# ASSOCIATION BETWEEN HUMAN LEUKOCYTE ANTIGEN HLA-B*13:01 AND DAPSONE-INDUCED HYPERSENSITIVITY REACTIONS IN NEPALESE LEPROSY PATIENTS

THE LEPROSY MISSION NEPAL

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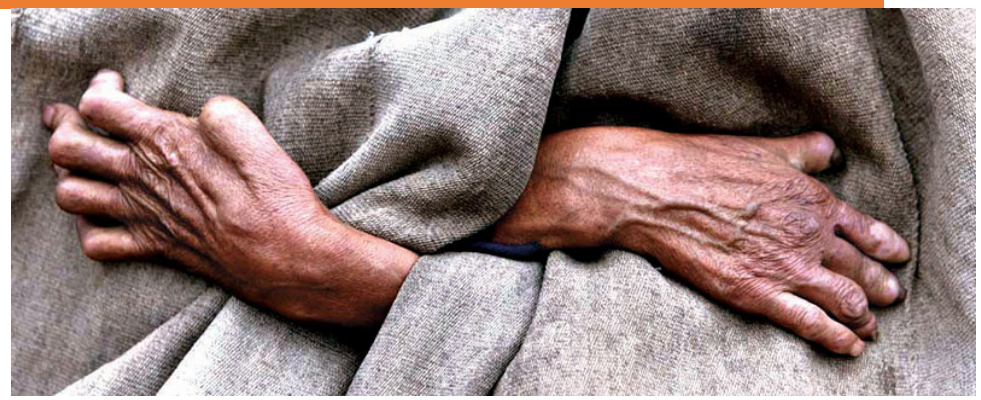
# Background and Objectives

- Leprosy: *Mycobacterium leprae* and *Mycobacterium lepromatosis*
- There are about 200,000 cases world-wide and 2-3,000 cases in Nepal annually
- The WHO-recommended treatment is MDT/multi-drug therapy (dapsone, rifampicin, and clofazimine).




Among these three drugs, dapsone has the highest potential for adverse drug reactions

- Adverse drug reactions (ADR) are **unintended** side-effects of drugs.
- ADRs account for up to 25% of outpatients (worldallergy.org).

**Adverse Drug Reaction**

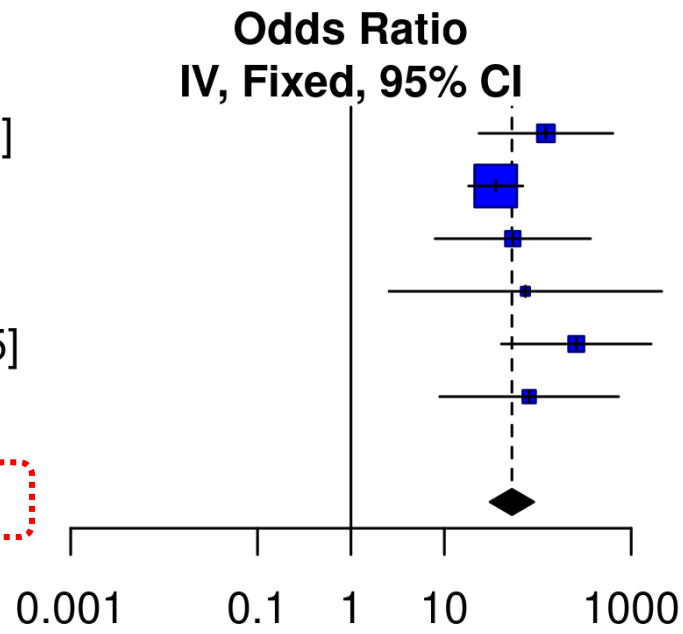
| Type I/ On-target | Type II/Off-target |
| --- | --- |
| > account 80% of ADRs<br>> Predictable by pharmacology | > account 20% of ADRs<br>> Non-predictable by pharmacology |

