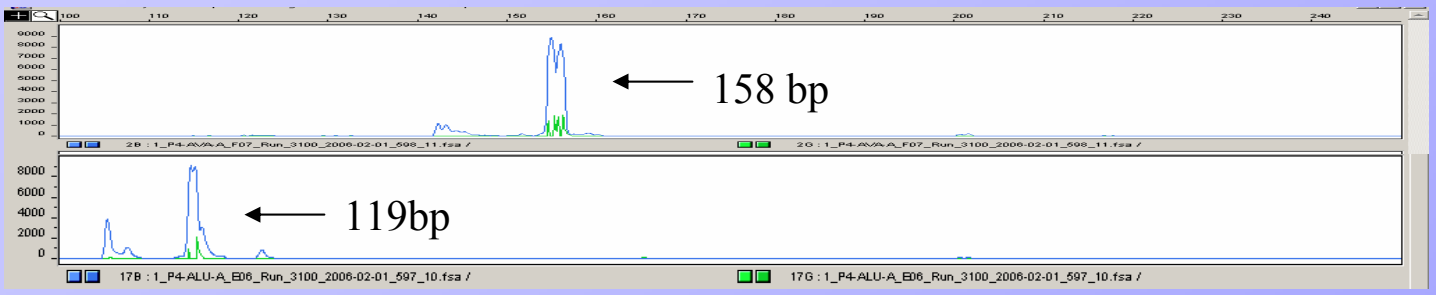
Alu I RE digest



Alu I RE digest

RE digestion of CLL patients

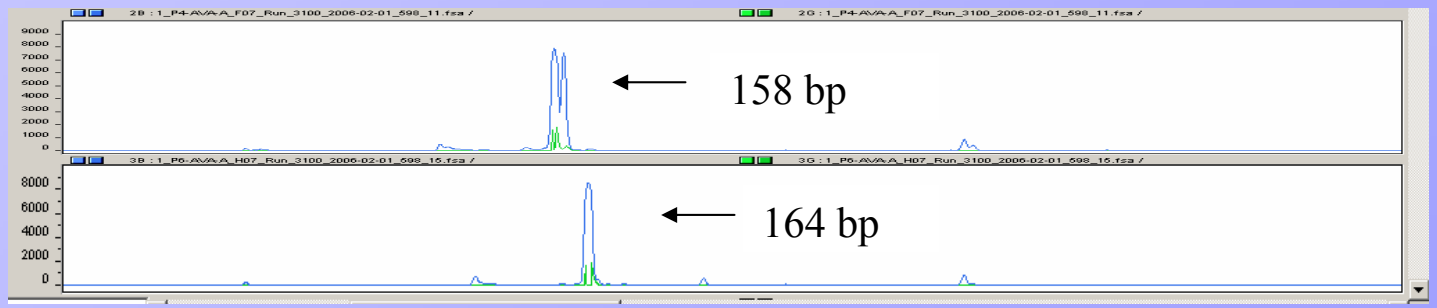
1



Ava II

Alu I

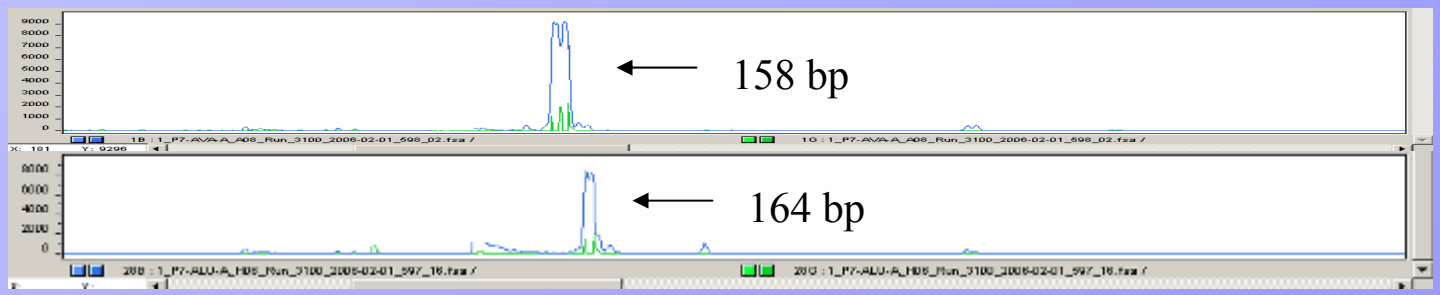
2



Ava II

Alu I

3



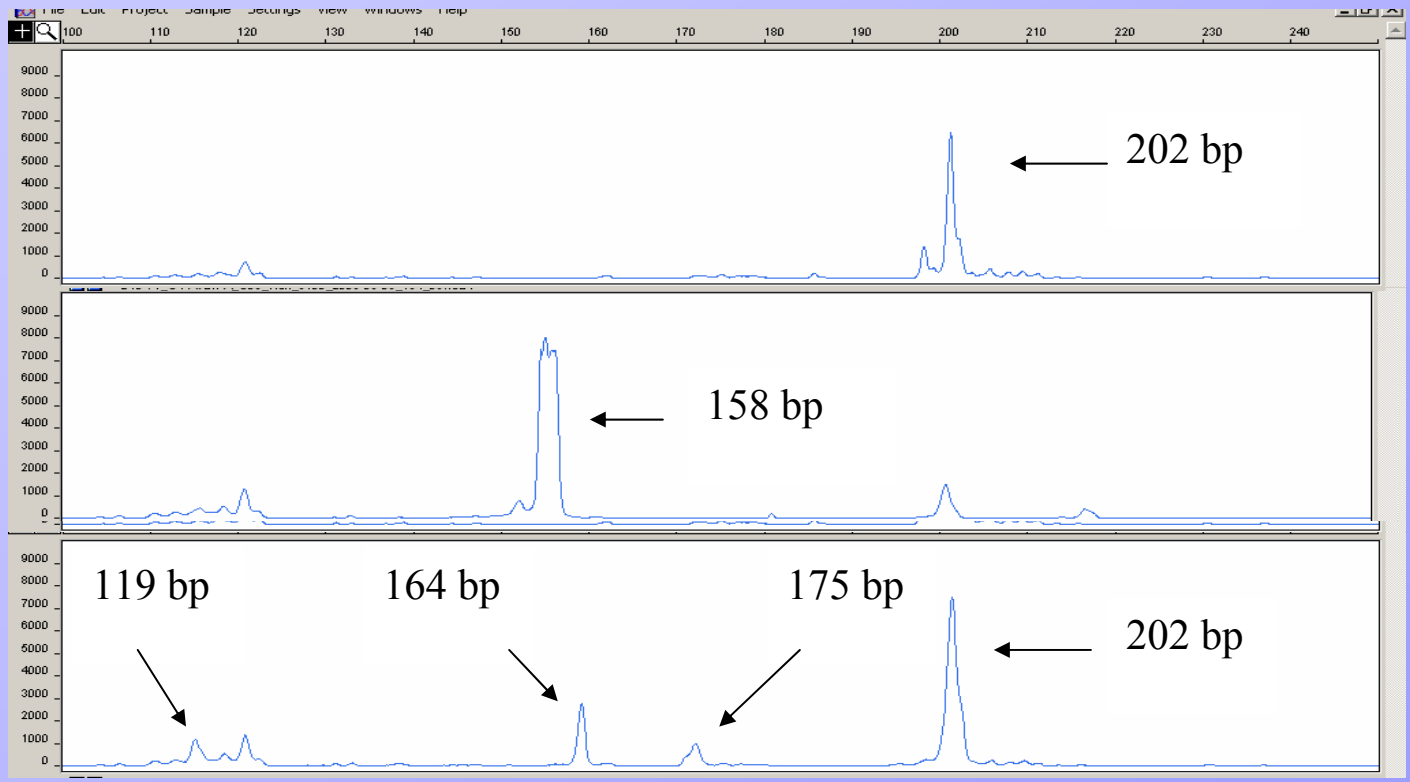
Ava II

Alu I

