Detection of SV40 in Patients with Kidney Disease

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Nephrotic syndrome

- Normal protein excretion: <150 mg/d

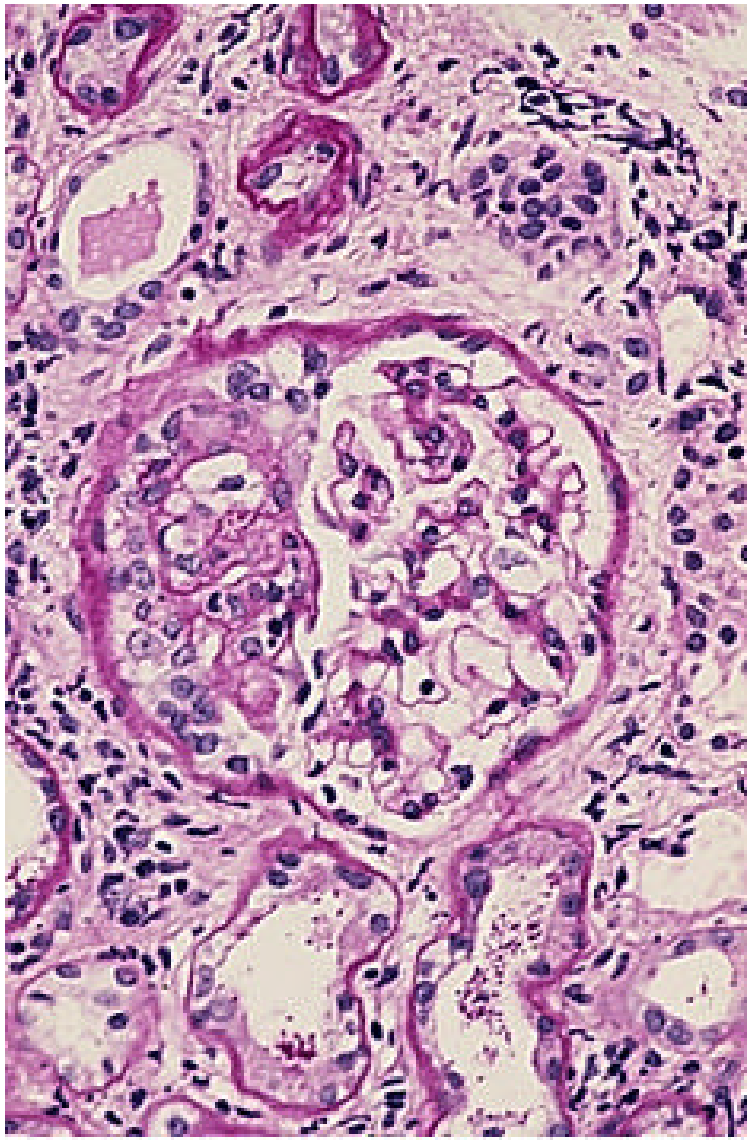
- Nephrotic syndrome: >3.5 g/d

Often with edema, hypoalbuminemia, hypercholesterolemia

- Systemic nephrosis: diabetes, lupus
- Primary nephrosis

Focal Segmental Glomerulosclerosis

- Idiopathic FSGS, described 1957, now the leading cause of 1° NS
- HIV-associated FSGS
- Collapsing FSGS, new disease ~1980
- Hyperfiltration injury
- Genetic/familial



Why suspect SV40 in FSGS?

- Other viruses can cause FSGS: HIV-1, possibly parvovirus B19
- Polyoma viruses are renotropic
- SV40 transgenic mice develop FSGS

  MacKay, Kidney Int 1987
- FSGS incidence has risen 2-3 fold in the past 25 years, suggesting a new environmental factor
- Collapsing FSGS, a new disease appearing about 1980