Not "IDIOSYNCRATIC" since 2000s
Associated with human leukocyte antigen

# HLA-B*13:01 and DHS Association (meta-analysis)

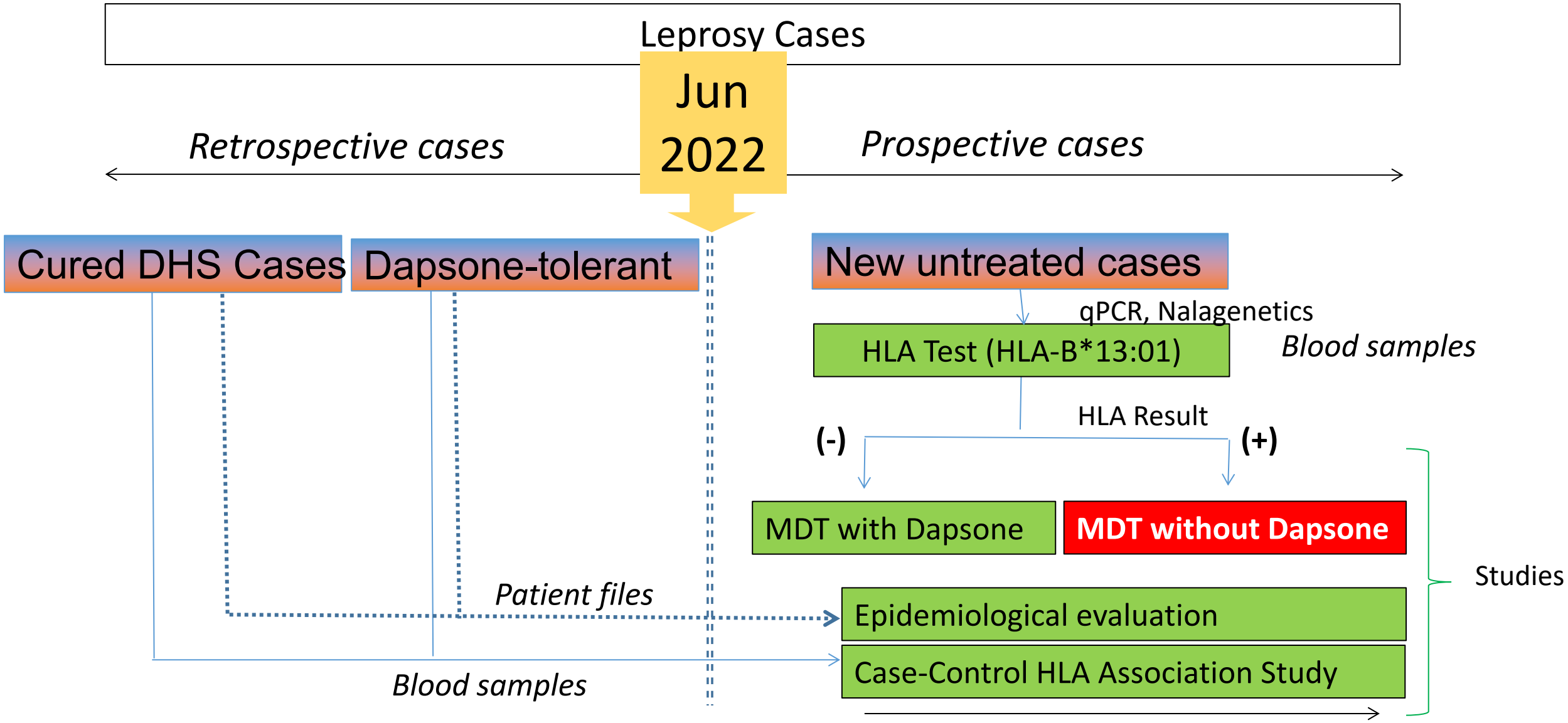
| Study | Experimental Events | Experimental Total | Control Events | Control Total | Weight | Odds Ratio IV, Fixed, 95% CI |
|---|---|---|---|---|---|---|
| Wang 2013 | 18 | 20 | 7 | 102 | 10.6% | 122.14 [23.45; 636.16] |
| Zhang 2013 | 65 | 76 | 119 | 833 | 64.6% | 35.45 [18.18; 69.14] |
| Tempark 2017 | 12 | 15 | 2 | 29 | 7.9% | 54.00 [ 7.96; 366.15] |
| Park 2020 | 6 | 7 | 0 | 8 | 2.6% | 73.67 [ 2.56; 2119.93] |
| Hana 2020 | 31 | 34 | 2 | 52 | 8.5% | 258.33 [40.85; 1633.85] |
| Chiramel 2019 | 7 | 8 | 9 | 113 | 5.9% | 80.89 [ 8.93; 732.35] |
| **Total (95% CI)** | | **160** | | **1137** | **100.0%** | **52.88 [30.92; 90.45]** |

Heterogeneity: $Tau^2 = 0.2592$; $Chi^2 = 5.39$, df = 5 ($P = 0.37$); $I^2 = 7\%$

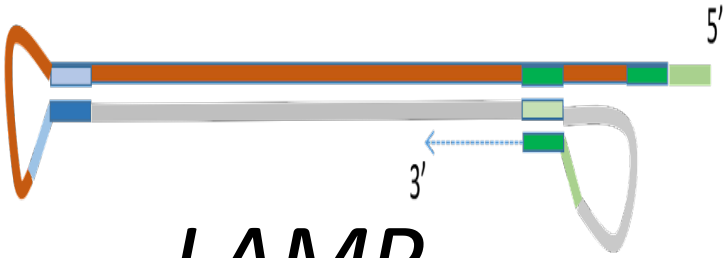



As in other drug allergy scenarios, person bearing a specific HLA (HLA-B*13:01) are more likely to suffer from dapsone allergy

