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Introducing Philippine-Origin Cynomolgus Macaque (*Macaca Fascicularis Philippensis*) As a Research Model in Drug Safety Assessment

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BACKGROUND INFORMATION

Supply shortages and increased demand for cynomolgus macaques (CM; Macaca fascicularis) in (bio)therapeutic drug development have increased the use of CM sourced from different geographical locations. A recent special issue of Toxicologic Pathology addressed concerns that genetic and environmental variability associated with different geographical origin CM could complicate the interpretation of nonclinical toxicity study results.

The main conclusions of this special issue support the use of different origin CMs (including Cambodia, China, Vietnam, Indonesia, and Mauritius) in drug development programs as long as scientific justifications and reference data are available and maintain animal origin consistency within the same program. **However, this special issue did not include Philippine-origin CM.**

OBJECTIVE

To fill this gap, this current study systemically compiles the 1) **Genomic background;** 2) **Disease susceptibility; and** 3) **Toxicological reference data** to assess the feasibility of using Philippine CM in drug development.

RESULTS

1) **Genomic background (underlying metabolism and immune system)**
Due to the crucial roles of metabolizing enzymes and immune sensitivities that can compromise drug safety and efficacy, the range of genetic variation in CMs specifically within proteins involved in the metabolism and immune response to the drug has been investigated and established.

Genetic and phenotypic background studies demonstrated the Philippine-origin CM exhibits similar variability or potentially lower genetic diversity for the **hepatic CYP450 isoforms (CYP1A/2A/2B/2C/2D/2E1/3A4), a cytokines/chemokines panel, and polymorphism of MHC class I (A, B, I, AG) and II (DRA, DRB)** genes compared to insular Asia CMs [1-4]. These genetic and expression heterogeneity data underscore the advantages of using the Philippine-origin CM in preclinical drug development and testing since it recapitulates the diversity and complexity of the human genome.

2) **Disease susceptibility (Helicobacter spp. infection)**
For disease susceptibility, **subclinical gastritis** is widespread in the Philippine-origin CM with a high incidence of **Helicobacter spp. infection in the digestive tract** [5,6].

3) **Toxicological reference data (in-house database vs. literature)**
Altasciences' established in-house database including **in life and clinical pathology** is comparable to the published Philippine-origin CM toxicological reference data [6] and more extensive CM (w/o origin) background data (The Laboratory of NHP 2018). The noteworthy spontaneous microscopic finding in the Philippine CM is **stomach antral mucosa** was generally more severely **infiltrated with lymphoplasmacytic cells** than the fundic mucosa [6].

CONCLUSION

The current study conducted a systemic literature review with a comprehensive understanding of genomic background, disease, and toxicological reference data of the Philippine-origin CM. These results intend to improve **scientific justifications for CM selection in drug safety assessment (ICH M3/S6) and provide criteria for evaluating nonclinical toxicity studies using the Philippine-origin CM**.

GENOTYPIC AND PHENOTYPIC BACKGROUND OF PHILIPPINE ORIGIN CM

CMs represent a significant percentage of the NHP used in the U.S. for biomedical research. Among all macaque species, the CM has the most diversified geographical area encompassing continental and insular populations. Based on mitochondrial DNA analysis, the CM species can be divided into three geographic subpopulations:

- Mainland or Indochinese CM (Cambodia, China, or Vietnam)
- Insular Asian CM (Philippines or Indonesia)
- Mauritius CM

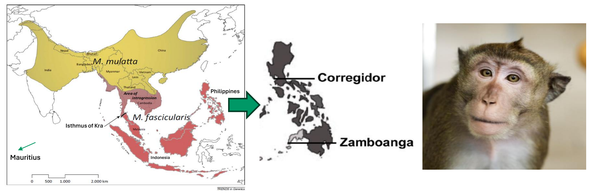


Figure 1. Natural distribution of cynomolgus and rhesus macaques and introgression area (adapted from Haus T, et al [7])

Genetic composition and diversity of different CM populations (from Cambodia, **Philippines-Corregidor and Zamboanga**, Indonesia-Sumatra, Singapore, and Mauritius) have been characterized based on 24 short tandem repeats (STRs) analysis (genetic variation marker) [8].