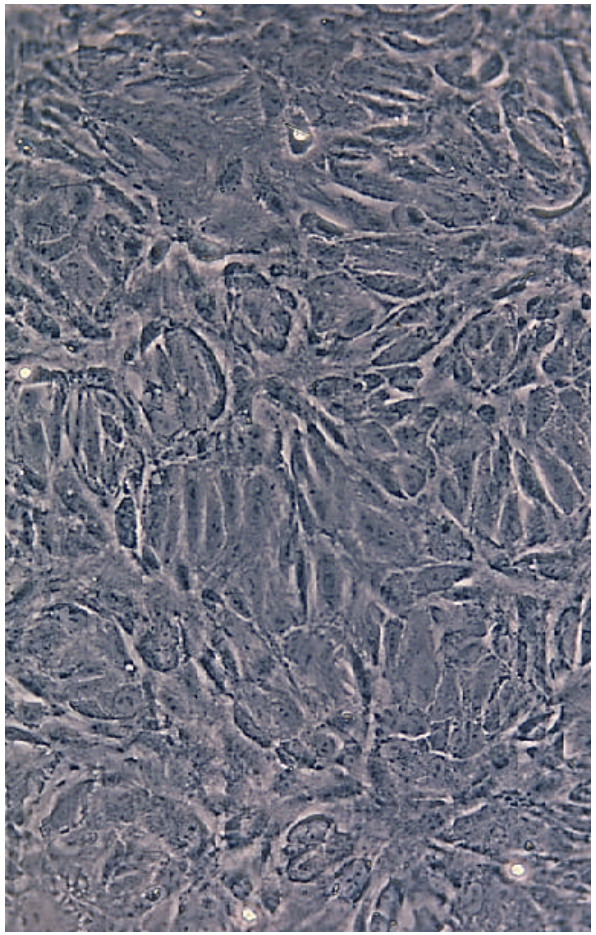
Methods

- Patients: FSGS, other kidney disease, healthy volunteers
- PBMC, urinary cells
- Co-cultures with susceptible CV-1 cells:
- T Ag staining, nested PCR of regulatory region, multiple clones sequenced

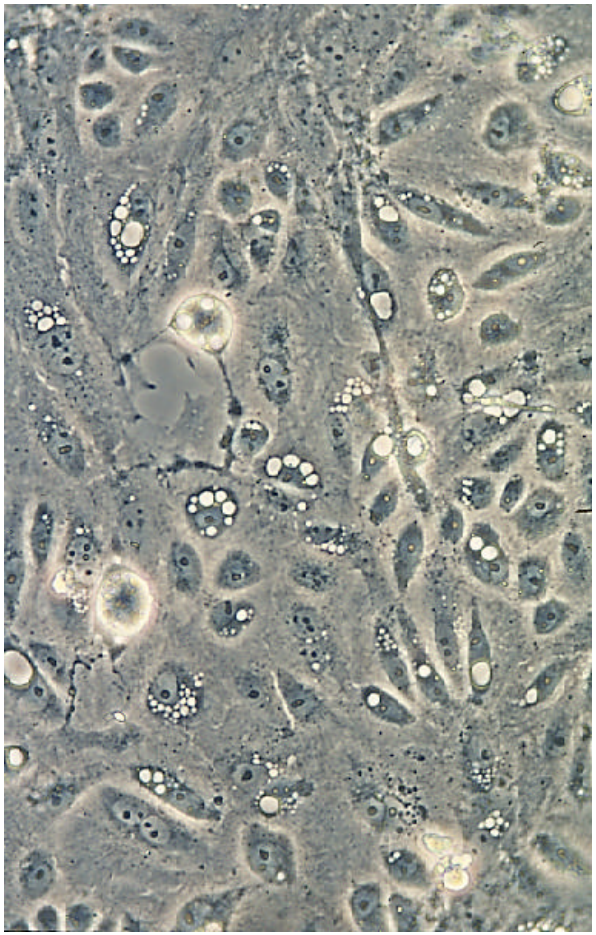
J Am Soc Nephrol, 9/02 (in press)

