Retrospective Genetic Analysis of Efficacy and Adverse Events in a Rheumatoid Arthritis Population Treated with Methotrexate and Anti-TNF-α

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Background

- Progress has been made in the treatment of rheumatoid arthritis (RA) but there remain a large number of patients who do not respond to therapy and/or experience drug-related adverse events (AEs).

- Literature presents many examples of association between gene polymorphisms and severity of disease, however, very little is known about genetic markers of efficacy or AEs

Importance of Genetic Biomarkers

- New tharapies present lack of efficacy or drug-related adverse events

  - Example: Infliximab (anti-TNF-alpha agent) showed a 25% dropoff in use after 2 years (Stem and Wolfe 2004), implying that a large number of patients would benefit from different or earlier and more aggressive therapy

- RA is a slowly-progressing disease

  - clinical trials last several months

  - substantial costs needed for evaluation of new therapeutic agents

  Use of genetic biomarkers results in more efficient clinical trials and cost savings

  - could be used to stratify/enrich clinical trial populations

  - used as covariates for analysis of therapeutic outcome data

  - used as covariates in the analysis of dynamic biomarkers

Objective

To identify genetic markers associated with efficacy and predisposition to adverse events during methotrexate (MTX) therapy or TNF-a blockade

Subjects

- the study cohort was selected from a large RA patient registry
- medication history, including current therapeutic regimen, was collected using a standardized self-report questionnaire

Table 1: Sample Size and Demographic Features

	Cases
Sample Size	346
Catchment Area	Boston, MA
Mean Age (Range)	58 (22-88)
Percent Female	84%
Osteoarthritis	108
Smoking (Ever)	154

Subjects

- RA registry patients were recruited at a major metropolitan rheumatology clinic and phenotyped using ACR diagnostic criteria
- All studies carried out using IRB-approved informed consent, questionnaire, and biological sampling protocols
- All individuals studied, self-described as being of European Caucasian descent

Subjects (cont.)

- **Non-responders**

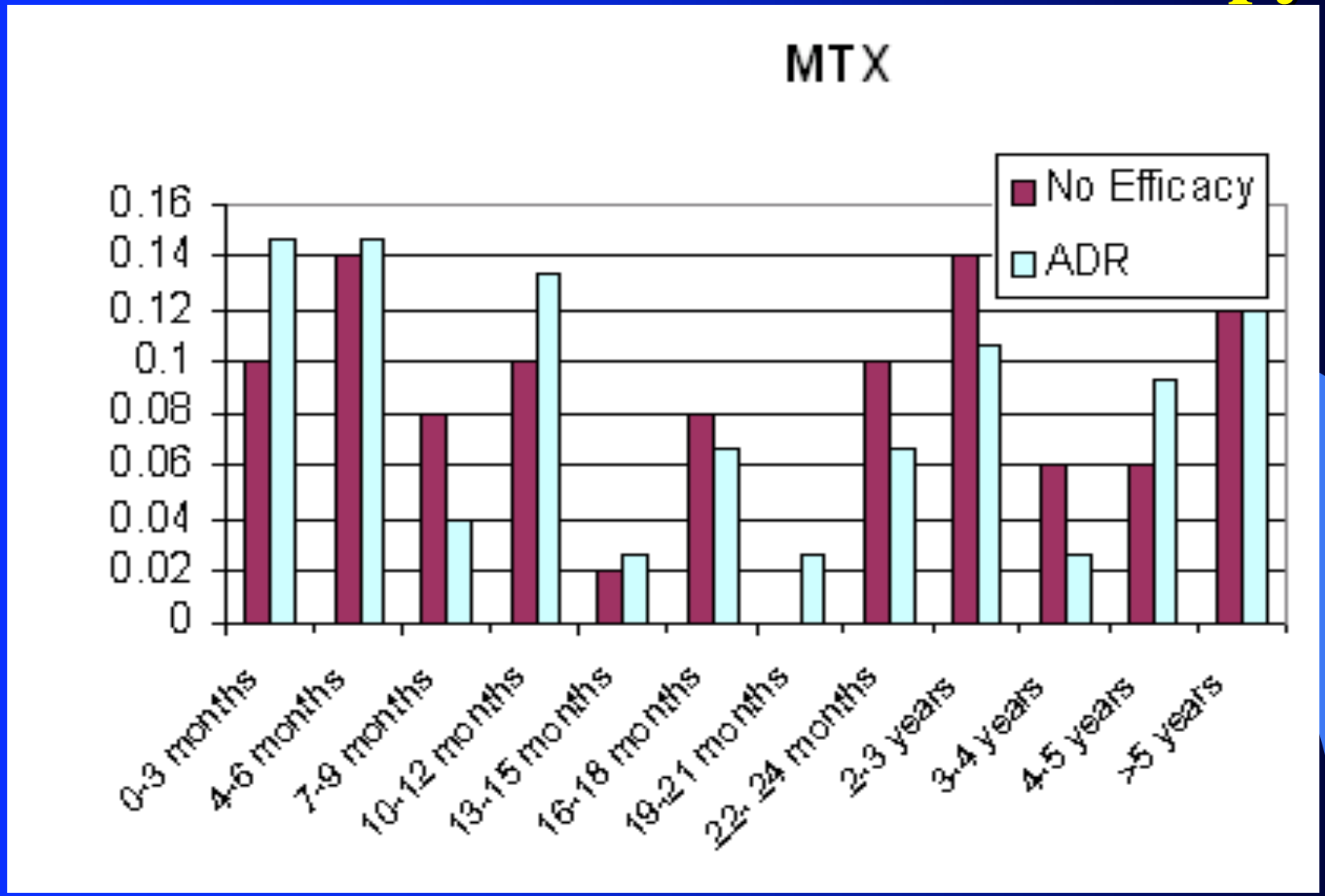
  - patients who discontinued therapy due to no efficacy after 3 to 18 months (MTX; N=21) or 1 to 18 months (anti-TNF-a; N=17)