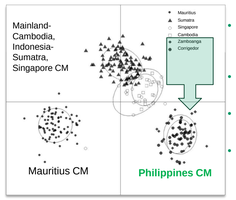


Figure 2. Genetic variance analysis identified three clusters of different geographic origins CM (quantitative identification of genetic clusters variance within- and) between-group)

- Cambodian CM exhibited the highest allelic diversity (interbreeding with rhesus macaques) while the **Mauritian and Philippines** populations exhibited the lowest.
- **Philippine and Mauritian** CMs show the most divergent from other populations (due to separation from mainland).
- No genetic differences between the two subspecies of CM co-exist in the Philippines (Corregidor and Zamboanga).
- **The Mauritian and Philippine-origin CMs are better suited to biomedical research due to these populations having fewer alleles than other geographic areas.**

For drug safety assessment, **metabolism profile (SM) and immunological responses (LM)** in nonclinical species (vs. human) are critical to characterizing in drug development.

- Genetic variation of hepatic metabolism enzymes (CYP450) identified [2] among different CM subpopulations, with no significant differences of major CYP450 isoforms (**CYP1A/2A/2B/2C/2D/2E1/3A4**) expression in the liver [1] (Fig .3)
- Transcript profiling of cytokine expression shows relative stable and comparable inter-animal variation in naïve CM with different origins [2] (Fig. 4)
- CMs show similar variability within their MHC (class I and II) genes [4] which influences the development of adaptive immune responses [3] (Fig. 5)
- Further metabolic and immunological validation studies with reference drugs are warranted.

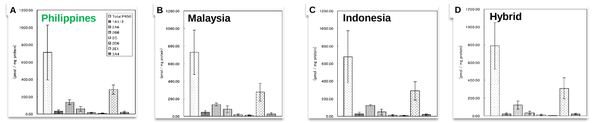


Figure 3. Total CYP450 content and key isozyme content in the livers of CM

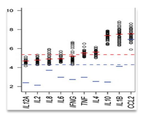


Figure 4. Variability in baseline gene expression of cytokines

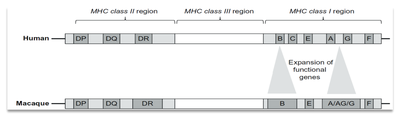


Figure 5. Genetic map of the human and CM MHC

BACKGROUND ORGAN WEIGHTS, MICROSCOPIC FINDINGS, AND DISEASE SUSCEPTIBILITY OF PHILIPPINE ORIGIN CM

All summarized data tables and figures (below) of animal terminal body weights, relative organ weights, and microscopic findings are adapted from the Drevon-Gaillot 2006 publication [6].

Table 1: Mean body weight and relative organ weight in control Philippines CMs

	Philippines CM	
	Males	**Females**
Body weight (kg)*	2.729 ± 0.42	2.474 ± 0.24
Relative organ weights (%)		
Kidneys	0.46 ± 0.06	0.51 ± 0.04
Heart	0.36 ± 0.03	0.34 ± 0.04
Liver	2.20 ± 0.28	2.38 ± 0.24
Spleen	0.25 ± 0.03	0.26 ± 0.05

Note: The terminal body weights are higher in males than in females, and selected relative organ weights are comparable to published data in CMs with different origins (Chinese, Indonesian, or Mauritian) [9].