CV-1 Cell Infection

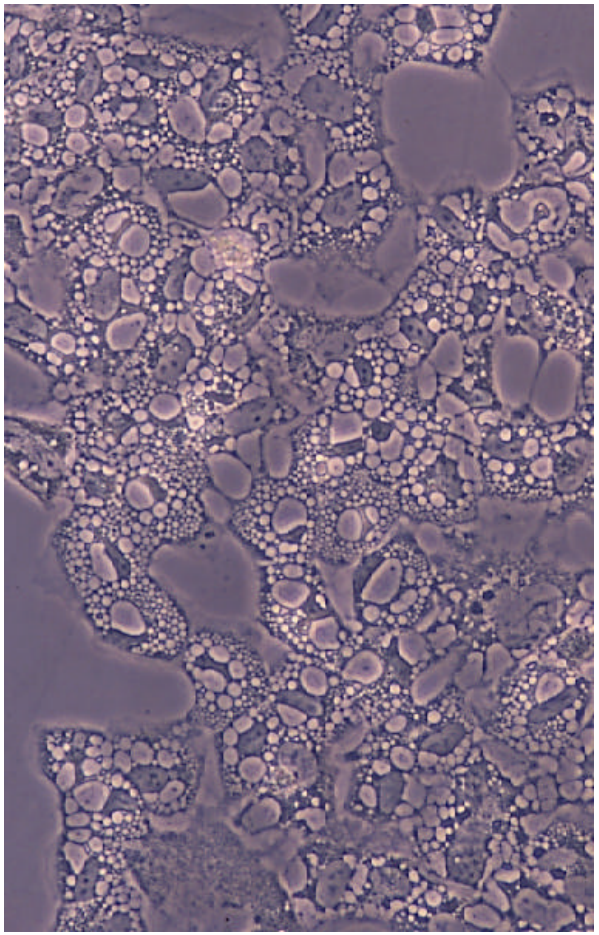
Volunteer d7



FSGS d7

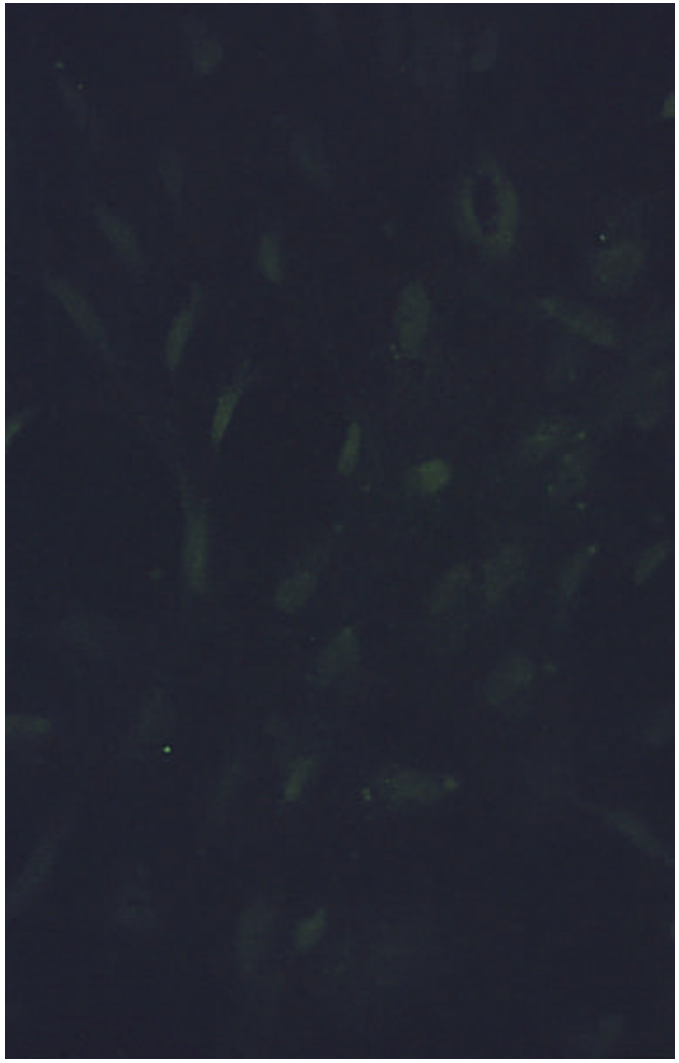