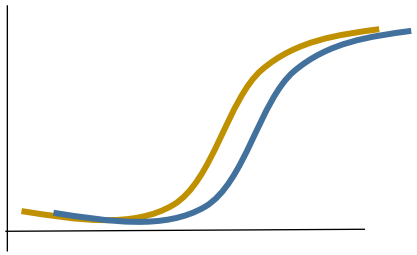
# Methodology



# Methodologies: wet lab

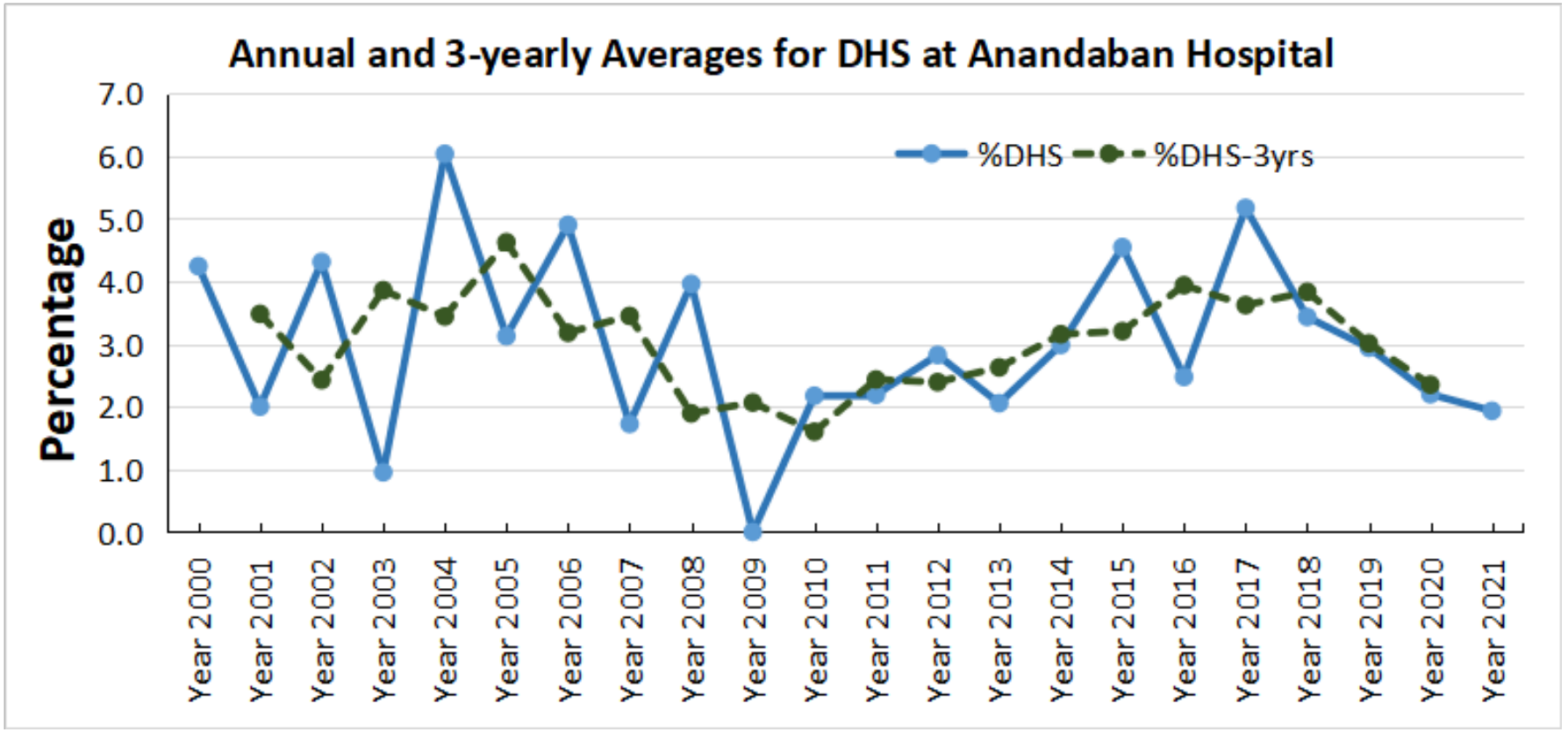

LAMP



## Lab Experiments

- Routine HLA Screening
  - *commercial Kit (Nalagenetics)*
  - *qPCR*
- Research Developments
  - *duplex endpoint PCR*
  - *LAMP*
- Co-infections
  - *HHV-5 and 6 tested (qPCR)*