Ala^{50} Ser^{35} Ser^{31}

Hot spots

RE digestion of a healthy control



Specific product

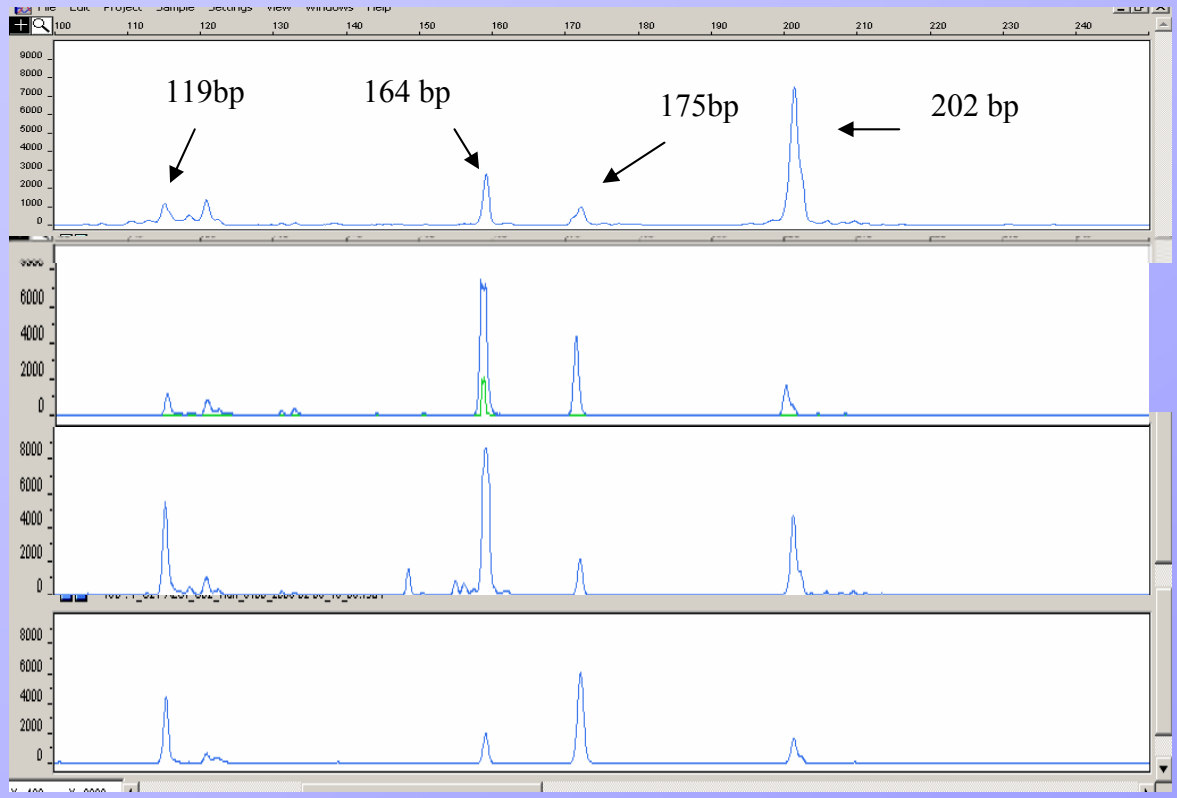
Ava II

Alu I

Ala^{50} Ser^{35} Ser^{31}

Hot spots

Alu1 digestion of MB0 CVID patients



Control

CVID 1

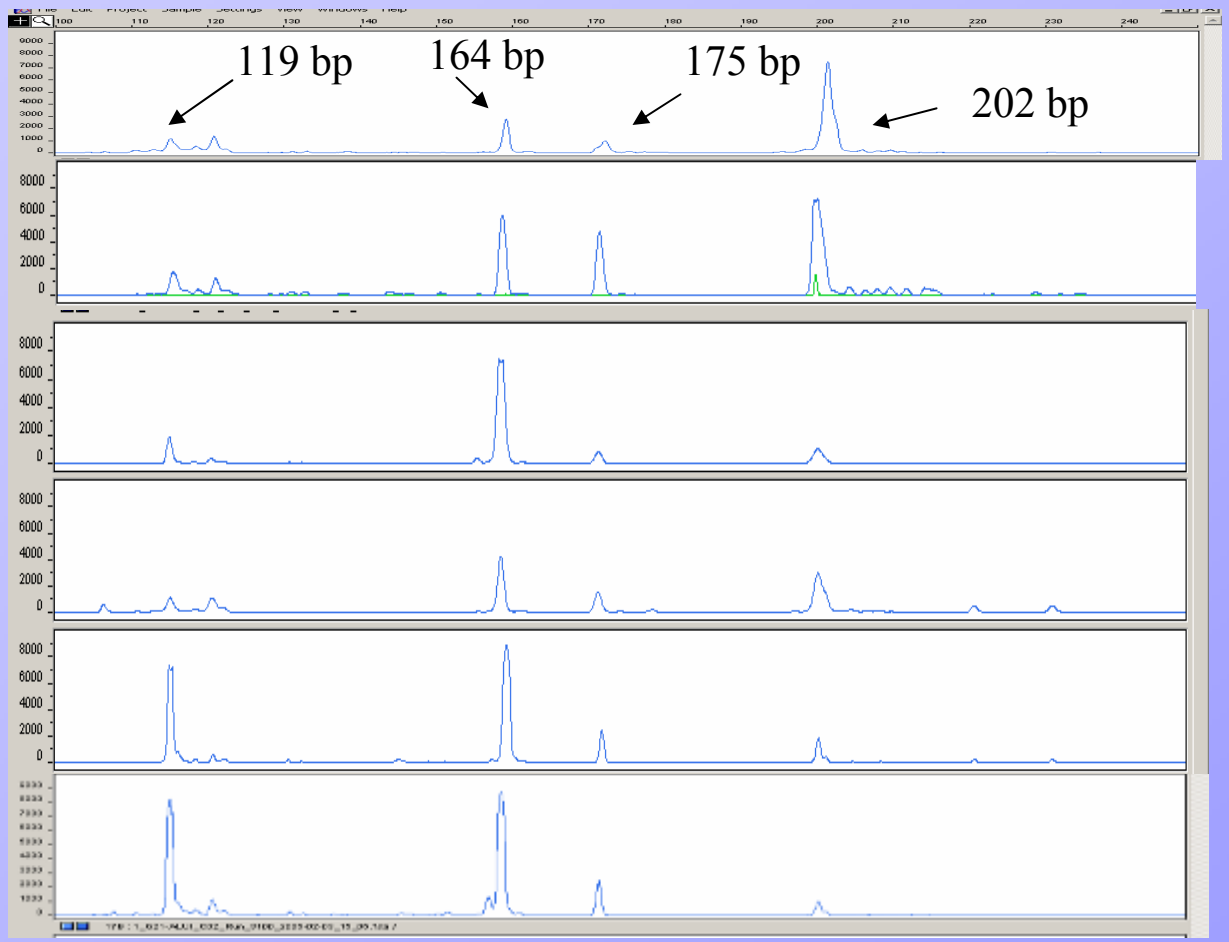
CVID 2