FSGS d15

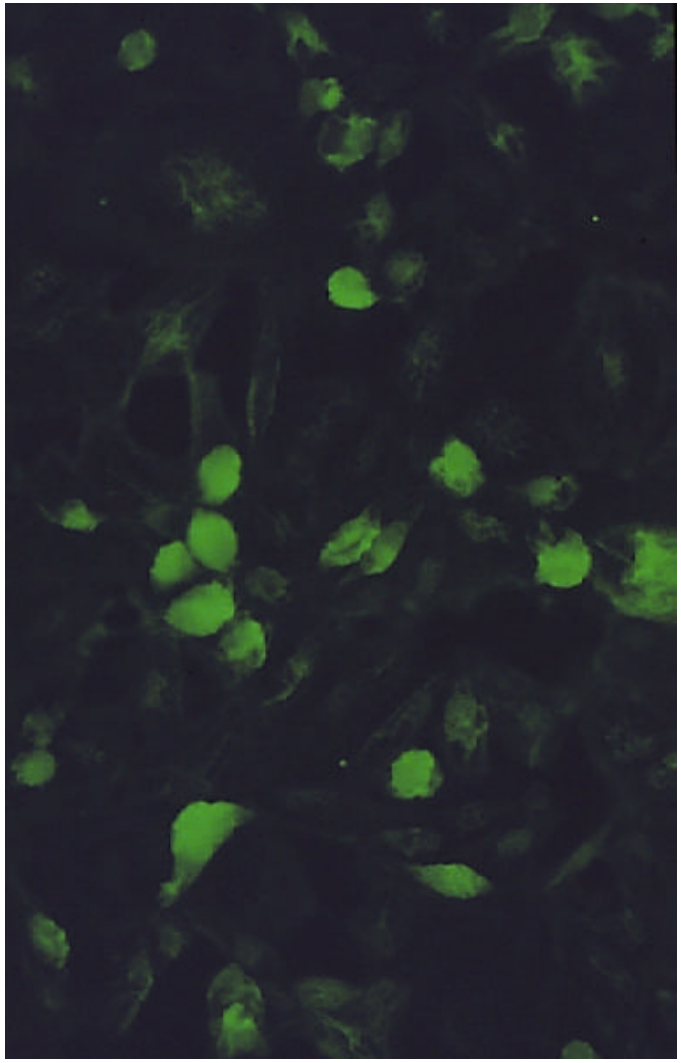


SV40-T Ag

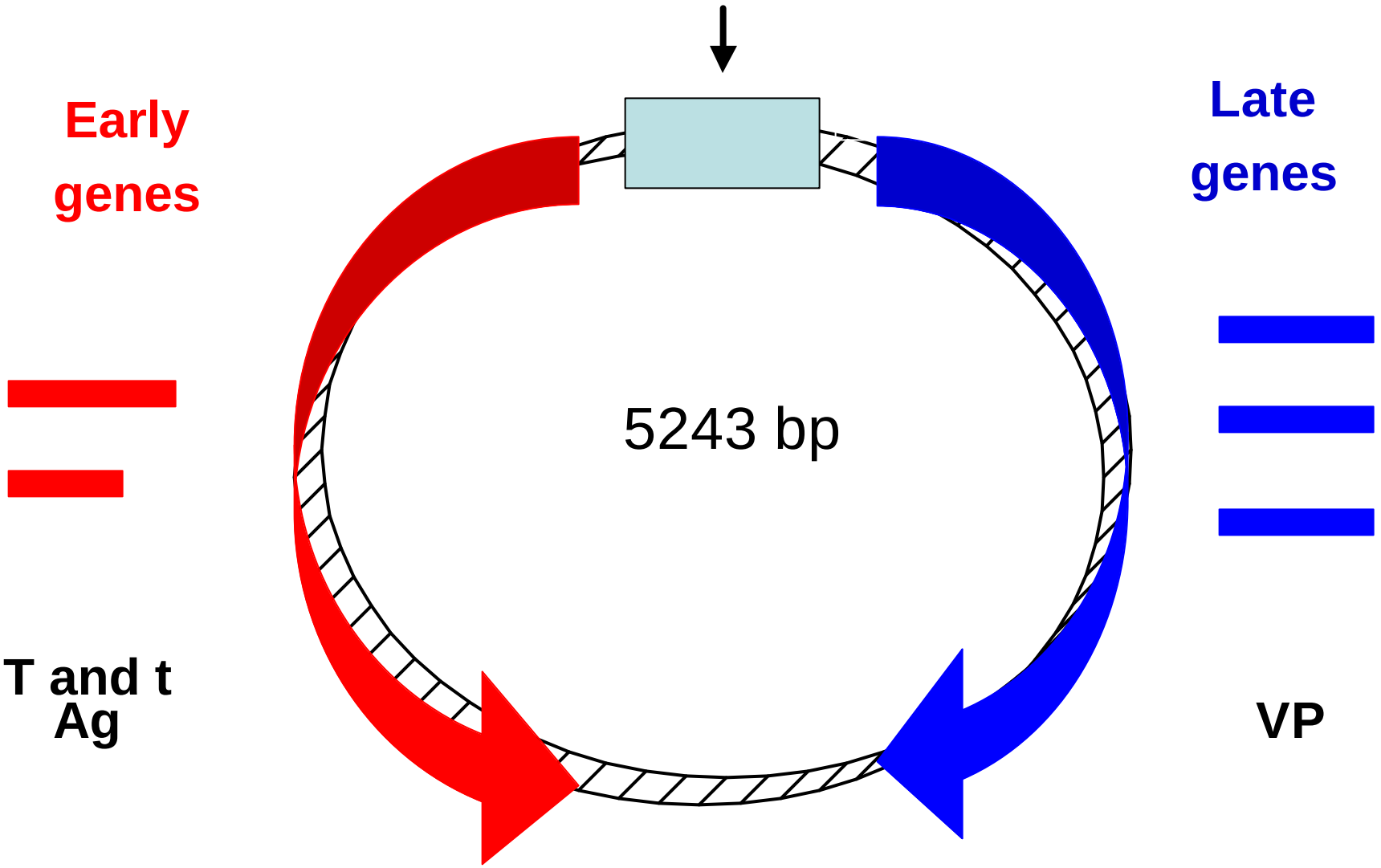
CV-1 + Volunteer Ur