  - an overview of the reasons given for discontinuing therapy across the entire patient cohort is shown in Figure 1

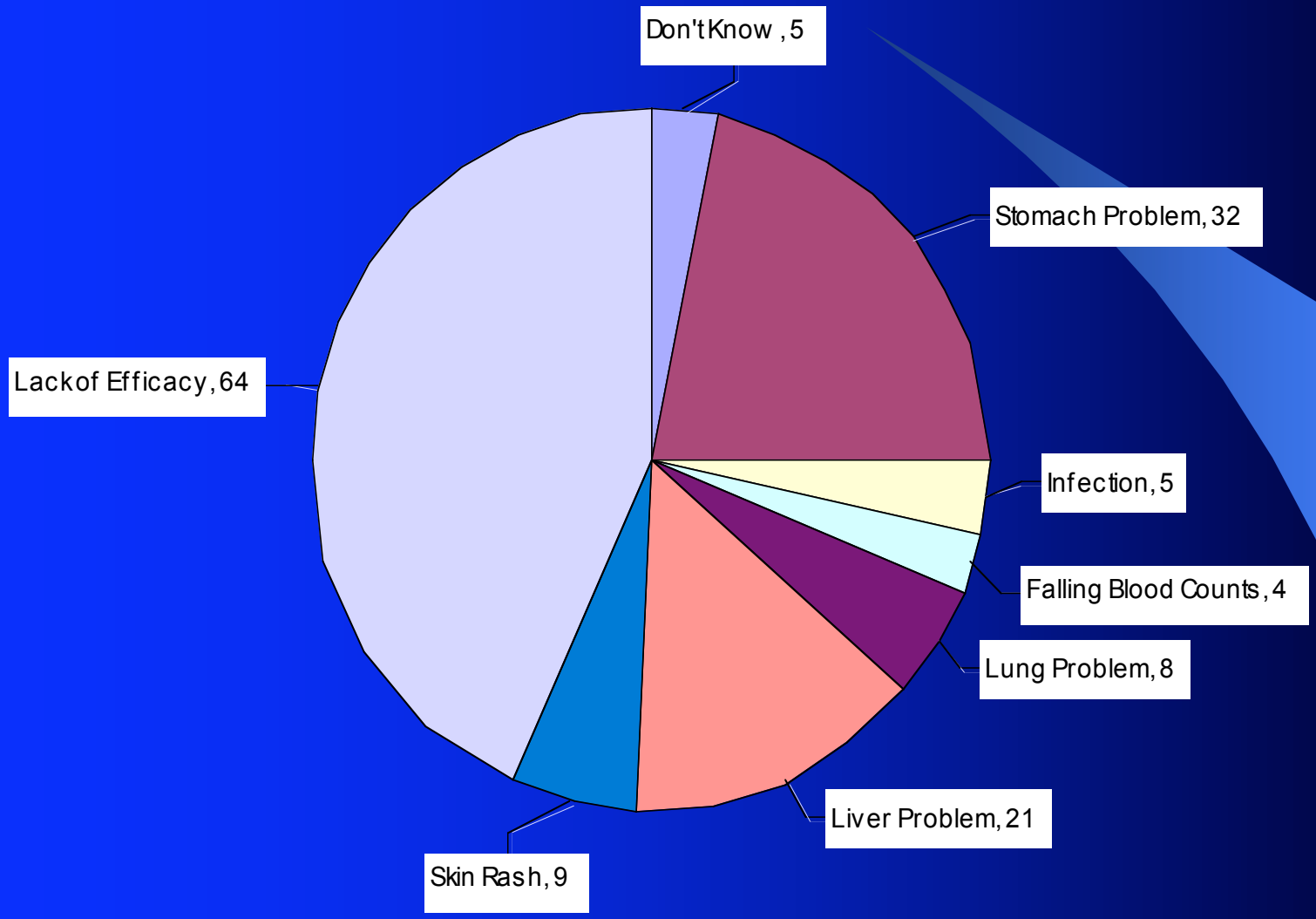
- **Controls**

  - currently treated patients who have been on therapy for at least 3 months (MTX; N=104, all anti-TNF-a naive) or 1 month (anti-TNF-a; N=124)

