Resequencing of 40 candidate genes in 960 individuals with higher and lower urinary calcium excretion suggests association with Claudin14

Authors: Hakan R. Toka[1,2], Giulio Genovese[1], David B. Mount[2], Martin R. Pollak[1] and Gary C. Curhan[2]

Dept. of Nephrology, [1]Beth Israel Deaconess Medical Center & [2]Brigham & Women's Hospital, Harvard Medical School, Boston, MA, USA

INTRODUCTION

1. Kidney stones affect ~10% of the population with increasing prevalence (men > women) and high recurrence rate (~50%)
2. Most common stone composition is calcium (Ca^{2+}) oxalate (~80% in adult men)
3. Several factors affect stone formation, Hyper-calciuria is the main factor, present in ~ 50%
4. Hypothesis: Do rare allelic variations in known candidate genes - associated with kidney stones - affect urinary Ca^{2+} excretion?

Table 1: Selected candiate genes

Candidate Genes	RefSeq ID	Exons
1. Aconitase	NM_001098	18
2. CASR	NM_000388	7
3. Citrate lyase	NM_001096	29
4. Claudin 2	NM_001171092	2
5. Claudin 8	NM_99328	1
6. Claudin 10	NM_001160100	5
7. Claudin 14	NM_001146077	3
8. Claudin 16	NM_006580	4
9. Claudin 19	NM_001123395	5
10. CLCN5	NM_000084	15
11. CLCNKA	NM_001042704	20
12. CLCNKB	NM_000085	20
13. FGF23	NM_020638	3
14. GCMB	NM_004752	5
15. Klotho	NM_004795	5
16. NHERF1	NM_004252	6
17. NHERF2	NM_001130012	7
18. NKCC2	NM_000220	27
19. PDZK1	NM_002614	10
20. PIK3C2G	NM_004570	32
21. PTH	NM_000315	3
22. PTHR	NM_000316	16
23. ROMK	NM_000338	4
24. SLC12A3	NM_000339	26
25. SLC13A2	NM_001145975	12
26. SLC13A3	NM_001193340	14
27. SLC25A1	NM_005984	9
28. SLC26A1	NM34425	4
29. SLC26A2	NM_000112	2
30. SLC26A6	NM_001040454	21
31. SLC34A1	NM_003052	13
32. SLC34A3	NM_001177316	13
33. SLC4A1	NM_000342	20
34. SLC4A2	NM_003040	23
35. SLC4A3	NM_005070	23
36. TRPV5 / CaT2	NM_019841	14
37. TRPV6 / CaT1	NM_018646	15
38. UMOD	NM_001008389	11
39. VDR	NM_000376	11
40. WNK4	NM_032387	19

METHODS

1.Study population: 960 individuals with higher and lower urinary Ca^{2+} excretion selected from Nurses Health Study (NHS I&II; >236,000 females) and Health Professional Follow-up Study (HPFS; > 51,000 males) based on availability of DNA, 24h urine collection data (Ca^{2+}, oxalate, citrate, phosphate) and medical history.
2. N = 40 candidate genes were selected based on disease-causing ability in human, feasibility to perform functional studies and preliminary genome wide association study (GWAS) data.
3. Re-sequencing technology: Target DNA enrichment utilizing RainDance (droplet-based) PCR technology (RDT) and next-generation sequencing (NGS) on the Illumina platform.

Table 2: DNA pooling of 960 subjects with higher vs. lower urinary Ca^{2+} excretion

Cohort	# of pools (sets of 15 + 20 samples)	# of individuals
NHS I, high urinary Ca^{2+}	7 (4 x 15 + 3 x 20)	120
NHS II, high urinary Ca^{2+}	6 (6 x 20)	120
HPFS, high urinary Ca^{2+}	13 (4 x 15 + 9 x 20)	240
NHS I, low urinary Ca^{2+}	7 (4 x 15 + 3 x 20)	120
NHS II, low urinary Ca^{2+}	6 (6 x 20)	120
HPFS, low urinary Ca^{2+}	13 (4 x 15 + 9 x 20)	240