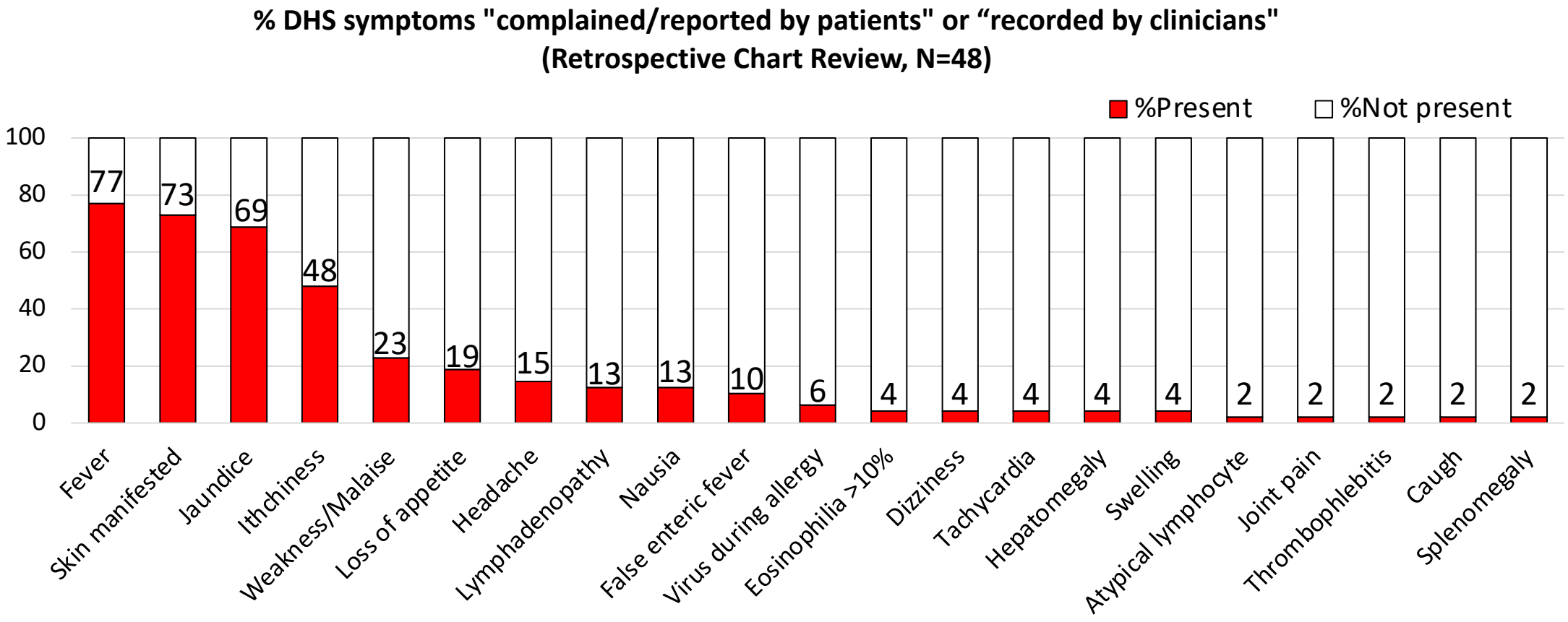
# Results: Chart review Data: 2000-2021



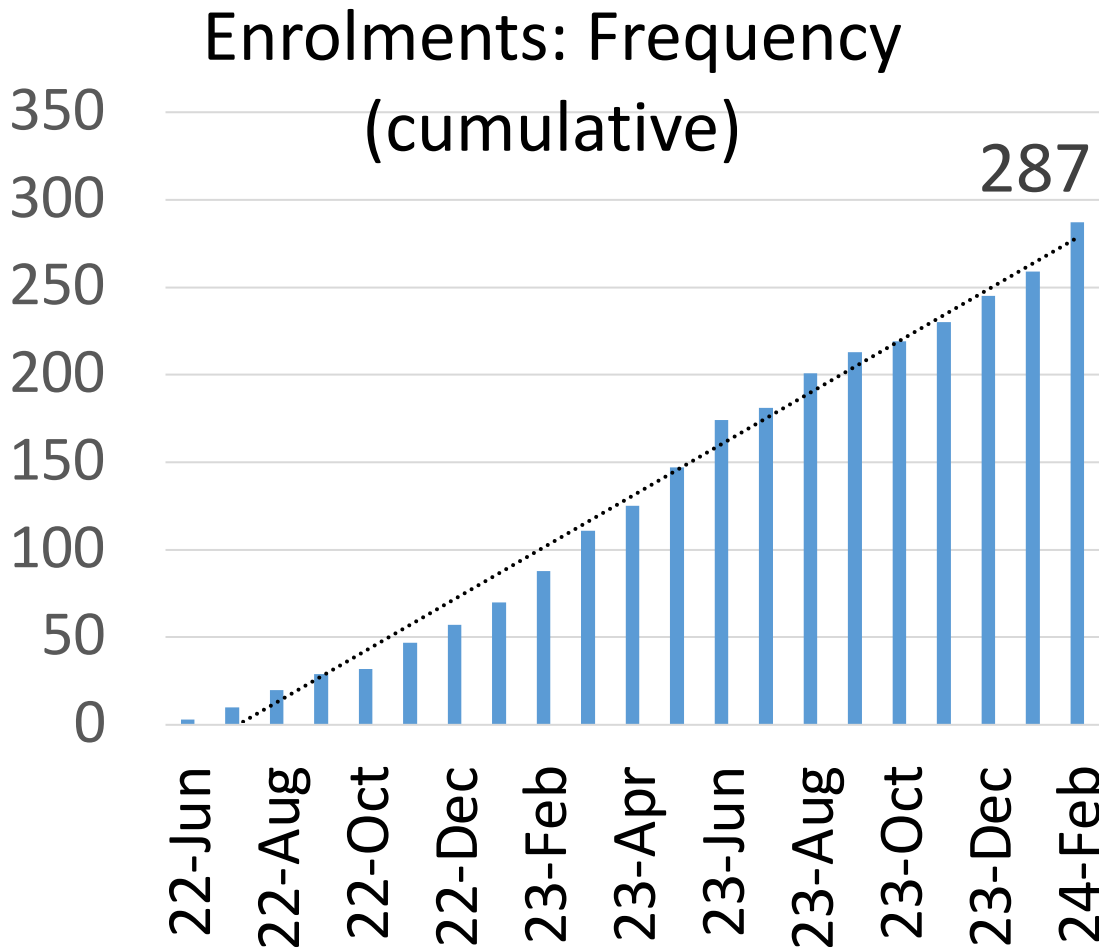

- Retrospective analyses of all newly diagnosed patients show ~3% of our patients suffer from some reasons to discontinue dapsone (inkling DHS, anemia, etc.)
- Previous study from Anandaban (Pandey 2007) showed 2%

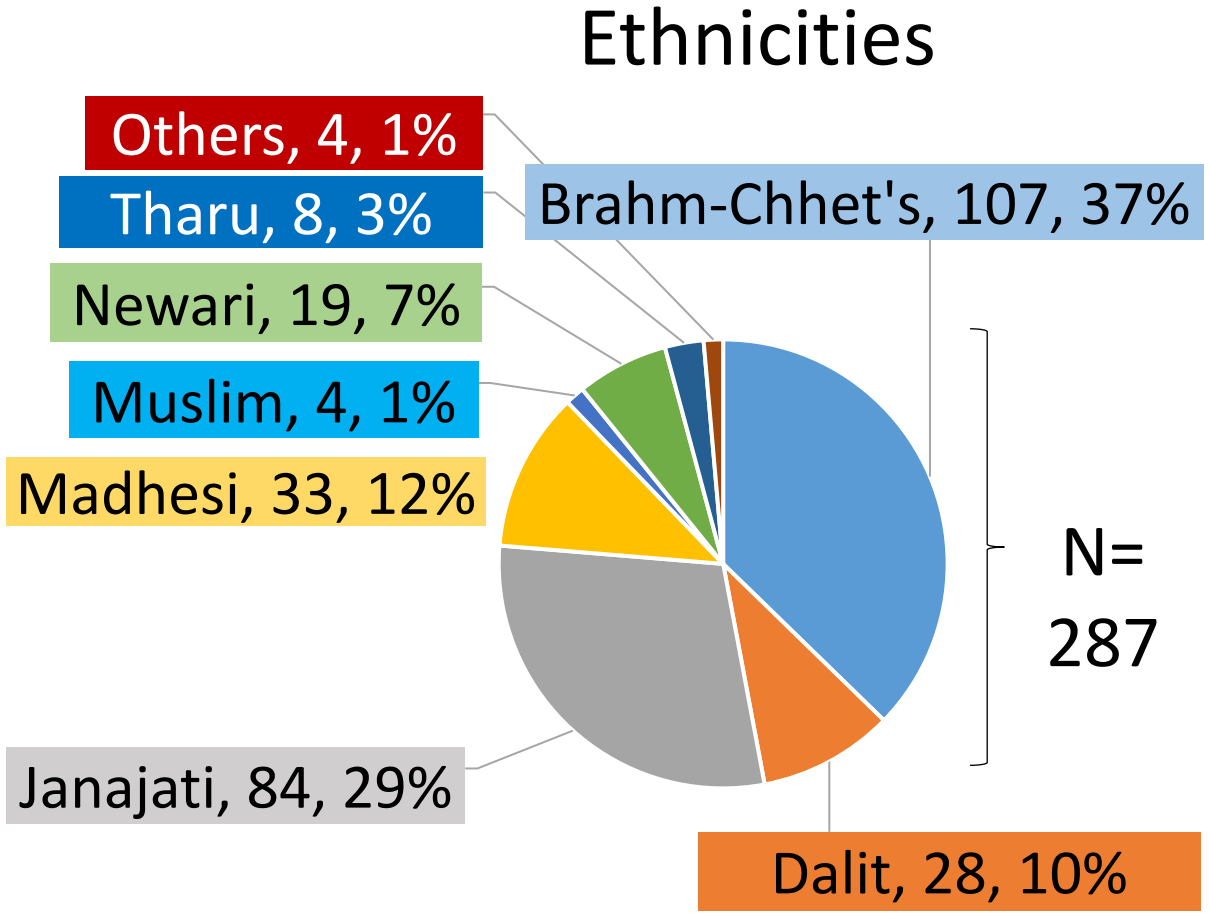
Pandey B. Trop Doct. 2007 PMID: 17716505 | Sapkota BR. Lepr Rev. 2008 PMID: 19274989.