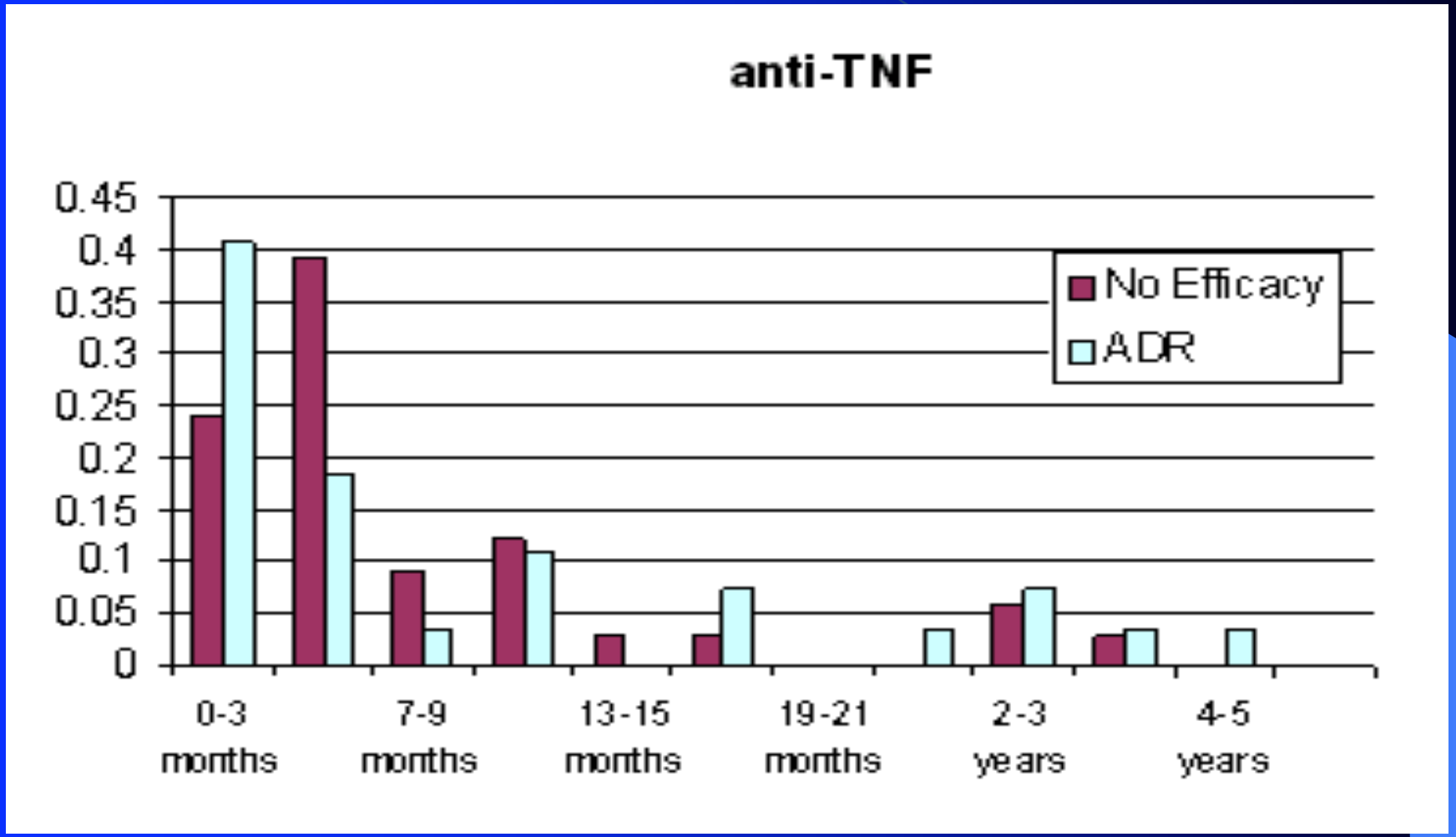
Length of Exposure Prior to Discontinuation of MTX Therapy



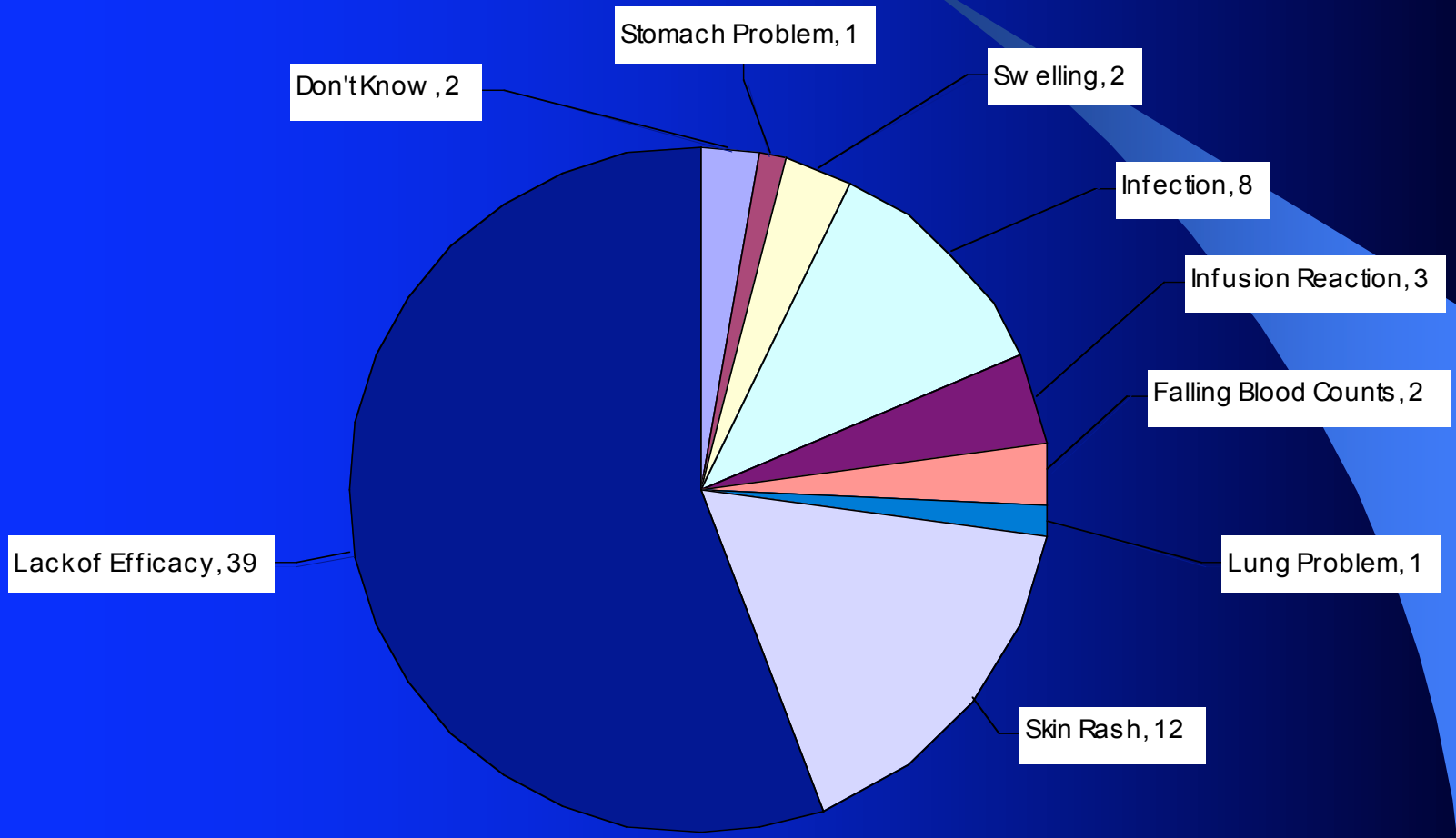
Reasons for Discontinuation of MTX Therapy



Length of Exposure Prior to Discontinuation of anti-TNF Therapy



Reasons for Discontinuation of anti-TNF Therapy



Subjects (cont.)