CVID 3

Ala^{50} Ser^{35} Ser^{31}

Hot spots

Alu1 digestion of MB1 CVID patients



Control

CVID 4

CVID 5

CVID 6

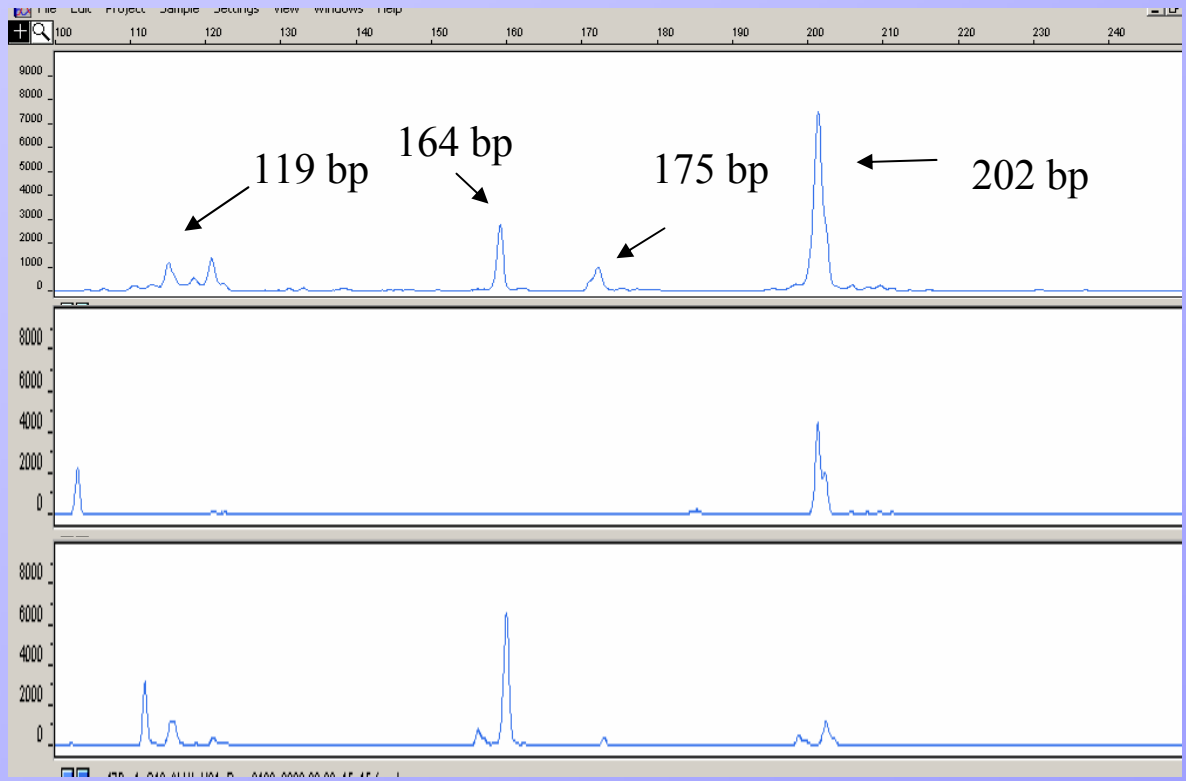
CVID 7

CVID 8

Ala^{50} Ser^{35} Ser^{31}

Hot spots

Alu1 digestion of MB2 CVID patients



Control

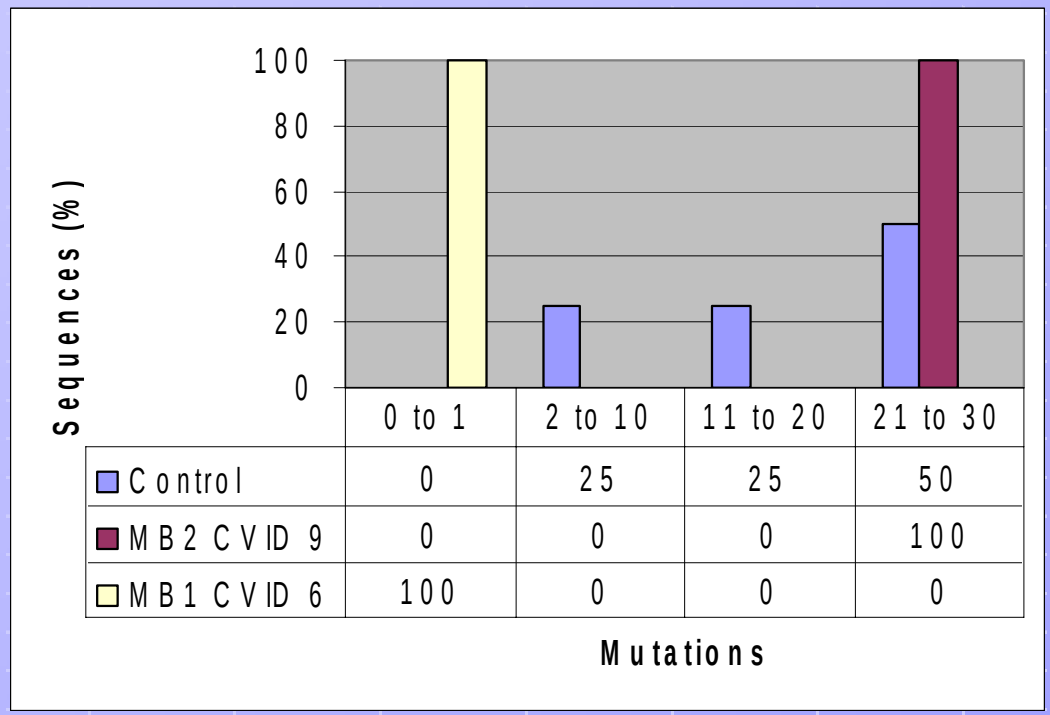
CVID 9

CVID 10

Ala^{50} Ser^{35} Ser^{31}

Hot spots

Mutational status by sequence analysis



Percentages of sequences with 0-1, 2-10, 11-20 and 21-30 mutations from a healthy control, an MB2 patient & an MB1 patient

Summary

- Rapid, non-radioactive screening method to look at SHM in CVID
- REHMA confirmed by sequence analysis
- REHMA patterns heterogeneous in the CVID subgroups defined by phenotype
- Correlation to clinical disease
- Investigation of IgM IgVH3-23 transcripts
- Use of PBMC

Acknowledgments

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