# Symptom profile: Determined in this study

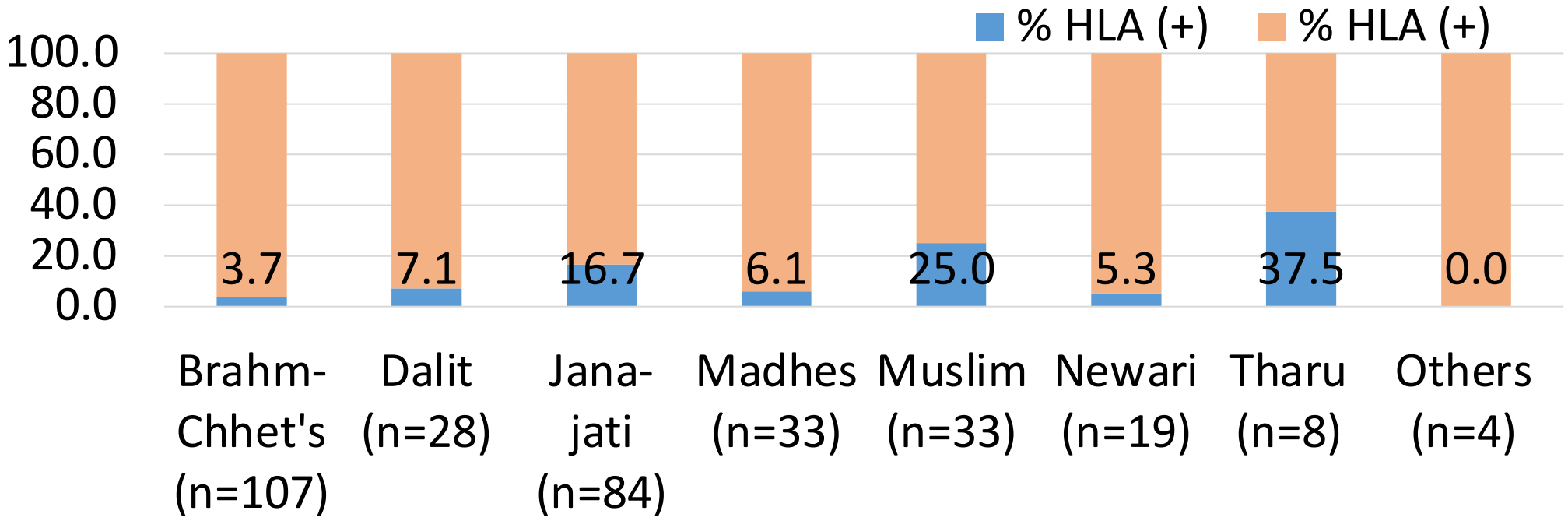


# Status of New case enrollment (until Feb 2024



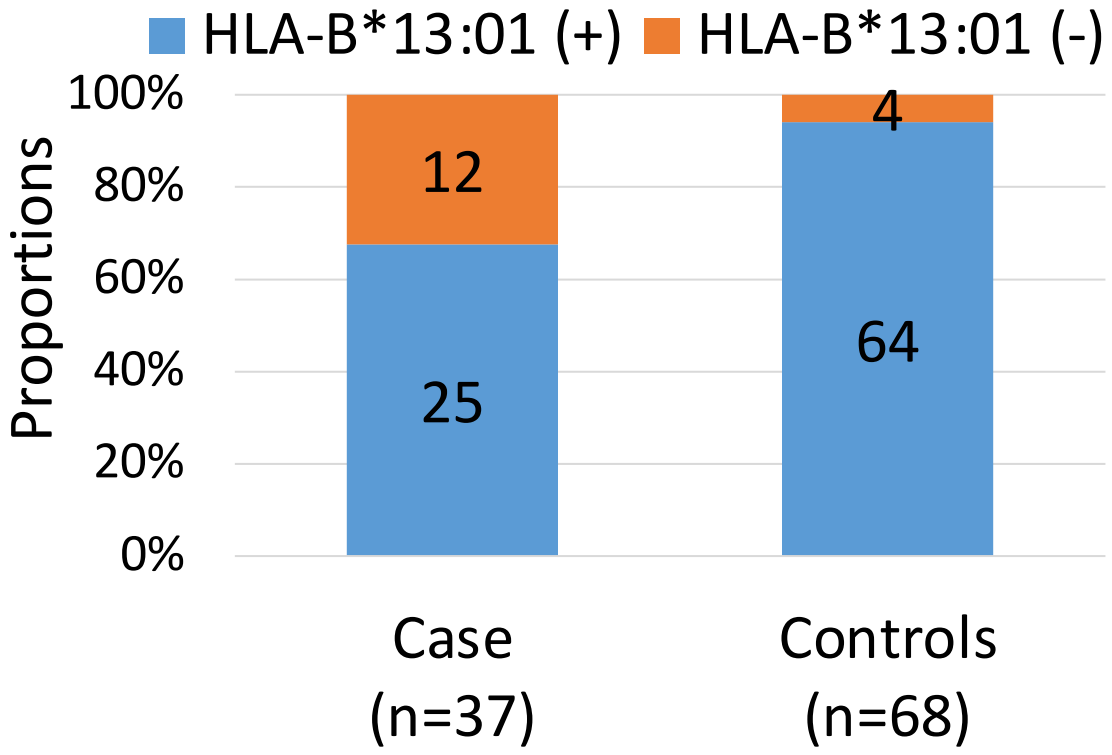
# HLA-B*13:01 positivity in our new cases: 9.2%




- Tharus have highest prevalence, ~38%. Tharus residing in Southern belt of Nepal and Northern India may have Ancient Tibeto-Burman or Ancient Austro-Asian influence.
- Janajatis (~17% prevalence) (comprising Tamang and Gurungs in majority) have obvious Ancient Tibeto-Burman ancestry
- Brahmins/Chhetris have 4% prevalence, are related to Indian Brahmins and have Ancient North Indian influence

Basu A et al. Proc Natl Acad Sci U S A. 2016 PMID: 26811443 | Cole AM. BMC Genomics. 2017 PMID: 28103797