-> 52 sample pools

Figure 1: Droplet-based target enrichment

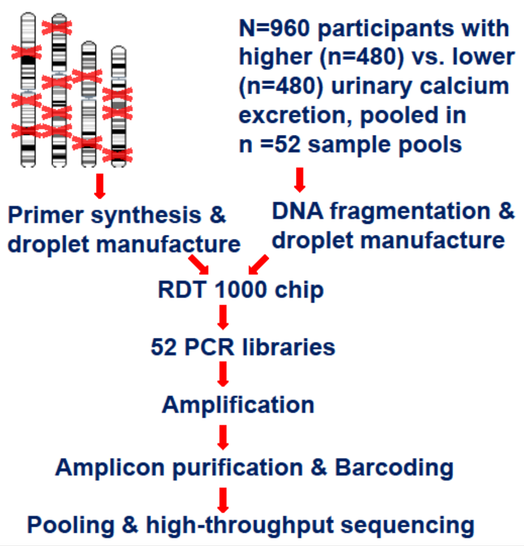
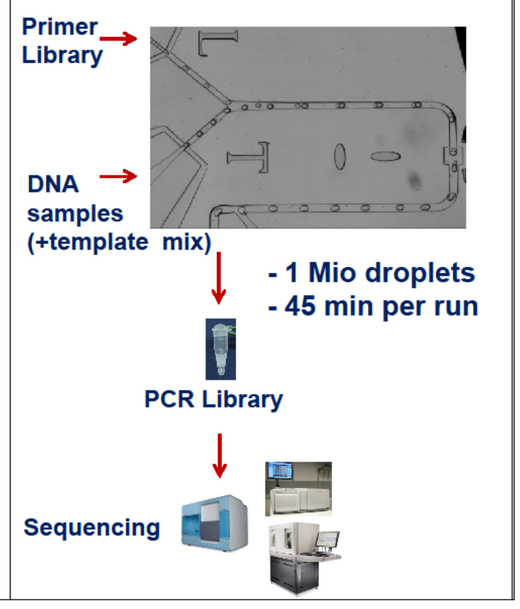


Figure 2: RainDance technology



RESULTS

Table 3: N = 615 samples with successful > 20x sequence coverage

	Higher urinary Ca^{2+} n=260 (210-465 mg/d)		Lower urinary Ca^{2+} n=355 (18-165 mg/d)	
	Stone, n=164	No stone, n=96	Stone, n=182	No stone, n=173
Ca^{2+} (mg/d)	319.5 57	309.4 55.9	99.7 36.7	100.9 35.2
Age	58.8 9.6	59.7 8.4	63.9 10.8	61.4 8.3
BMI	27.7 5.3	27.0 4.8	27.7 6.0	26.0 4.5

Figure 3: Total of 1,572 sequence nucleotide variants (SNVs) identified with Szygy software

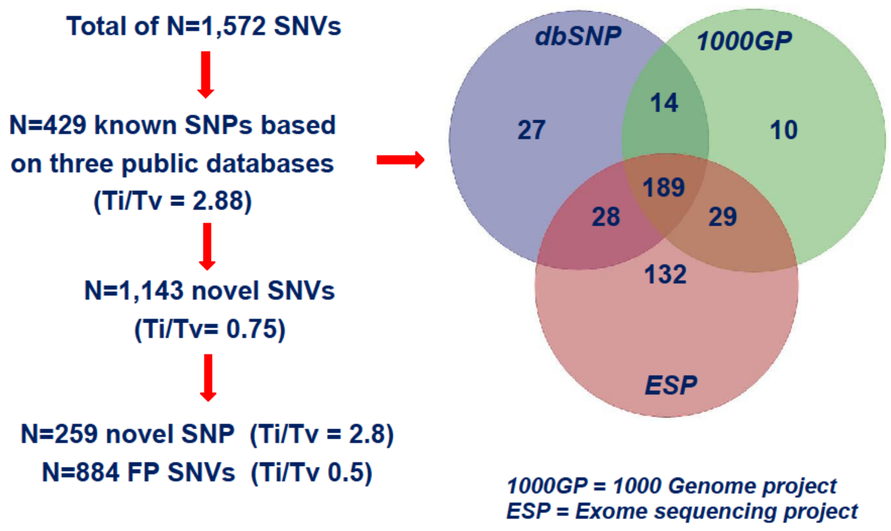
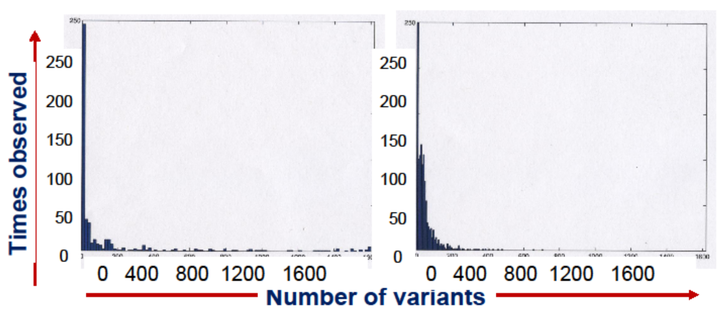


Figure 4: Known and novel SNVs



429 known variants
Ti/Tv ratio: 2.88

1,143 novel variants
Ti/Tv ratio: 0.75

1. Transition (Ti) = Mutation of a purine to another purine (A<->G) or a pyrimidine to another pyrimidine (C<->T)
2. Transversion (Tv) = Substitution of a purine for a pyrimidine or vice versa
3. In nature, Ti are more common than Tv -> Ti/Tv ~ 3:1
-> Observed Ti/Tv ratio is mixture of true-pos. (TP) and false pos. (FP) SNVs
4. If all detected SNVs are true, Ti/Tv will be ~3; if all SNVs are false Ti/Tv = 0.5
-> Common novel variants are most likely artifacts of sequencing, therefore novel variants were only considered in our study if observed 1, 2 or 3 times in the whole dataset (n=259; Ti/Tv -> 2.8). Remaining variants (n=884) had a Ti/Tv ratio of 0.5.