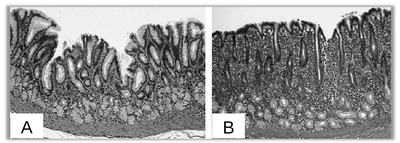
The notable and main microscopic findings are **lymphoplasmacytic cell infiltrates (LPCI) in the digestive tract, kidney, and other selected organs.**

Table 2. Microscopic findings and incidence (%) in control Philippines CMs

Philippines CM		Animal number (N=28)
Digestive tract	**Stomach: LPCI / chronic diffuse gastritis**	**100%**
	Colon: LPCI / chronic diffuse colitis	71%
	Caecum/colon: *Balantidium coli* in the lumen	75%
Kidney	LPCI / interstitial nephritis, focal, chronic	61%
	Cytoplasmic inclusions in the urothelium	**100%**
Heart	LPCI	50%
Liver	LPCI	75%
Spleen	Increased cellularity of white pulp	54%
Lung	LPCI / pneumonitis, focal, chronic	14%

Digestive Tract:
Subacute to chronic gastritis characterized by a diffuse LPCI of the lamina propria, which could be related to intestinal Helicobacter spp infection and parasitic load with Ciliated protozoan.

A moderate to marked diffuse infiltration of the antral mucosa was reported, with variations for the mean infiltration grade (±SD) in the two gastric regions (**antral mucosa 2.93±0.81** vs. fundic mucosa 0.86±1.04) and colon (0.89±0.69).



(A) Non-increased cellularity

(B) Markedly increased cellularity

Figure 6. Stomach gastric antral mucosa (HE stain X40)

Kidney:
Interstitial nephritis (IN) characterized by an inflammatory infiltrate with a disrupted tubular basement membrane and epithelial cell degeneration

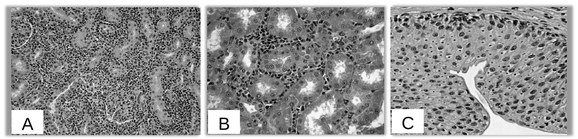


Figure 7. Kidney (HE stain X200). (A) IN, chronic, minimal, focal; (B) LPCI with the infiltrated lymphocytes exclusively in the interstitium; (C) Eosinophilic intracytoplasmic inclusions in the urothelial cells

TOXICOLOGY REFERENCE DATA OF PHILIPPINE ORIGIN CM

NHP models exhibit a wide range of variation both between species and within species in their basic biological and physiological parameters as well as clinical pathology values, due to many potential sources of variability including geographic origins. However, many available sources of historical normative or reference data in the literature mix data across species and subspecies.

Recently, great efforts have been taken to compare similarities and differences in toxicological relevant data between CMs with different geographic origins (Toxicologic Pathology 2022). For more accurate toxicology study data interpretation, Altasciences Pathology is establishing normative reference values for our in-house Philippine-origin CM colony, including standard clinical pathology and immunophenotyping panels. Results of these panels are generally similar amongst the subpopulations compared in the literature.

Table 3. Altasciences Philippine-origin CM database vs. published clinical pathology reference values