- **AE cases**

- patients who reported discontinuing therapy due to any AE (MTX, N = 64; anti-TNF-a, N = 19)

➢ **severe AEs** (liver or pulmonary toxicity, anemia, neutropenia, and infections)

➢ **mild AEs** (headaches and alopecia)

- MTX, N = 29; anti-TNF-a, N = 7)

- **controls**

- patients who are currently receiving therapy without reported AEs

  - MTX, N=180, mean exposure 58 months, SD = 64
  - anti-TNF-a, N=132, mean exposure 25 months, SD = 20

Methods

- 31 genetic loci selected (including HLA-DRB1), all implicated in either risk for or severity of RA in at least 2 published studies
- Series of genetic markers, both VNTRs and SNPs, selected to characterize these genes in a recently-recruited RA registry
- Analyses made using contingency tables and multivariate logistic regression techniques

Methods (cont.)

- 60 SNPs, 9VNTRs and the HLA-DRB1 locus were genotyped

  * microsatellite (VNTR) genotyping was carried out using fluorescently-labeled PCR primers and standard capillary electrophoresis protocols (AB 3100)
- SNP genotyping was performed at Genaissance Pharmaceuticals (New Haven, CT) using single-base extension and the Mass Array™ detection platform (Sequenom).
- HLA genotyping was conducted using AS-PCR methods based on those of Kotsch et al. (1999), followed by DNA sequencing where required to resolve SE and D-70 copy number

Methods (cont.)

- All VNTRs were collapsed to two-allele markers following published reports of allele-specific association
- Significance of single marker associations with lack of efficacy or Aes was assessed using Fisher's exact test.
- All markers were evaluated assuming dominance

  - for markers with minor allele frequency greater than 10%, a recessive model was also tested

Evaluation

- Single-marker associations with lack of efficacy or adverse events were evaluated using contingency table analysis
- All markers that exhibited nominally significant evidence for association were included in construction of multimarker models – these used multivariate logistic regression

Results

Table 2: Summary of Results

Phenotype Cohort	Drug Regimen	Locus	P-value
Lack of efficacy	MTX	CTLA4	0.0334
		IL1B	0.0079
		TNF	0.0217
		RUNX1	0.0034
		SLC11A1	0.0084
	TNF	FcGR2A	0.0176
		IL1RN	0.0086
		IL4R	0.0456
Adverse Events	MTX	IL1B	0.0140
	TNF	HLA-DRB1	0.0373
		IFNG	0.0495
		IL3	0.0405
		SLC19A1	0.0432
Severe Adverse Events	MTX	HLA-DRB1	0.0331
		CCR5	0.0077
	TNF	IL3	0.0072
		TNF	0.0148
		IL4R	0.0228
		PADI4	0.0192
		SLC19A1	0.0326
		SLC22A4	0.0496

Table 3: Analysis of Response vs. SE Status

	Responders	Non-Responders
SE+	37	6
SE-	129	19
	Odds Ratio	0.9
	95% C.I.	0.347 - 2.366