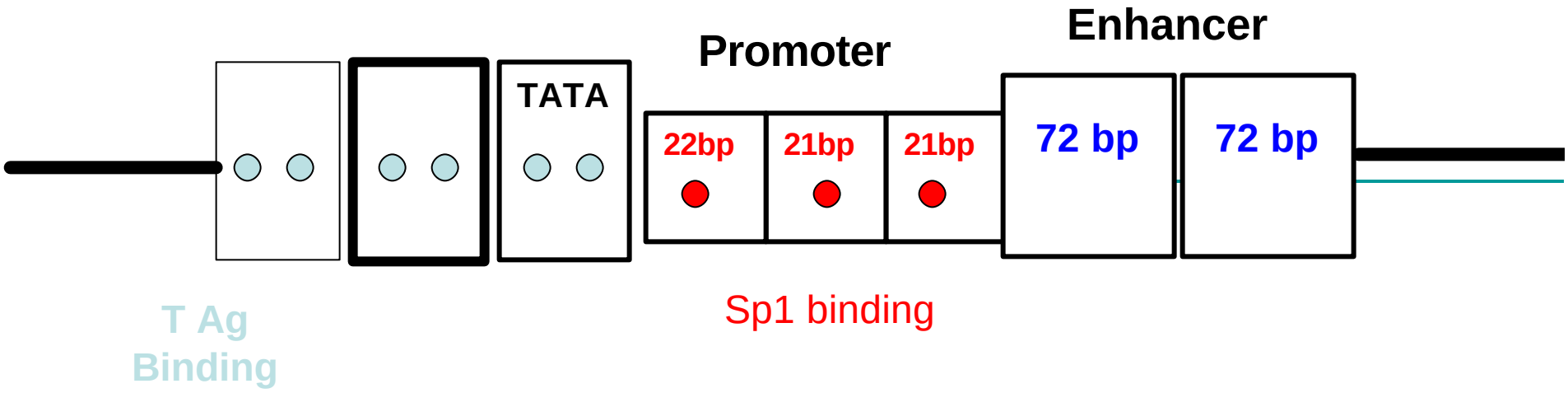
CV-1 + FSGS Ur



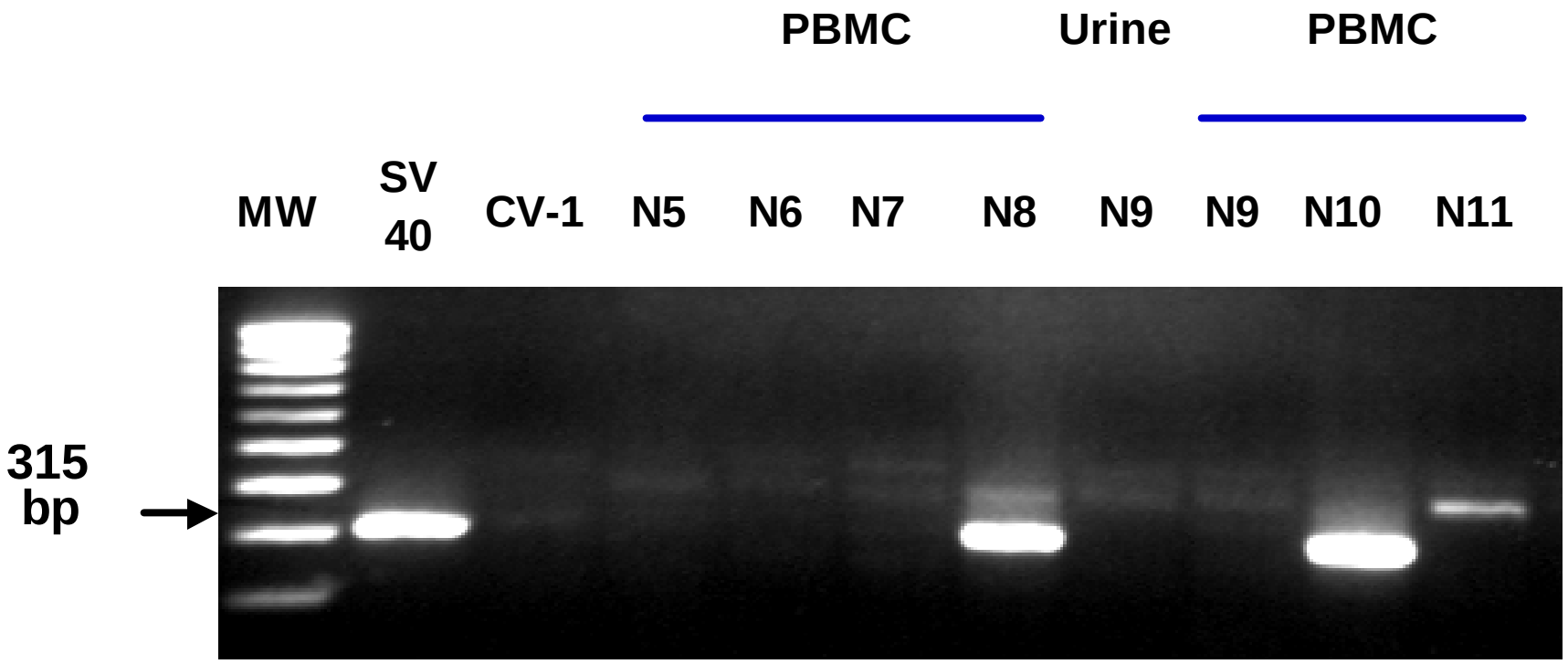
SV40 Regulatory Region