Animal number		14M/20F		N/A		14M/14F	
Stand Animal Screening		negative for SIV, B virus, simian retroviruses type D, rabies, simian T-cell leukemia virus (STLV), measles, with additional tuberculin, bacterial and parasitological tests		N/A		negative for rabies, herpes B, and tuberculosis tests, and treated against internal parasites	
Acclimatization period		>2 weeks		N/A		>6 weeks	
Age (years)		1.2 to 1.8		5 to 6		1.8 to 3.1	
BW (kg)		M: 1.9±0.1; F: 1.8±0.1		Adult M: 4.7–8.3; Adult F: 2.5–5.7		M: 2.729±0.42; F: 2.474±0.24	
Sexual maturity (years)		No		F: 3.0–4.0; M: 4.0–5.0		F: at 4; M: at 6	
Test	**Units**	**Philippines-origin (Mean ± SD)***		**mixed-origin (Mean ± 2SD)**		**Philippines-origin (Mean ± SD)**	
CLINICAL CHEMISTRY		**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Alanine Aminotransferase	ALT (U/L)	**42 ± 12**	**36 ± 9**	42.6 ± 16.9	48.5 ± 29.1	38 ± 19	38 ± 14
Aspartate Aminotransferase	AST (U/L)	**38 ± 7**	**39 ± 7**	46.3 ± 12.6	42.2 ± 18.3	33 ± 6	34 ± 9
Alkaline Phosphatase	ALP (U/L)	**695 ± 162**	**744 ± 157**	423 ± 192	249 ± 117	1887 ± 323	1684 ± 616
Blood Urea Nitrogen	BUN (mg/dL)	**19.4 ± 3.3**	**20.0 ± 2.5**	17.1 ± 2.6	17.8 ± 3.5	0.49 ± 0.07 (g/L)	0.48 ± 0.08 (g/L)
Creatinine	CRN (mg/dL)	**0.4 ± 0.1**	**0.5 ± 0.1**	1.01 ± 0.22	0.78 ± 0.11	6.9 ± 0.6 (mg/L)	6.8 ± 1.0 (mg/L)
Calcium	CA (mg/dL)	**9.9 ± 0.3**	**10.4 ± 0.4**	10.9 ± 0.6	10.6 ± 0.6	97 ± 2.7 (mg/L)	101 ± 7.7 (mg/L)
Inorganic Phosphorus	IP (mg/dL)	**6.9 ± 0.5**	**7.5 ± 0.7**	7.3 ± 1.6	6.2 ± 1.2	74 ± 11.3 (mg/L)	73 ± 8.9 (mg/L)
HEMATOLOGY		**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Red Blood Cells	RBC (^6/µL)	**6.7± 0.3**	**6.9 ± 0.4**	6.90 ± 0.34	5.75 ± 0.41	6.91 ± 0.24	6.64 ± 0.51
Hemoglobin	HGB (g/dL)	**12.5 ± 0.5**	**12.7 ± 0.5**	13.6 ± 0.7	12.9 ± 0.8	12.9 ± 0.3	12.3 ± 0.9
Mean Corpuscular Volume	MCV (fL)	**64.6 ± 2.6**	**64.5 ± 2.7**	70.7 ± 2.2	70.4 ± 3.6	65.5 ± 3.1	65.7 ± 1.9
Mean Corpuscular HGB	MCH (pg)	**18.5 ± 1.0**	**18.4 ± 1.0**	22.8 ± 0.9	22.4 ± 1.3	18.8 ± 0.7	18.4 ± 0.9
White Blood Cells	WBC (^3/µL)	**10.5 ± 3.5**	**9.3 ± 2.8**	11.8 ± 2.9	10.3 ± 3.3	13.79 ± 3.62	11.15 ± 3.73
Neutrophils (Absolute)	NEUT (^3/µL)	**5.3 ± 3.3**	**5.4 ± 2.6**	7.19 ± 3.58	6.92 ± 3.16	31.5 ± 11.1 (%)	37.5 ± 11.9 (%)
Lymphocytes (Absolute)	LYMPH (^3/µL)	**4.6 ± 0.9**	**3.5 ± 0.8**	3.10 ± 1.84	3.65 ± 4.66	59.9 ± 10.4 (%)	52.3 ± 11.5 (%)
Eosinophils (Absolute)	EOS (^3/µL)	**0.2 ± 0.2**	**0.1 ± 0.1**	0.14 ± 0.20	0.12 ± 0.20	3.5 ± 2.7 (%)	4.5 ± 2.8 (%)

*for these data, mean values are rounded to a whole number or one significant digit.

SUMMARY

- Compared to other geographic-origin macaques, Philippine-origin macaques are of a more limited genetic variation and are a more homogenous population with fewer spontaneous physiologic and anatomic abnormalities thereby providing less variability and lower incidences of background pathology. These minor differences are most likely due to isolation on the island of Philippine Archipelago.
- The results of this study support the interchangeable use of Philippine-origin CM **in pharmaceutical safety assessment programs.**
- **Although differences in background data have been identified,** with the use of a consistent source, robust pre-study screening, and concomitant controls**, none of these identified differences would be sufficient to exclude the use of different origin macaques in preclinical toxicology studies.**

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