SUPPLEMENTARY APPENDIX

Brief Communication

Renal hypodysplasia associated Wnt4 variant reveals molecular mechanism leading to aberrant canonical Wnt signaling.

[a,b]Asaf Vivante M.D., [b]Michal Mark-Danieli Ph.D., [g]Miriam Davidovits M.D, [b]Harari-Steinberg O Ph.D, [b] Omer D M.Sc., [b] Gnatek Y M.Sc., [g] Roxana Cleper M.D., [h]Daniel Landau M.D. [g,k] Yael Kovalski M.D., [i]Irit Weissman M.D., [j]Israel Eisenstein M.D., [e] Michalle Soudack M.D., [l] Haike Reznik Wolf, Ph.D, [m] Naomi Issler MD, [c]Danny Lotan M.D., [d]Yair Anikster MD, Ph.D., and [a,b,c] Benjamin Dekel M.D, Ph.D.

[a] Department of Pediatrics, Talpiot Medical Leadership Program, [b] Pediatric Stem Cell Research Institute, [c] Pediatric Nephrology Unit, [d]Metabolic Disease Unit, [e] Pediatric Imaging, Edmond and Lily Safra Children's Hospital, , [l] Genetic institute, Sheba Medical Center, Tel Hashomer , Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv. [f]Department of Molecular Genetics, MIGAL—Galilee Technology Center, Kiryat Shmona and Tel-Hai Academic College, Israel. [g]Department of Pediatric Nephrology, Schneider Children's Medical Center of Israel, Petah Tiqwa 49202, Israel [h] Department of Pediatrics A and Pediatric Nephrology, Saban Children's Hospital, Soroka Medical Center, Ben Gurion University, Beer Sheva, Israel. [i] Nephrology Unit, Western Galilee Hospital, Nahariya, Israel.[j] Pediatric Nephrology Unit, Meyer Children's Hospital, Rambam Health Care Campus; Faculty of Medicine, Technion – Israel Institute of Technology; Haifa, Israel . [k] Children's Medical Center, Ramla, Israel. [m] Centre for Nephrology & ICH, University College London, London, UK.

List of contents:

Contents…….…….1

Supplementary Methods……..……...2
Fluorescence-activated cell sorting (FACS) analysis…….……2
Cell treatments……2
Clonogenic potential evaluation…...……...2

Supplementary Results……..……..……....3-4
Genetic and clinical characterization of families with *PAX2* and *HNF1B* mutations……...……..……3-4

Supplementary Tables……..…..……5
Supplementary Table 1: Renal phenotype of 20 probands with isolated familial RHD……5

Supplementary Figures..……..…….……..…6-8
Supplementary Figure 1: Pedigree Structures of the Twenty Families with Familial Renal Hypodysplasia Studied ……..……6
Supplementary Figure 2: Pedigree of Family 1…….……....…7
Supplementary Figure 3: Pedigrees of Family 2 and Family 3…….…….8

References…….9-10

SUPPLEMENTARY METHODS

Fluorescence-activated cell sorting (FACS) analysis

Cells were re-suspended in FACS buffer consisting of 0.5% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis) and 0.02% sodium azide in PBS. The surface antigens were labeled by incubation with fluorochrome conjugated primary antibodies (HumanCD56-PE Backman coulter, HumanCD34-FITC Backman coulter, HumanCD45-APC Sino Biological Inc.) at a concentration of 1µg primary antibody per 10^6, for 45min in the dark at 4°C to prevent internalization of antibodies. All samples were stained to 7-amino-actinomycin-D (7AAD; eBioscience, San Diego, CA) for viable cell gating. All washing steps were performed in FACS buffer. Quantitative measurements were made from the cross point of the IgG isotype graph with the specific antibody graph.

Cell treatments

HFK-PC Cells were treated for 72 hours with growth medium supplemented with 3µg/ml DKK1 (R&D systems), 7µg/ml sFRP1 (R&D systems) or with 10µg/ml WIF (R&D systems).

Clonogenic potential evaluation

To assess the stem/progenitor functional potential of the HFK-PC cells we have characterize the clonogenic abilities of the cells. Our lab performs on a regular basis single-cell clonogenicity assays for human kidney-derived cells (previously described by Pode-Shakked N, *J Cell Mol Med* 2008). Briefly, 6 cells at limited-dilution concentration are plated in matrigel (BD) - coated 96-well micro well plates in culture media and are further expanded. The number of colonized wells is recorded after 3-4 weeks.

SUPPLEMENTARY RESULTS

Genetic and clinical characterization of three families with *PAX2* and *HNF1B* mutations: The three families with *PAX2* and *HNF1B* mutations found in our study were from different medical centers. All were originally labeled as "non-syndromic" isolated RHD by their nephrologists, and the genetic etiology of their condition was not suspected on clinical grounds.