Figure 3: Genotype Distributions of Selected Markers

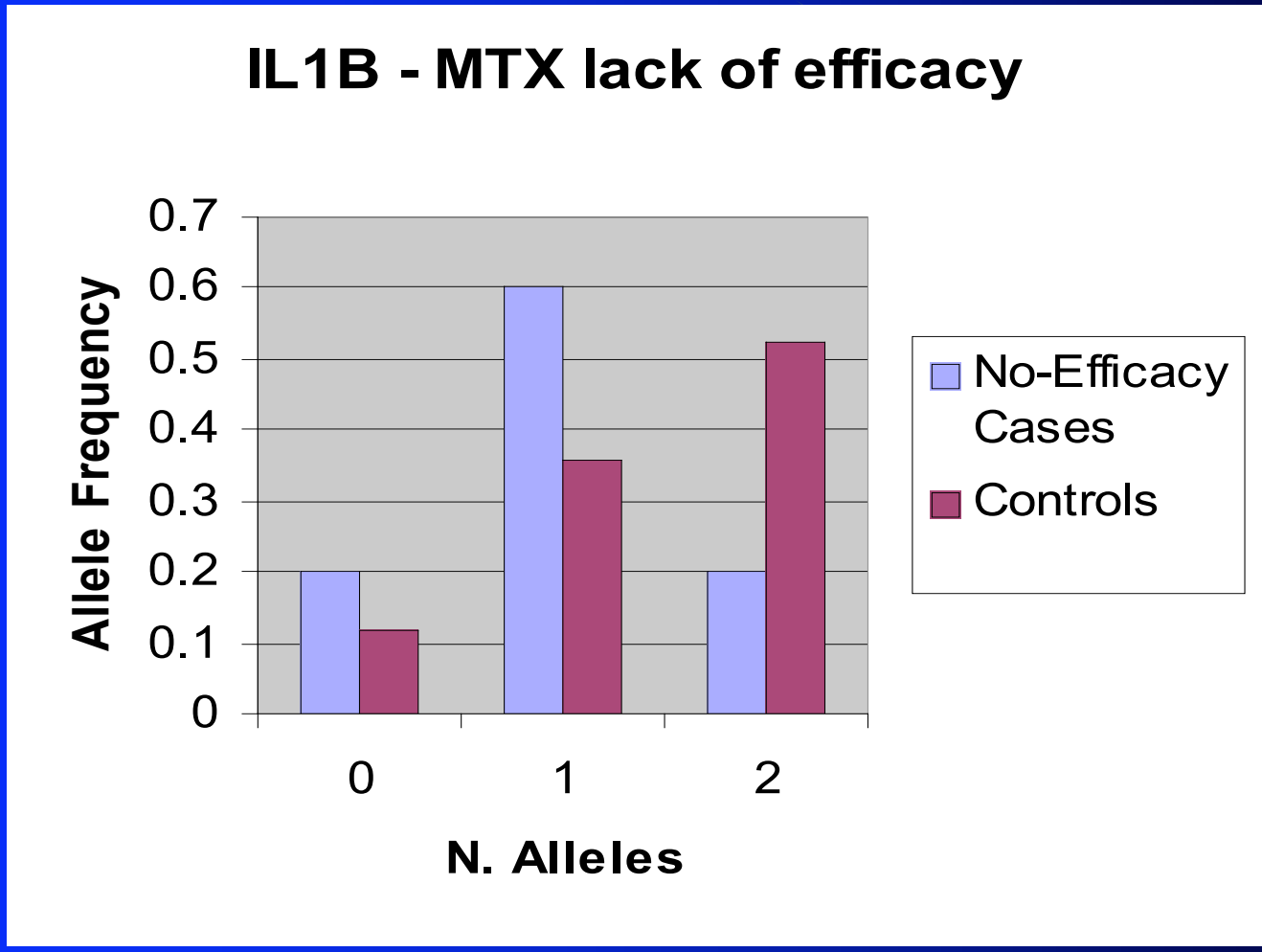
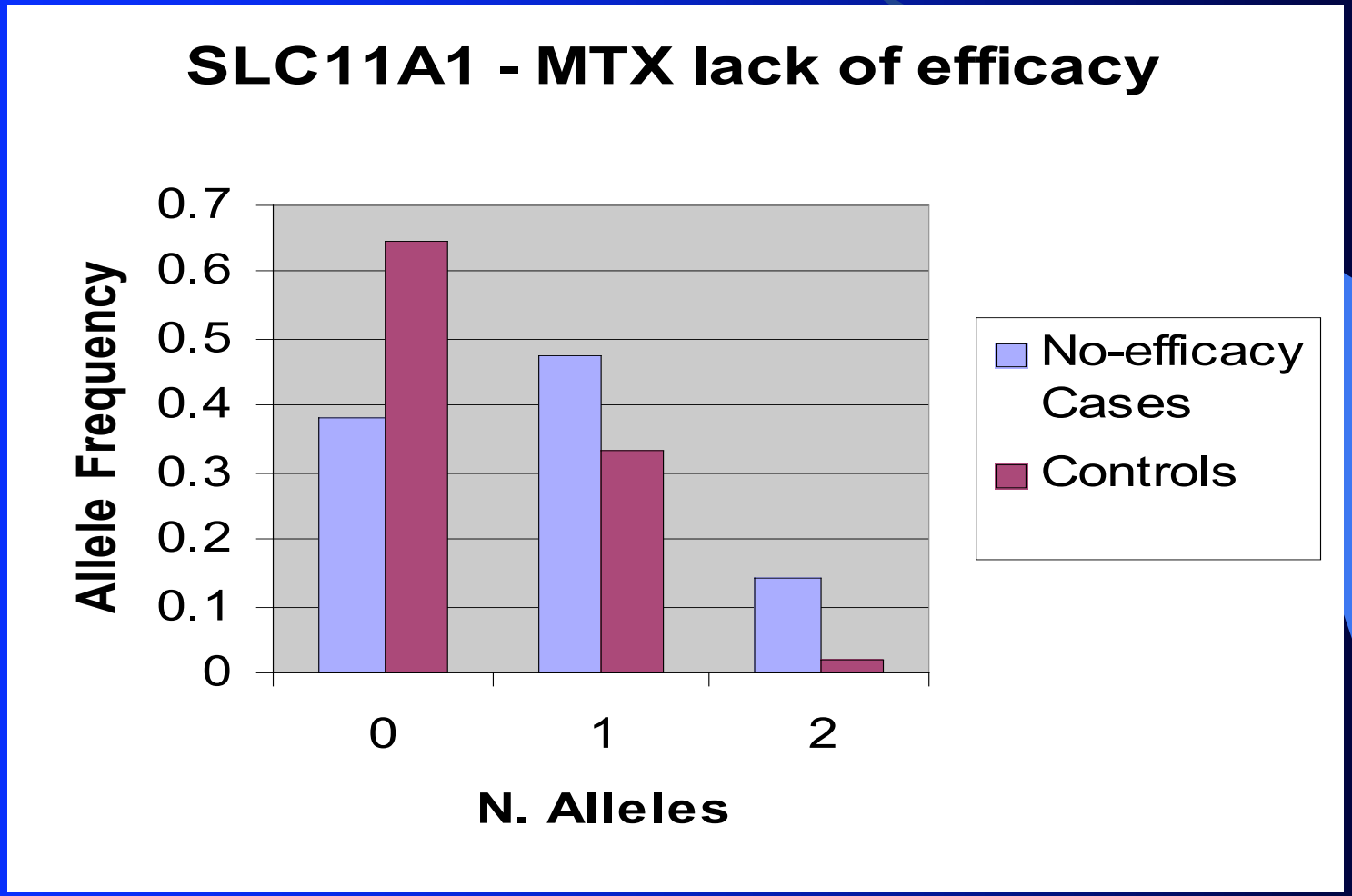