# Genotype-phenotype association and stat parameters



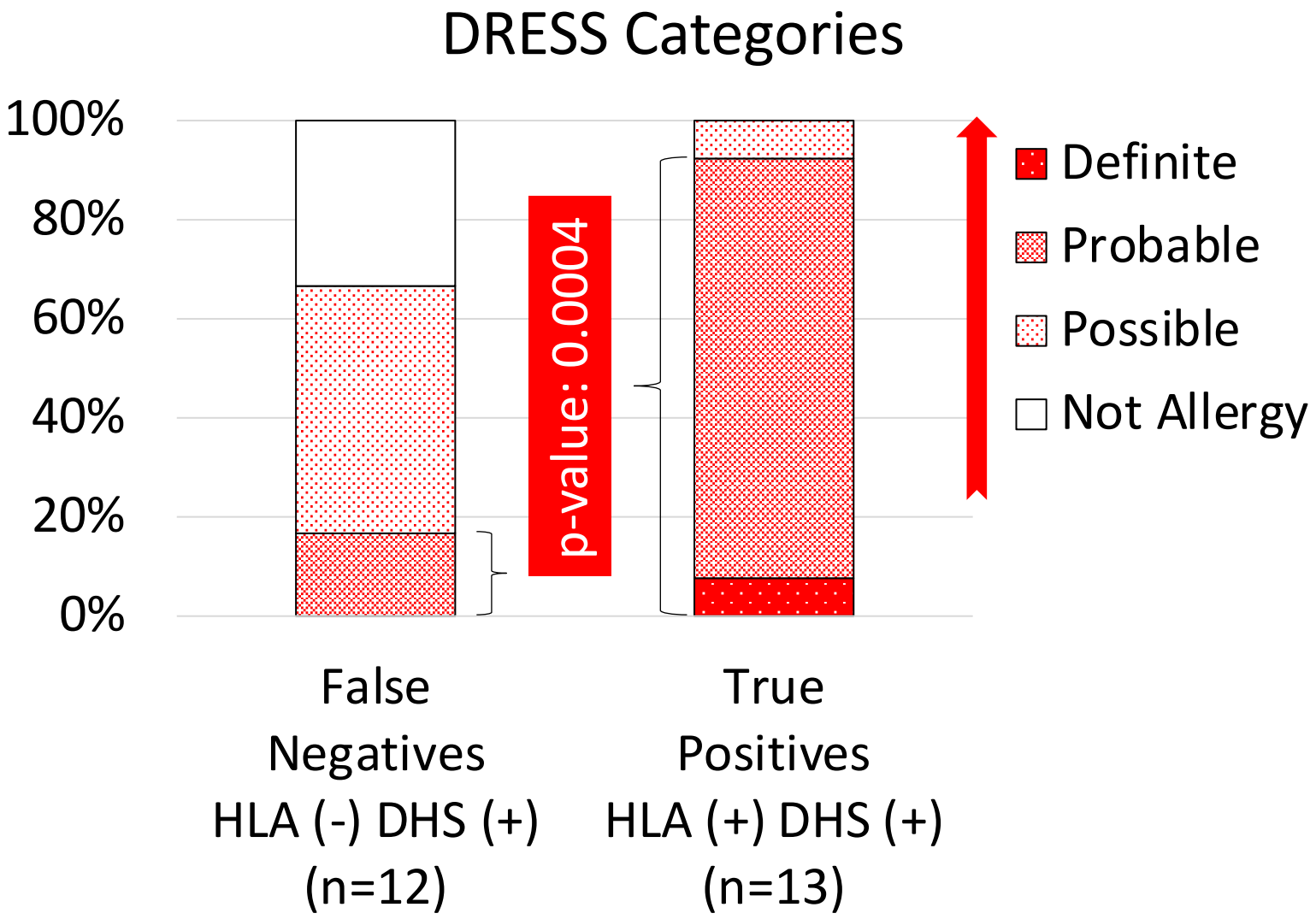

| Parameters | Values |
|---|---|
| Sensitivity (among cases, n=19) | 67.5% |
| Specificity (among controls, n=50) | 94.1% |
| DHS Incidence (Retrospective data) | 2.5% |
| Positive Predictive Value | 22.8% |
| HLA-B*13:01 Prevalence in New | 9.4% |

Odds Ratio for association: 33.3 (95% CI: 9.8 to 113.2).