Figure 5: Distribution of SNVs seen ≤ 3 times versus > 3 times

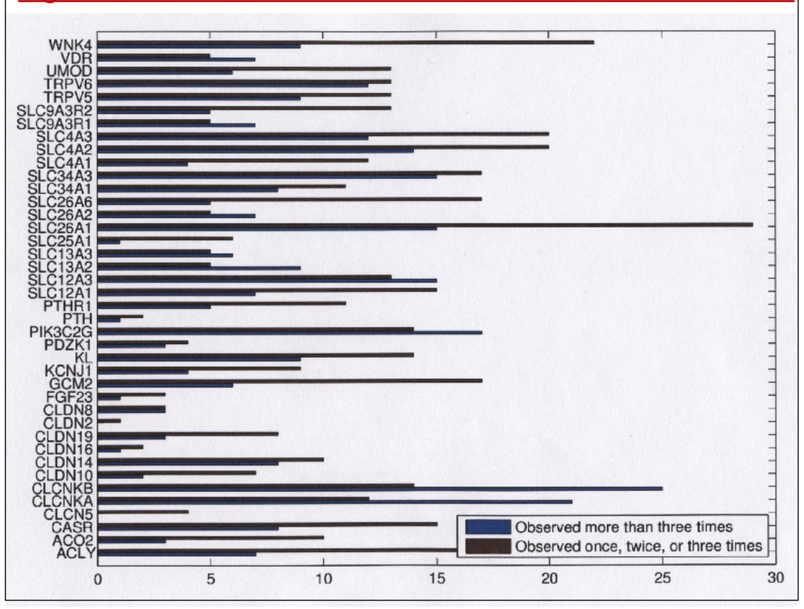


Table 4: Claudin14 SNPs with unadjusted Chi- square P values; rs 113831133 suggests association with lower urinary Ca^{2+}

SNP ID (rs..)	Class	Nucleotide	Lower Urine Ca^{2+}	Higher Urine Ca^{2+}	P value
-	Miss.	c.664G>T	2.2%(16/710)	1.5%(8/520)	0.41
219779	Silent	c.243C>T	24.2%(172/710)	23.8%(124/520)	0.89
113831133	Miss.	c.11C>T	4.1%(29/710)	1.1%(6/520)	0.003
74934405	Silent	c.333A>C	4.1%(29/710)	4.4%(23/520)	0.77
219780	Silent	c.687G>A	18.4%(131/710)	18.9%(98/520)	0.82

SUMMARY

1. Analysis of rare allelic variants (frequency<2%) in 40 genes associated with Ca^{2+} based kidney stone disease did not produce any significant results; no difference of SNP frequencies was observed in lower vs. higher urinary Ca^{2+} excretion groups in the tested population

2. Unadjusted analysis of more common variants (frequency 2-5%) suggested association with Claudin14 (CLDN14) SNP rs113831133 (P value = 0.003). When corrected for multiple comparison, this finding didn't reach statistical significance.

3. Two common synonymous SNPs (rs219779, rs219780) in CLDN14 were associated with kidney stones and bone mineral density in a large Icelandic GWAS (Thorleifsson et al, 2009); these SNPs did not show any association in our study. Furthermore, CLDN14 has been shown to be an important regulator of paracellular Ca^{2+} transport and re-absorption in the thick ascending limb. Our data combined with these studies suggest an important role for CLDN14 in urinary Ca^{2+} excretion.

4. Additional genes may need to be re-sequenced in appropriate sample sets to test the hypothesis that the combination of rare coding allelic variants determine predisposition to urinary Ca^{2+} excretion and/or kidney stones.

REFERENCES

-Saigal et al, Kidney Int, 2005; 68:1808
-Stamatelou et al, Kidney Int, 2003, 63: 1817
-Taylor et al, JAMA 2005; 293(4):455
-Gillen et al, Am J Kidney Dis, 2005, 46(2)
-Auge et al, Endo Metab Clin , 2002; 31:1065
-Daudon et al, Drugs 2004, 64:245
-Coe et al, JCI, 2005, 115:2598
-Curhan et al, Kidney Int, 2001, 59(6):2290
-Marangella et al, Urol Int, 2004; 72(suppl1):6
-Goldfarb et al, Kidney Int, 2005; 67(3):1053
-Loredo-Osti et al, Kidney Int, 2005; 68: 966
-Curhan et al, NEJM, 1993, 328(12): 833
-Curhan et al, Ann Intern Med, 1997, 126:497
-Curhan et al, Arch Intern Med, 2004, 164:885
-Moe and Bonny, JASN, 2005:729
-Thorleifsson et al, Nat Genet, 2009, 41(8):926
-Toka et al, JASN, 2012, 23(11):1879-90
-Gong et al, EMBO J, 2012 Feb 28;31(8

50ERA-EDTA
53--MP
Genetic diseases and molecular genetics
Hakan Toka
Poster presented at: ERA-EDTA 50th Congress Istanbul, Turkey May 18-21, 2013
PosterSessionOnline.com