Development of an Array-Comparative Genomic Hybridization (aCGH)-Based Algorithm to Assist Renal Tumor Subtyping in Needle Biopsies and Resected Specimens

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OBJECTIVE

- To develop an algorithm that can accurately classify major renal cortical neoplasms based on copy number alterations (CNA) detected in biopsy DNA by aCGH.

INTRODUCTION

- Image-guided, percutaneous biopsy of kidney tumors is increasingly utilized in the initial diagnosis, particularly in patients at higher risk of adverse outcomes.
- Biopsy results may facilitate decision-making in the management of small renal masses.
- Despite improved biopsy techniques, low yield and disrupted tissue architecture may make histologic diagnosis of biopsy samples challenging.
- Specific genetic alterations have been identified in kidney tumors.[1-5]
- Accurate detection of genetic alterations may improve the diagnostic capabilities of percutaneous kidney biopsy.
- Selected patients may avoid extirpative treatment if benign or indolent tumors are determined by biopsy.

MATERIALS

Specimen acquisition:

- Percutaneous 18-22 Gauge core biopsies from 44 renal masses prospectively collected from 40 patients (9/2011 – 9/2013)
- Excluded 5 cases:
  Clinical data (1 patient); Cystic fluid only (1 patient), needle below mass (1 patient), Benign cyst (1 patient), insufficient material (1 patient)
- Technique:
  - 1-4 core biopsies/tumor (median: 2)
  - 1-2 cores: DNA extraction for aCGH

Patient Characteristics:

- Men : Women= 1.2: 1.0

Histologic Analysis:

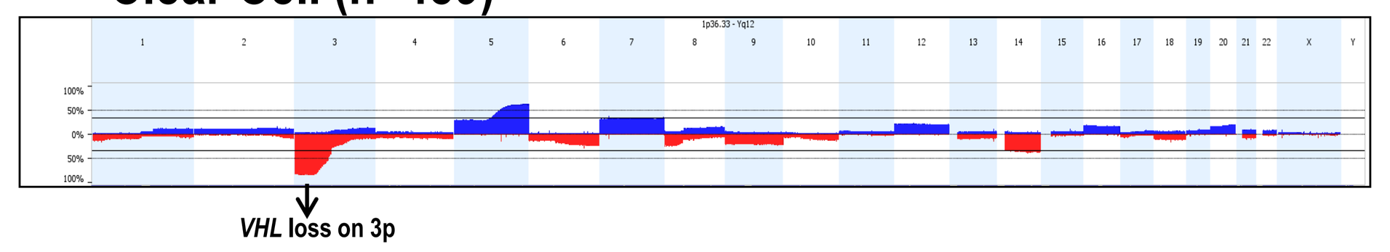
- Diagnosis from pathology reports of biopsy tissue
- Surgical pathology assessment used when available

METHODS AND RESULTS

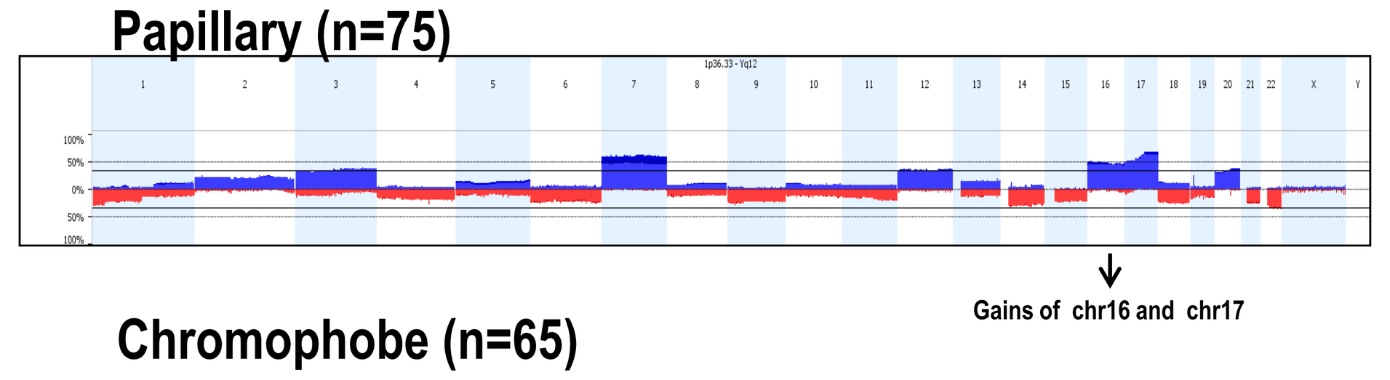
Build an algorithm based on the following:

- aCGH data from TCGA (analyzed using Nexus 7.0 algorithm):
  489 clear cell RCC (ccRCC)
  75 papillary RCC (pRCC)
  65 chromophobe RCC (chrRCC)

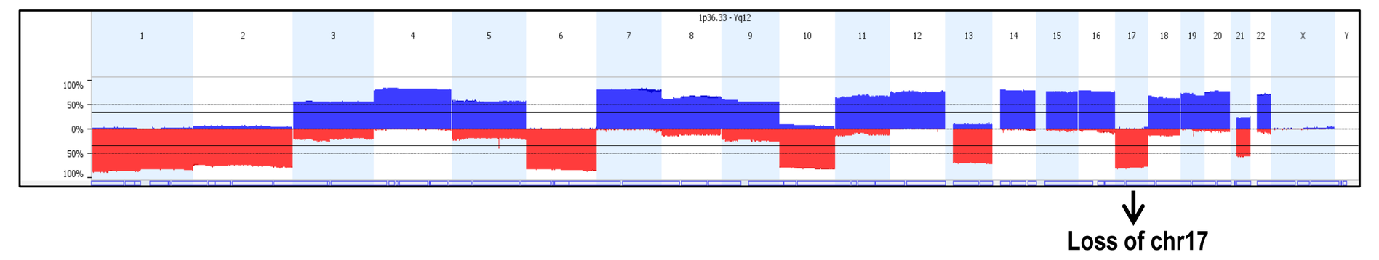
Clear Cell (n=489)



Papillary (n=75)



Chromophobe (n=65)