SV40-776 Regulatory Region



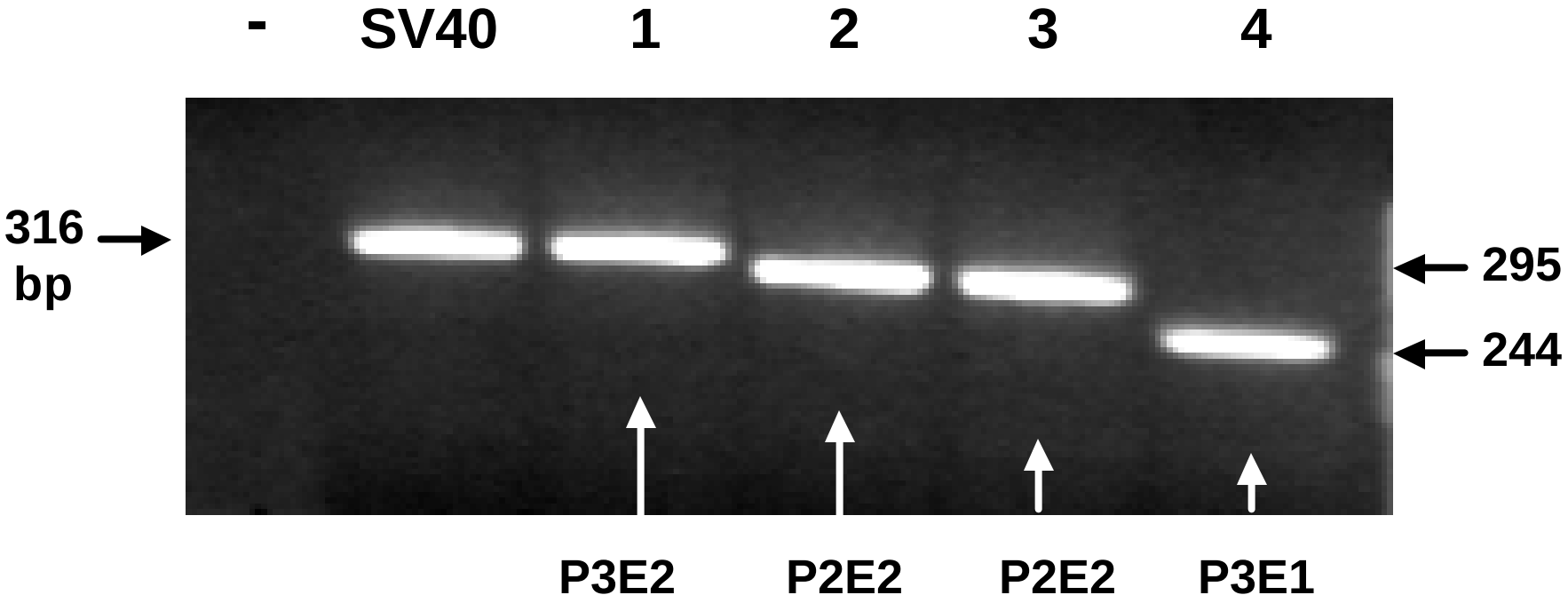
Nested PCR from Healthy Volunteer Co-cultures