Figure 3(cont.): Genotype Distributions of Selected Markers



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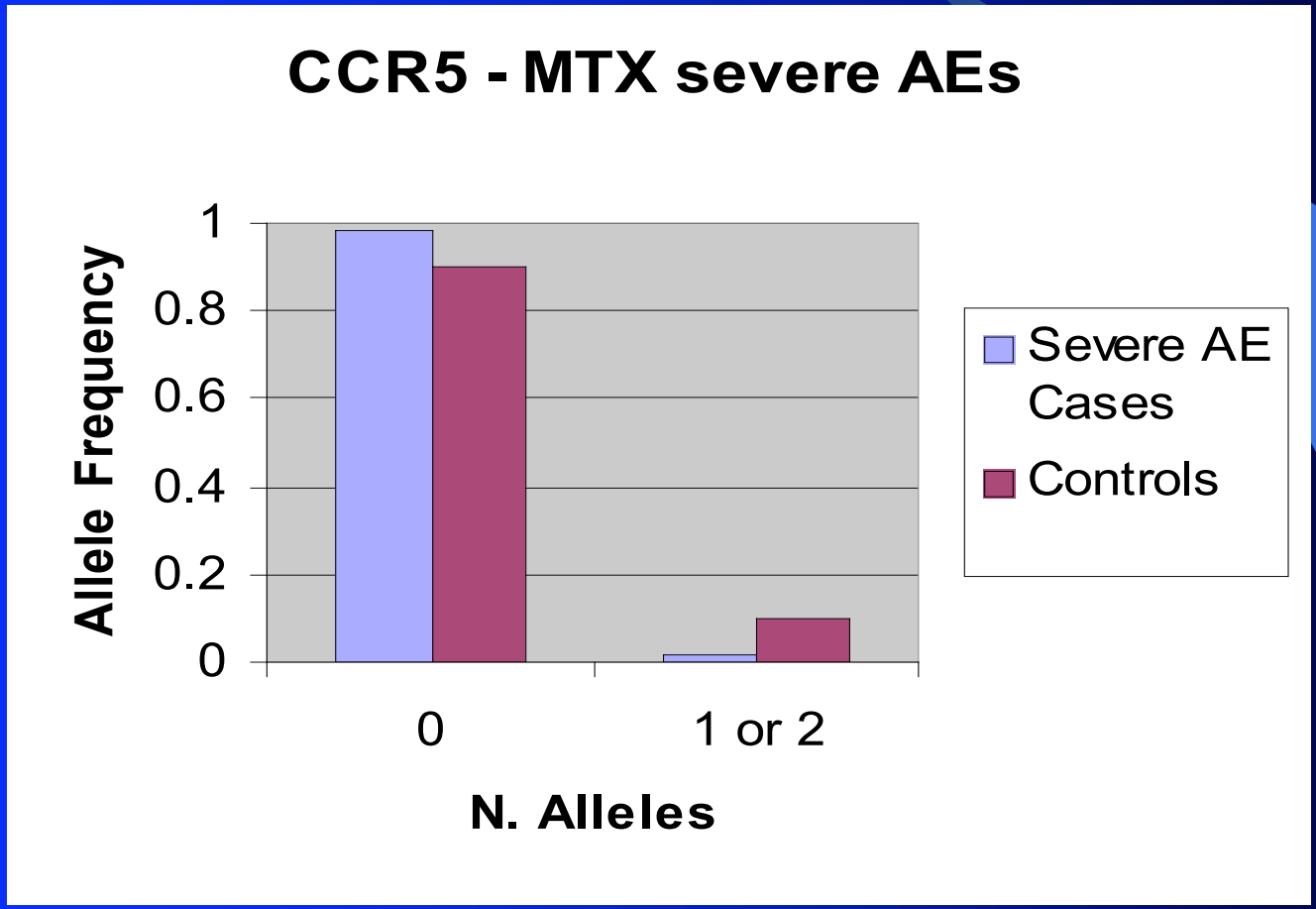