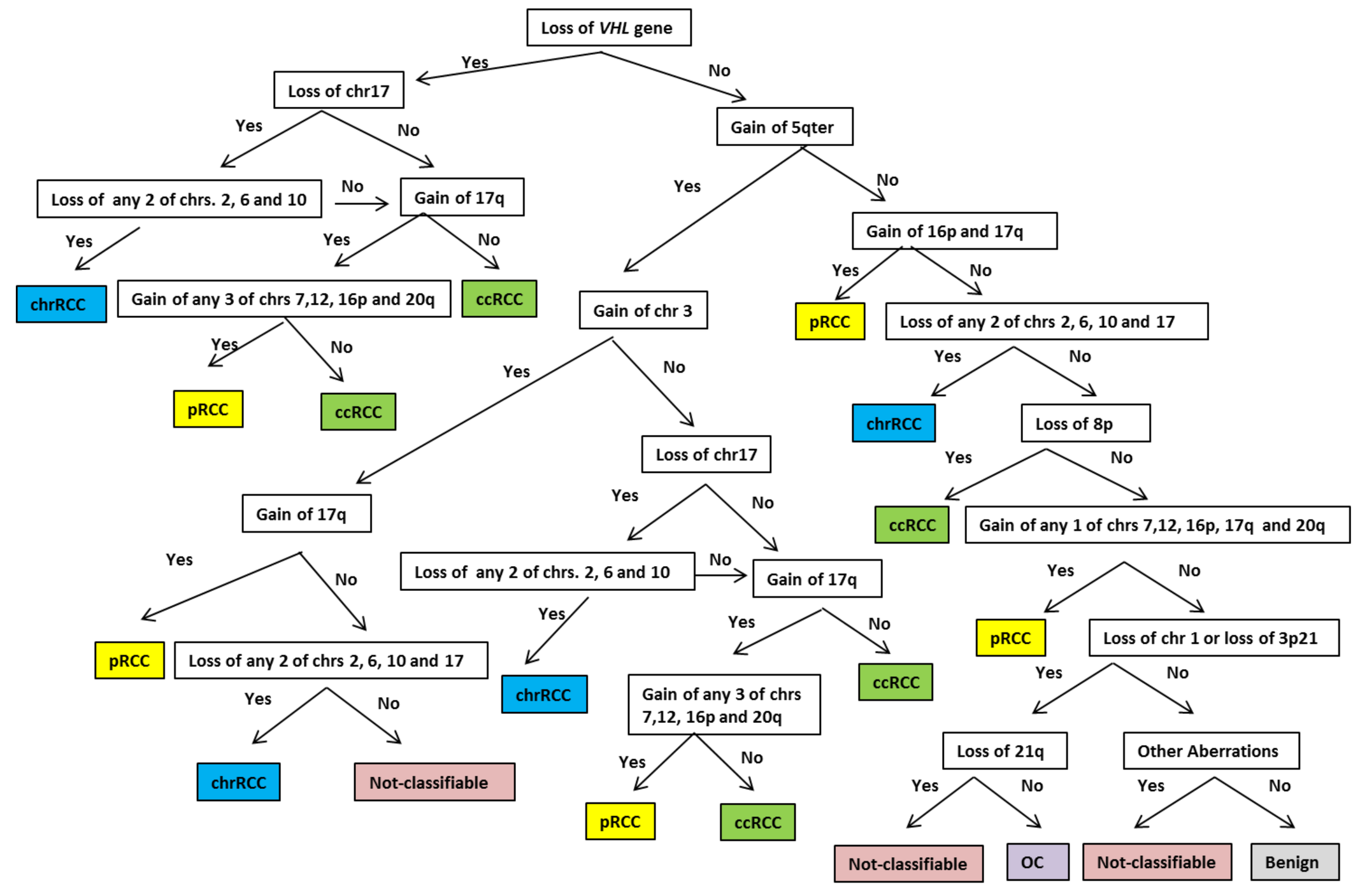


- In house FISH study:
  127 renal cortical neoplasms needle biopsies (*ex vivo*)
- Literature Search

KidneyPath™

A decision-tree algorithm based on 16 genomic aberrations

Loss									Gain						
VHL (3p25)	1	2	6	8p	10	17	21q	3p21	3	5qter	7	12	16p	17q	20q