Human SV40 Isolates: Regulatory Region Diversity

	1	11	21	31
SV40	GCCGAGGCGG		CCTCGGCCTC	
FSGS20 P		TGCATAAATA	AAAAAAATT	
FSGS10 U	----------	----------	----------	----------
FSGS10 U	----------	----------	----------	----------
FSGS01 P	--T-------	----------	----------	----------
FSGS07 P	----------	----------	----------	--G-------
FSGS08 P	--G-CCT-A-	----||||-|	|---------	---------- --G-
	CCT-A-	----||||-|	|---------	----------

PCR of Positive Clones from an FSGS Patient PBMC



SV40 Regulatory Region Variation

- P3E2 (Non-archetypal): no mutation or 1-3 NT mutations
- P3E2: 10-12 bp mutations in T Ag binding core region
- P3E1 (Archetypal): 72 bp enhancer element deletion
- P2E2 (Novel): 21 bp promoter element deletion

SV40 Isolation

Diagnosis	N	PBMC	Urine
FSGS	40	14 (35%)	15/36 (42%) *
Other kidney dx	20	3 (15%)	2 (10%)
Volunteer	22	5 (23%)	1 (5%)

* P<0.001*

Detection of other polymomaviruses by direct PCR

Virus	PBMC	Urine
BK	3/63	7/61
JC	2/63	9/61

Lack of age effect in SV40 detection among all study subjects

Birth date	PBMC	Urinary Cells
<1964	13/50 (26%)	10/47 (21%)
=1964	9/32 (28%)	8/31 (26%)

P=NS

Immunosuppression does not account for SV40 recovery in urine

Group	ImmSupp	Not ImmSupp
FSGS	7/16	8/24
Other	2/15	0/5
Healthy	0/0	1/22

Immunosuppression: HIV-1 or immunosuppresive Rx in the preceding 3 mos

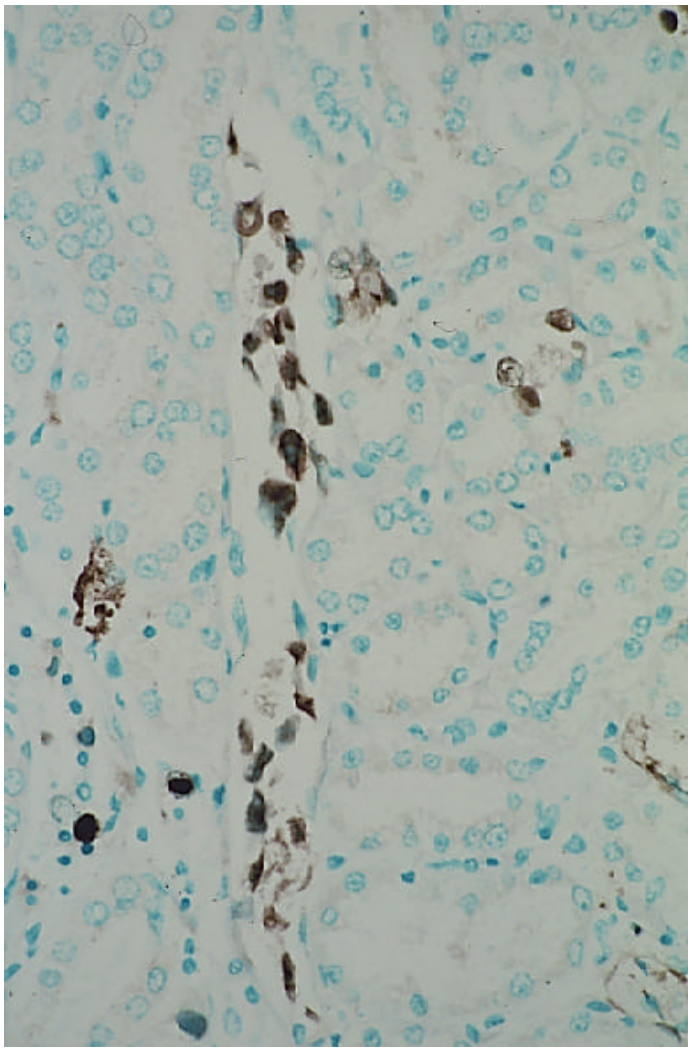
SV40 Detection in Renal Biopsies

Diagnosis	N	SV40+
FSGS	10	5
Collapsing FSGS	10	6
HIV FSGS	10	6
All FSGS		57%
Membranous nephropathy	10	2
Minimal change disease	10	2
		20%