*Odds Ratio by meta-analysis: 43-53*

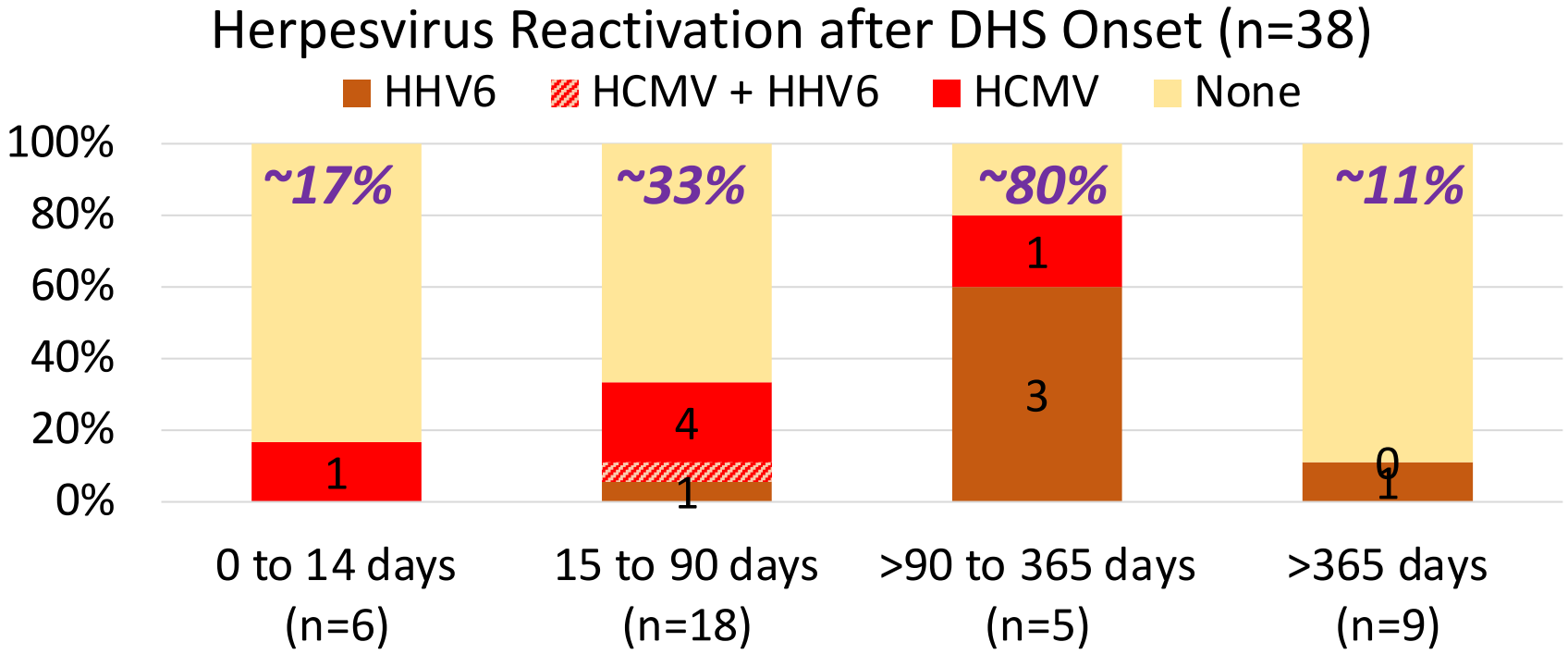
*(next slide for possible reasons)*

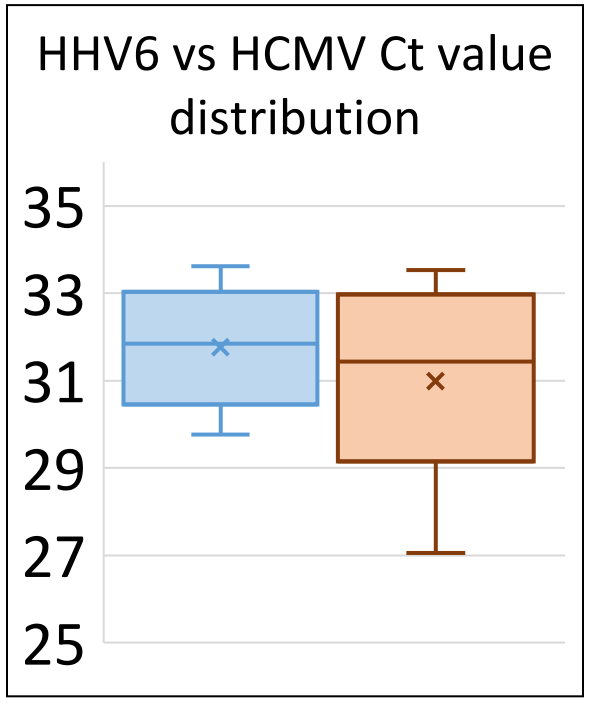
# Why False Negatives?




- As 32% of enrolled DHS cases were HLA-negative (**FALSE NEGATIVE**), we doubted some of the cases could be just “Dapsone intolerant” and not DHS in true sense (**TRUE POSITIVE**)
- We used RegiSCAR DRESS (Kardaun 2013) criteria to score the DHS cases
- 92% of **true positives** were classified as probable or definite allergic compared to only 17% of the **false negative** cases