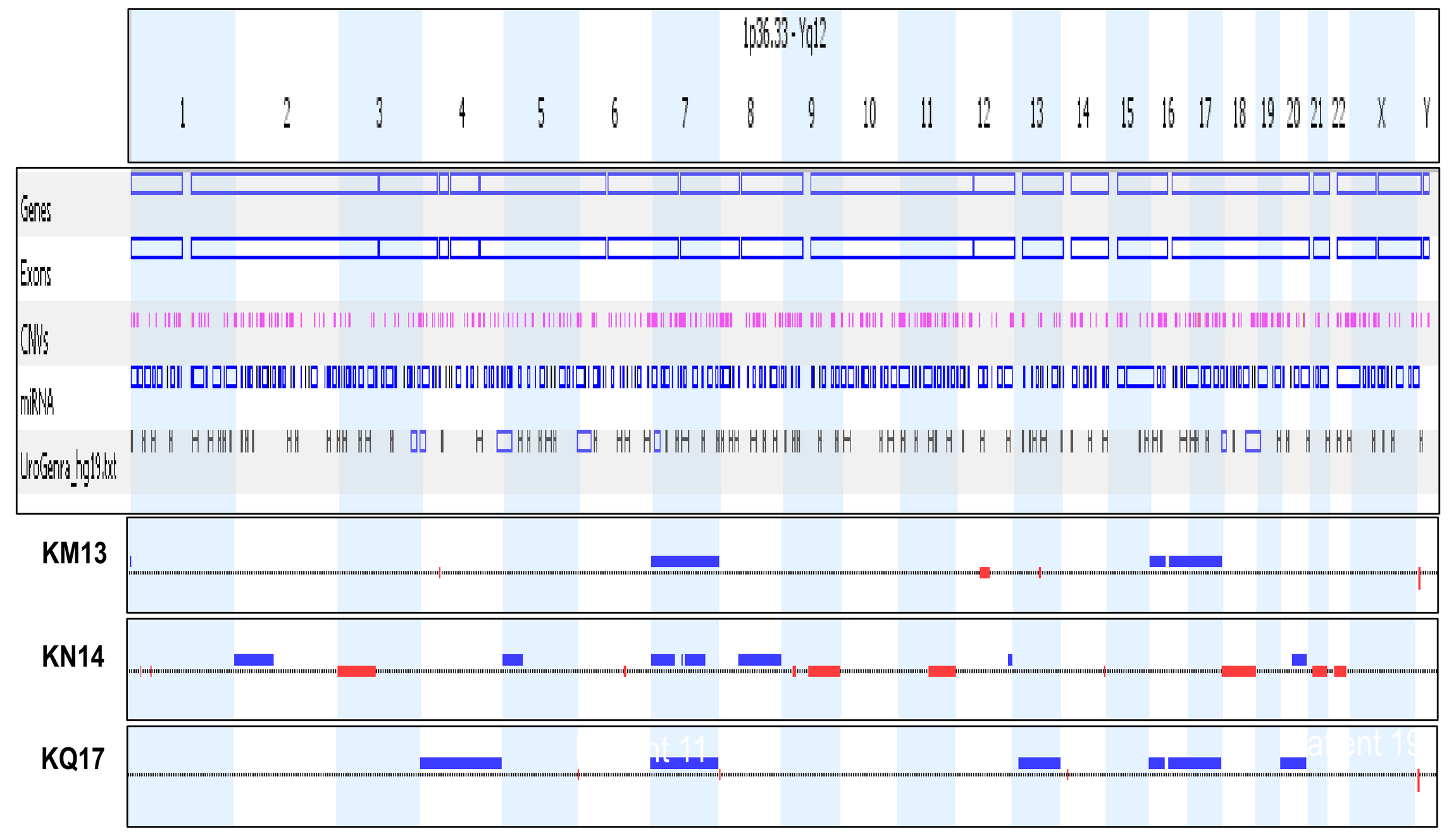


ccRCC = Clear Cell RCC; OC = Oncocytoma
pRCC = Papillary RCC; Not-classifiable = Aberrations detected but cannot be classified into a specific subtype
chrRCC = Chromophobe RCC; Benign = No aberration detected

METHODS AND RESULTS

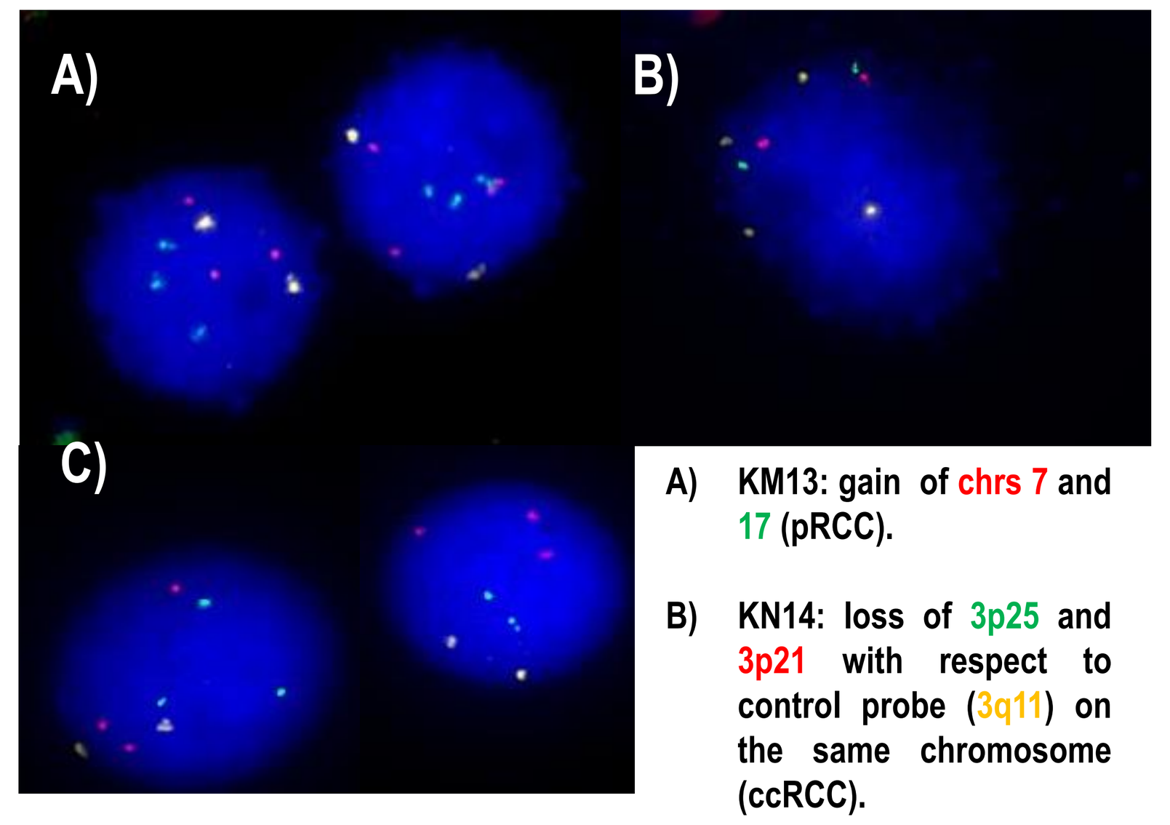
Array-CGH Analysis:

- DNA extraction resulted in yields >200ng after QC
- Reference DNA: Sex-matched normal DNA (Promega)
- Digested and labeled DNA hybridized to either targeted oligonucleotide microarray UroGenRA™ (biopsies) or whole genome 244k array (resected specimens) and analyzed according to manufacturer (Agilent)
- Identification of genomic aberrations
  Nexus Copy Number Analysis 6.1 (BioDiscovery Inc.)
  (at least 8 consecutive probes with a median log ratio greater than 0.15 or less than -0.15)



FISH (FReCaD™) Analysis:

- 7-Probe combinations used
  Used to confirm aberrations detected by aCGH in 11 cases



A) KM13: gain of chrs 7 and 17 (pRCC).