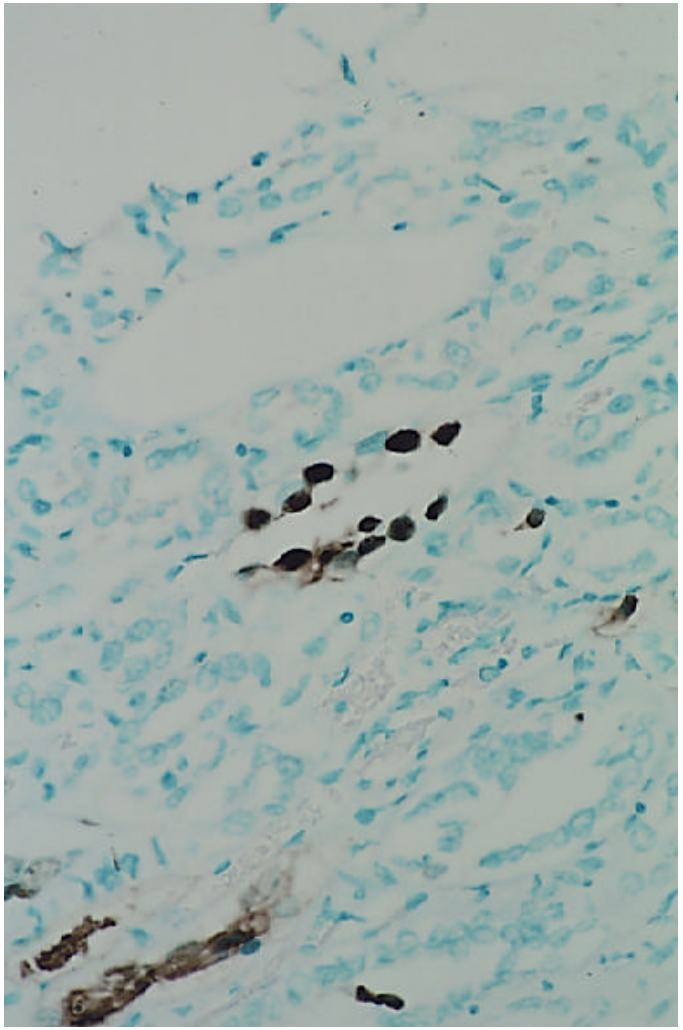
P<0.02

ISH: FSGS Kidney Biopsies

FSGS



HIV-FSGS



Other studies of SV40 in volunteers

- Methods: PCR, Southern blot
- Blood

Martini, Cancer Res 1996	16/70 (23%)
David, Cancer Letters 2001	18/115 (16%)
Butel, Lancet 2002	0/100
Shivapurkar, Lancet 2002	0/12

- Urine

Shah, J Infect Dis 1997	0/166

SV40 detection following renal transplant

- Butel, J Infect Dis 1999 4/20 pediatric tissues + SV40, including 1 Wilm's tumor and 3 renal transplants

- NIH 5/71 renal transplant recipients developed PV nephropathy

	PBMC	Urine
BK	2/5	5/5
SV40	3/5	2/5

Summary

- Isolation of infectious SV40 from PBMC and urine cells
- Sensitive techniques, including nested PCR and sequencing - but also risk of contamination
- Regulatory region heterogeneity argues against laboratory contamination
- SV40 recovered more frequently from urine cells of kidney patients, particularly FSGS and PV nephropathy

Conclusions

- **Possible roles of SV40 in kidney**

1. Cause of glomerular injury (FSGS) and tubulitis (PV nephropathy)

2. Bystander, activated by immunosuppression or cytokines, or more readily detected due to excretion of renal cells with injury

3. Bystander, but the resulting tubulitis contributes to renal damage

- **Frequent recovery argues for relatively common infection in the general population**

Acknowledgements

FSGS	**Ronald Falk** **Charles Jennette**	**Rui-Mei Li**
Renal transplant	**Roslyn Mannon** **Allen Kirk**	**Rui-Mei Li**