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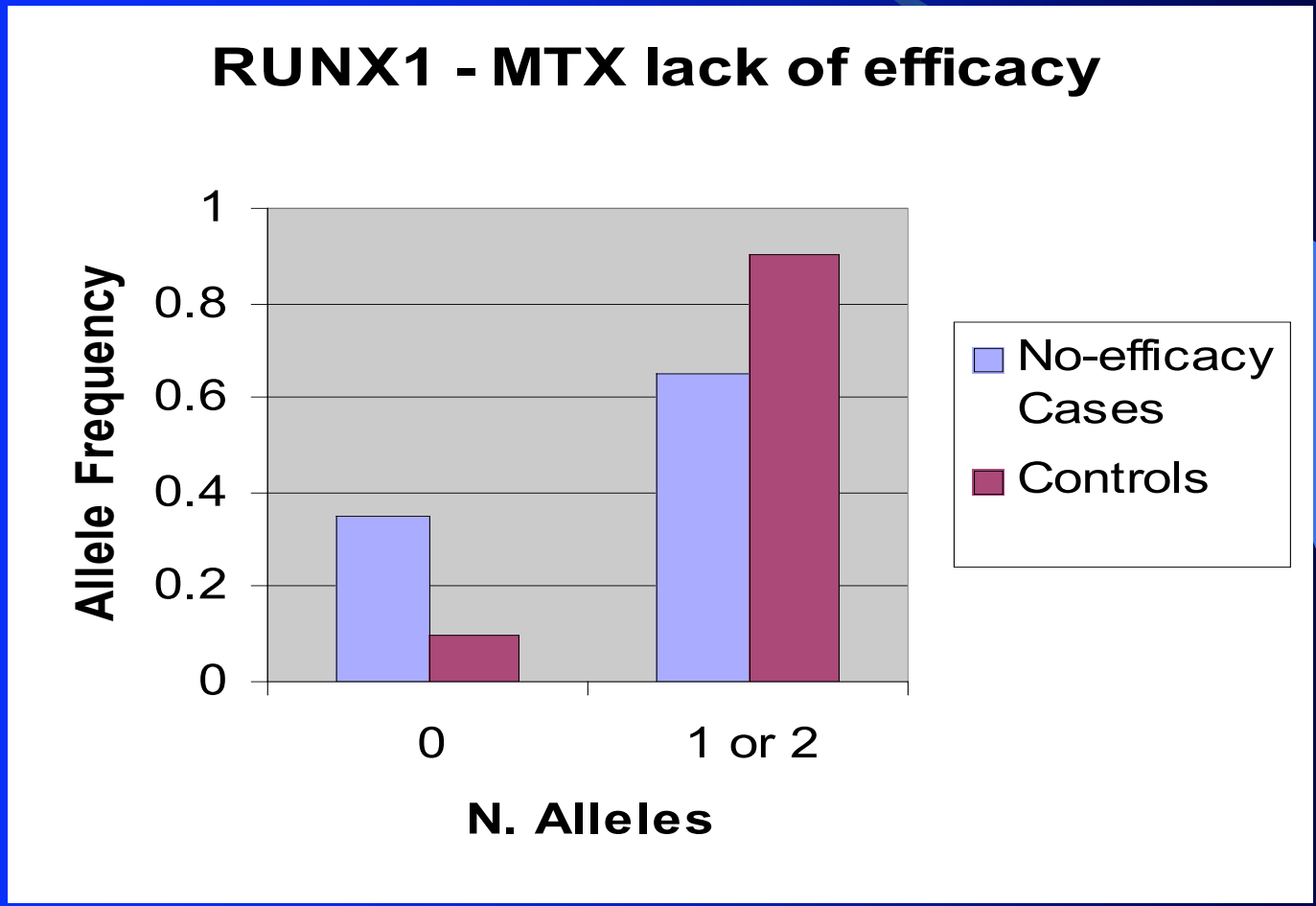

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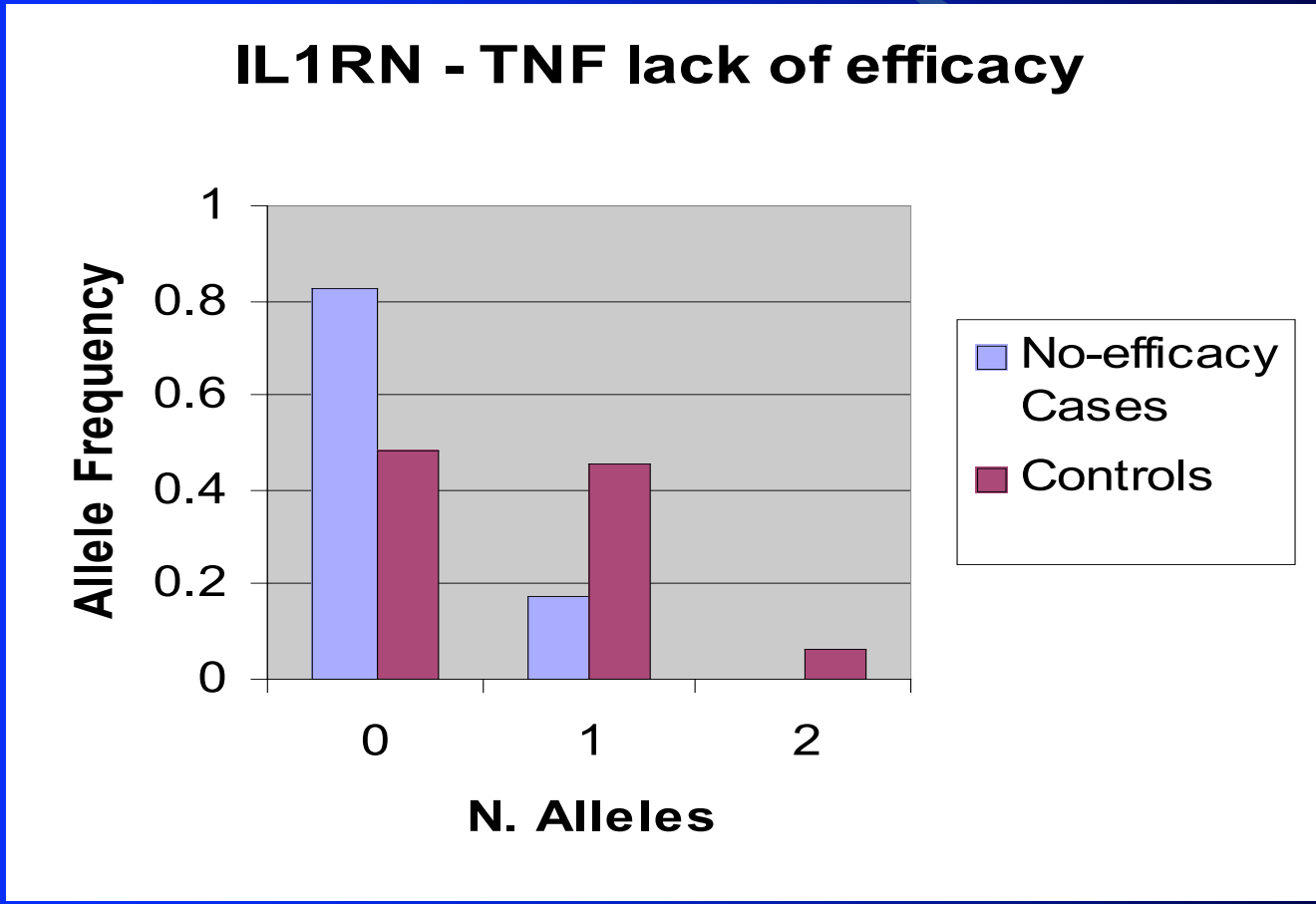