B) KN14: loss of 3p25 and 3p21 with respect to control probe (3q11) on the same chromosome (ccRCC).

C) KQ17: gain of chrs 7 and 17 (pRCC).

RESULTS

39 biopsies
- 36 Biopsies: analyzable by aCGH
- 3 Biopsies: non-diagnostic (ND) by aCGH
  Low DNA yield or
  Poor array quality

36 diagnostic biopsies
- 6 Biopsies classified as Benign
- 8 Biopsies classified as ccRCC
- 13 Biopsies classified as pRCC
- 2 Biopsies classified as chrRCC
- 1 Biopsy classified as OC
- 6 Biopsies Not-classifiable

Sample ID	Biopsy Histology (Resected Histology)	Biopsy-aCGH
KB2	ccRCC	ccRCC
KE5[1]	ccRCC	Benign
KG7	ccRCC	ccRCC
KH8	ccRCC (ccRCC)	ccRCC
KN14	ccRCC (ccRCC)	ccRCC
KO15	ccRCC (ccRCC)	ccRCC
KT20	ccRCC (ccRCC)	Not-classifiable
KL38	ccRCC	Benign
KM39[2]	ccRCC	Benign
KS45	ccRCC (ccRCC)	ccRCC
KT46	ccRCC	Benign
KR18	Unclassified (ccRCC)	ccRCC
KK11[2]	pRCC	pRCC
KM13	pRCC	pRCC
KP16	pRCC (Unclassified RCC)	pRCC
KQ17	pRCC	pRCC
KW23[3]	pRCC	pRCC
KX24	pRCC	pRCC
KN40	pRCC	pRCC
KQ43	pRCC (pRCC)	pRCC
KG33	pRCC	pRCC
KY25	pRCC	pRCC
KO41	chrRCC	chrRCC
KJ36	chrRCC (chrRCC)	chrRCC
KS19	Unclassified	pRCC
KF6[1]	High grade RCC	Not-classifiable
KK37	Poorly differentiated, favor RCC	ccRCC
KD4	OC	OC
KI9	Benign AML	Benign
KD30	Benign AML	Benign
KV22[3]	Benign	Benign
KF32	Low grad oncocytic neoplasm and AML	Benign
KA27	Low grade oncocytic renal cortical neoplasm	pRCC
KH34	Low grade smooth muscle neoplasm favor AML	pRCC
KU21	High grade urothelial carcinoma	ND
KB28	ccRCC	ND
KJ10	OC	ND
KC29	ND	Not-classifiable
KI35	ND	Benign

Biopsies from the same patient are indicated with the same superscript numerical number

RESULTS

Diagnostic Yield

Histology- 37/39 (95%)

KidneyPath™- 36/39 (92%)

Diagnostic Accuracy

- 28 specimens that have clear subtype classifications in both histology and aCGH, the concordance is 23/28 (82%).
- 6 specimens histological examination did not give clear subtype classifications:
  3 (unclassified , high grade, and poorly differentiated) have high malignant potential and aCGH detected genomic aberrations in all 3.
  3 (2 low grade oncocytic neoplasms and 1 low grade smooth muscle neoplasm) have low malignant potential and aCGH detected genomic aberrations in 2/3.

In an independent study of 126 resected specimens, 97/126 (77%) were correctly classified by KidneyPath™ (data not shown).

CONCLUSIONS

- Kidney biopsy can yield sufficient material for aCGH studies.
- In this initial experience, the aCGH method provided a robust and interpretive assay for tumor subtyping. Larger experience with these novel diagnostic tools is needed to determine their utility for the genomic classification of kidney tumors from kidney needle biopsies.
- Reflex to FISH analysis for *CCND1* rearrangement (associated with oncocytoma) should be considered for specimens that are benign by aCGH.
- Mutation analysis of the *VHL* or *PBRM1* genes can be combined to increase the accuracy in the future.

CONFLICTS OF INTEREST

C.M., B.G., and J.H. are full time employees of Cancer Genetics, Inc.

INTELLECTUAL PROPERTY

CGI Patent 11/932,422; Panel for the Detection and Differentiation of Renal Cortical Neoplasms

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