# Herpesvirus Reactivation Scenario (qPCR)



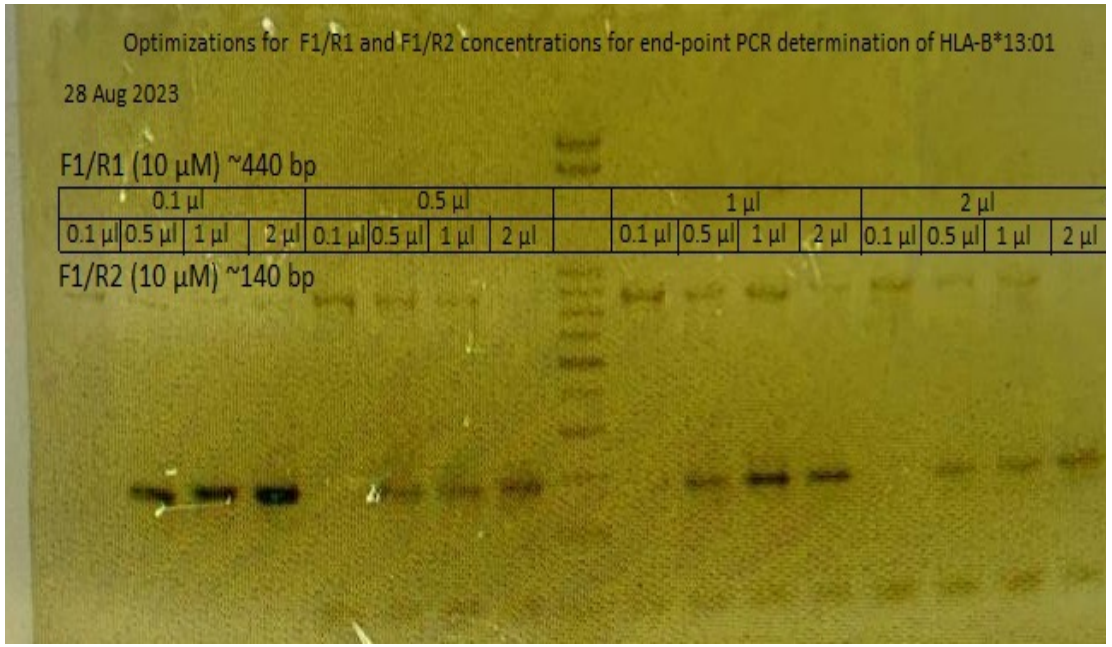



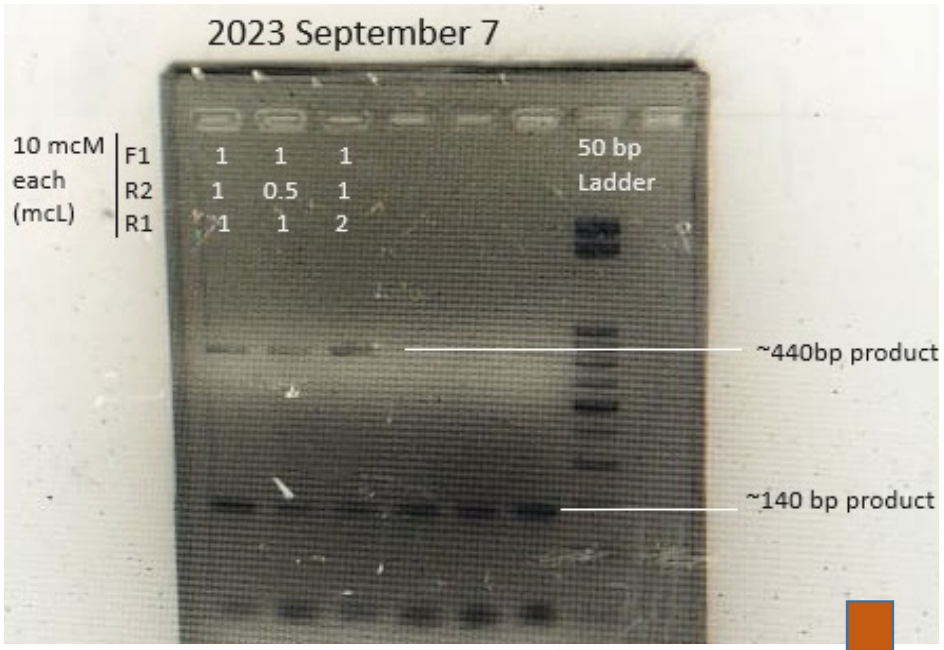

- Japanese Consensus group on drug-induced hypersensitivity use HHV6 reactivation as a distinguishing criteria.
- HCMV is recognized as severity factor in drug-induced hypersensitivities.
- At least (tested) HHV-5 (CMV) and HHV-6 (Roseola) infections recorded

Shiohara T. Allergol Int. 2019 PMID: 31000444.| Mizukawa Y. J Am Acad Dermatol. 2019 PMID: 30240780.

# Developing/Optimizing an endpoint PCR

- We are developing/optimizing of a new indigenous endpoint duplex-PCR that can help make the test affordable in poor communities like Nepal



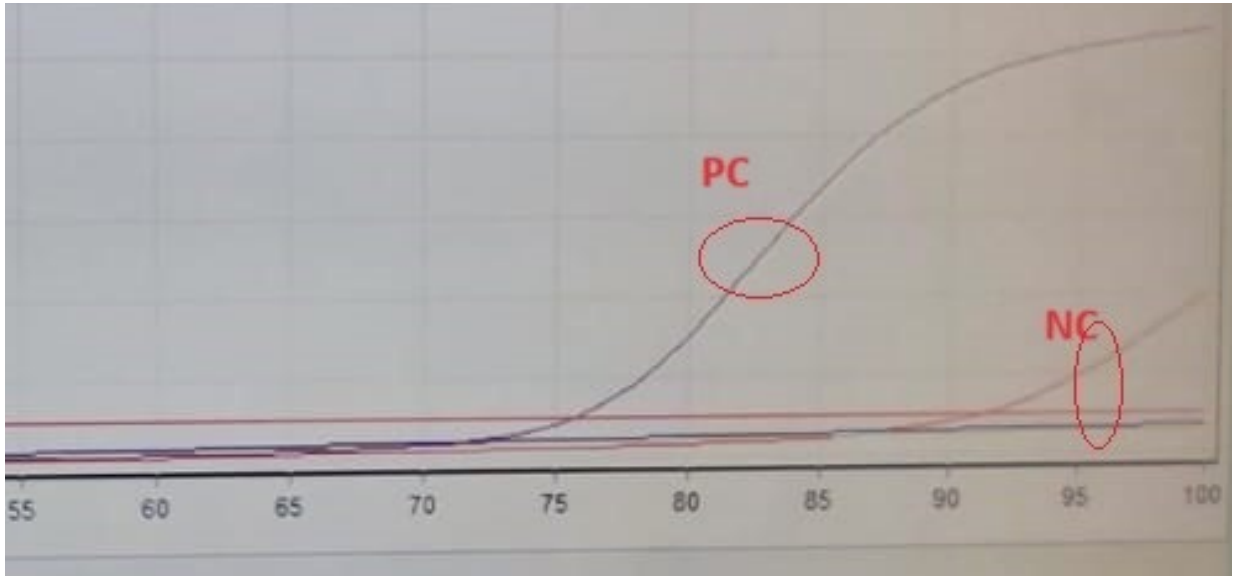

| | Commercial Standard qPCR | | | |
|---|---|---|---|---|
| Endpoint PCR | | Pos | Neg | Total |
| | Pos | 5 | 0 | 5 |
| | Neg | 0 | 20 | 20 |
| | Total | 5 | 20 | 25 |

Validation at work!

Sensitivity: 100%
Specificity: 100%

# Developing/Optimizing a LAMP technique

- Loop-mediated isothermal amplification (LAMP) is a relatively new point-of-care technique that can be done with water bath or heat block. We are optimizing the method with positive results.




| | Commercial Standard qPCR | | |
|---|---|---|---|
| LAMP | Pos | Neg | Total |
| Pos | 2 | 0 | 2 |
| Neg | 0 | 20 | 20 |
| Total | 2 | 20 | 22 |

Sensitivity: 100% Specificity: 100%

- Thanks Eiken Chemicals for free software for LAMP primer design
- Eiken, also original developers of LAMP

Validation at work!

# Acknowledgement and Funding


- The study was possible due to kind-hearted leprosy patients who provided consent for blood samples.
- Staffs of Mycobacterial Research Laboratory including Aratee Shrestha, Laxman Khadka, Sabina Khadka, Suman Shrestha, Anuja Mahat, Krishus Nepal and Dr. Bishwanath Sapkota played vital roles in recruiting and collecting samples.
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The Leprosy Mission International

# intro

- Mr. Divya RSJB Rana hails from the commercial city of Birgunj and pursued MSc in China as scholarship student
- He has worked more than 10 years at Mycobacterial Research Laboratory, Anandaban Hospital, The Leprosy Mission Nepal