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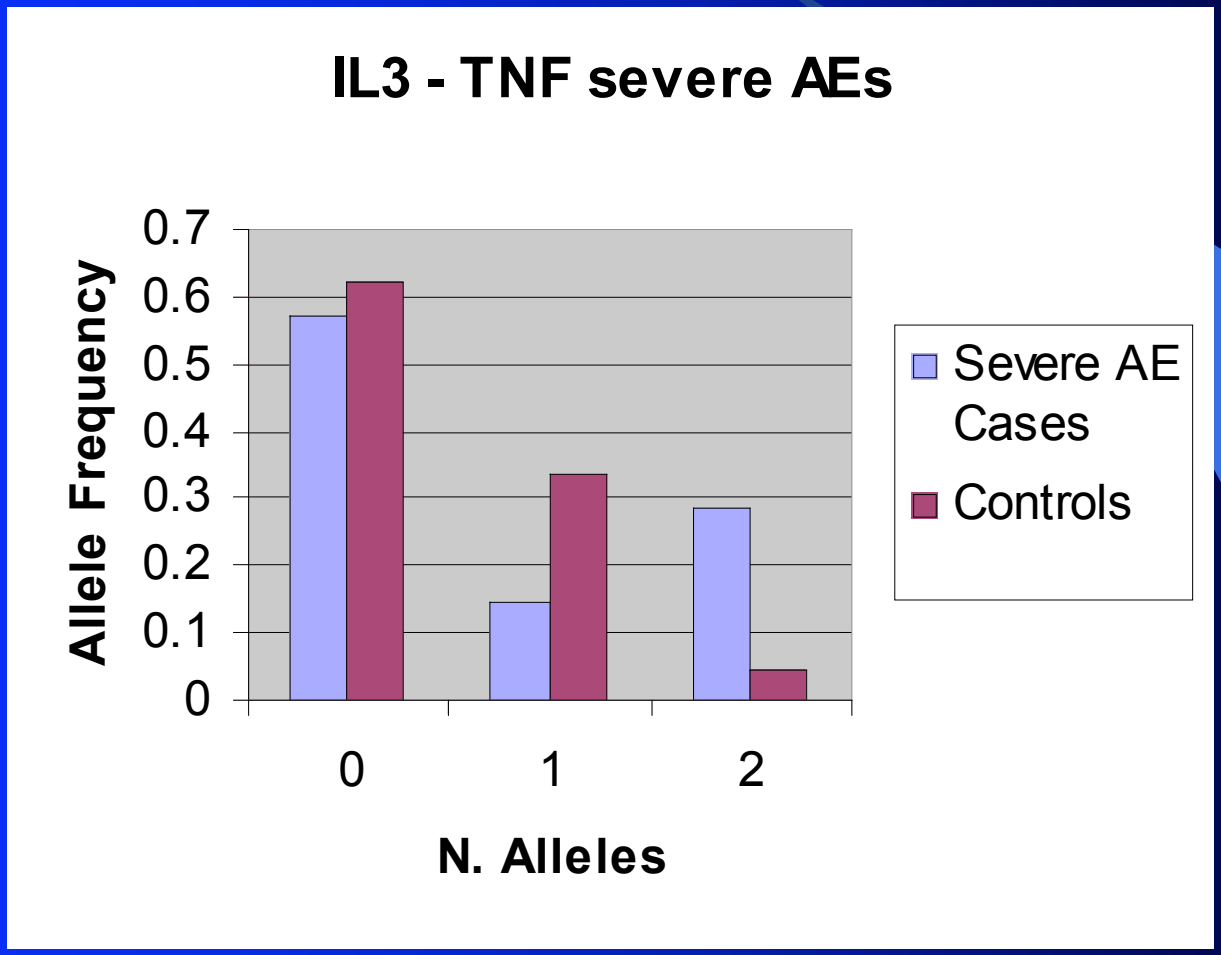

Discussion

- **results show several loci potentially associated with lack of response to either MTX or anti-TNF therapy**

  - The lack of overlap between the two groups suggests that while there is likely to be a genetic component to therapeutic response in RA, this can be expected to be a complex set of interactions specific to the type of therapy administered.

Discussion (cont.)

- Interestingly, we were unable to replicate previous reports of association between the -308 TNF polymorphism and response to anti-TNF-a therapy (Mugnier 2003, Padykulov 2003

- We also did not observe any association between the HLA-DRB1 Shared Epitope (SE), and response to therapy, in contrast to a recent study by Criswell et al (2004) which has showed a trend towards association between response to MTX therapy and homozygosity for the SE, albeit statistically nonsignificant (OR 1.4, 95% CI 0.6-3.1), and a definite association between SE homozygosity and response to high-dose (25mg) Etanercept therapy.

- Analyses of the adverse event groups yielded a greater number of nominally significant results when more stringent inclusion criteria were used

  - This may be due to a confounding effect from lower grade, non-specific AEs that lack a uniform, therapy-specific genetic component.

Discussion (cont.)

- Overall, our results suggest that a wide variety of genetic loci may be involved in clinical response to RA therapy, and in consequent adverse events.
- In the future, analysis of a set of genetic markers may provide a useful tool for enriching and stratifying clinical trial populations and analyzing clinical trial data in RA.
- Such markers may also be useful in making decisions among therapeutic alternatives in clinical practice.

Conclusion

- Results indicate a significant genetic component to the efficacy and toxicological profiles of two common RA therapies
- The non-overlapping sets of efficacy-associated genes suggest the potential for therapy-specific markers
- Our results also imply a central role for cytokines and their receptors in RA pharmacogenetics.

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