***PAX2* mutation**

Two brothers with severe bilateral RHD were found to have a previously reported heterozygous *PAX2* nonsense mutation (c.75InsG - p.fs52X, Family #1),[1] responsible for renal-coloboma syndrome (RCS), which includes eye coloboma and hearing impairment. As a result these patients were referred for further clinical evaluation, which revealed mild retinal coloboma for both and mildly abnormal hearing status for one. Interestingly, both parents were negative for the mutation and exhibited a normal ultrasonographic renal phenotype, suggesting germline mosaicism in this case (Supplementary Figure 2).

***HNF1B* mutations**

Nine affected subjects from two unrelated families were found to harbor two different novel *HNF1B* heterozygous mutations (family #2 and #3). Family #2 had a novel frame shift mutation, c.del 983C - p.fs375X. The mutation was fully segregated among affected and unaffected family members but demonstrated variable expression: One subject had only MODY type 5 with normal renal US, while all other affected individuals had isolated RHD of differing severity (Figure 3S). Family #3 had a novel missense mutation, c.a398g - p.N133S, fully segregated among family members. Affected subjects presented variable expression with differing severities of RHD and hyper-uricemia, characteristic of *HNF1B* mutations[2-4] (Supplementary Figure 3).

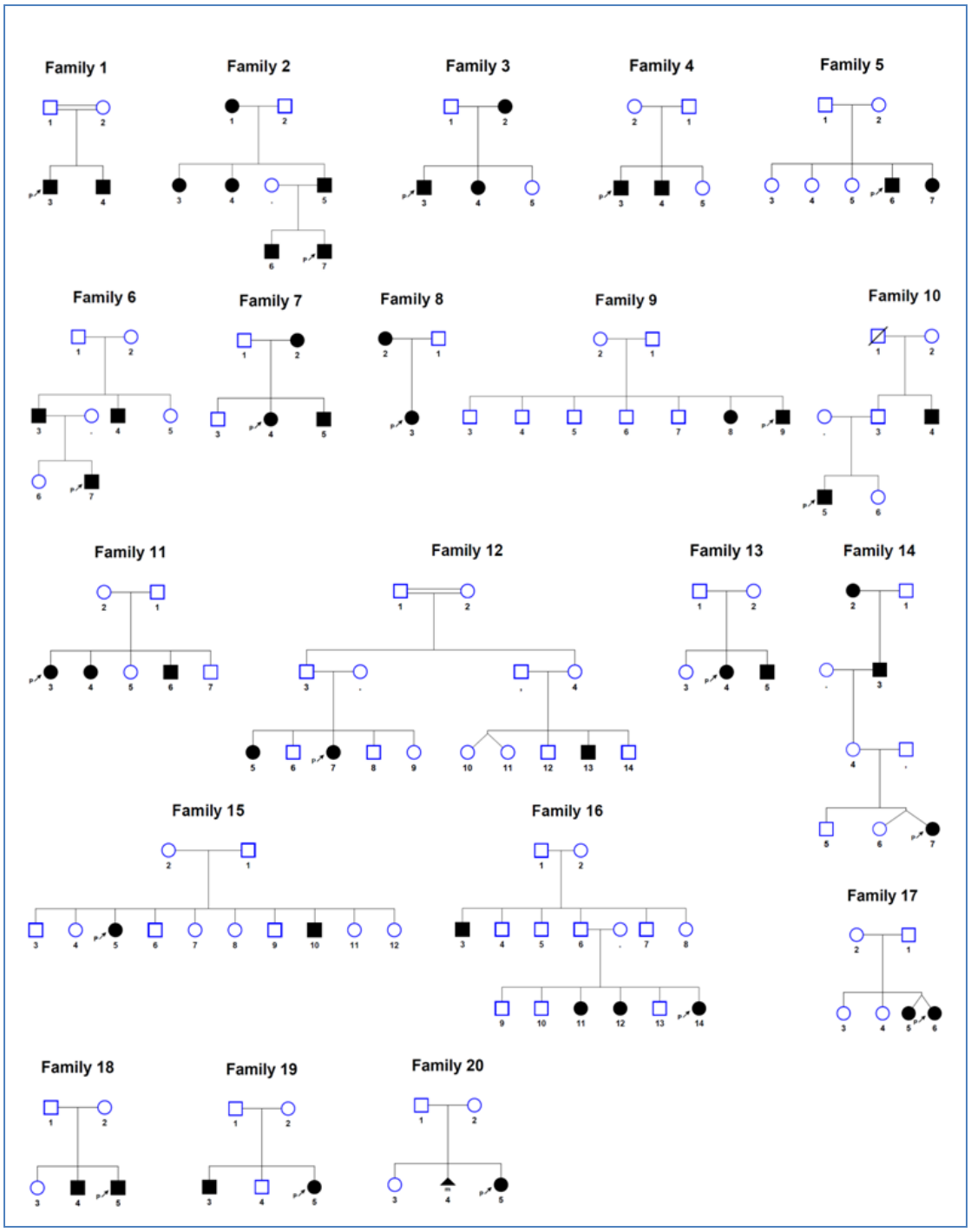
Eighteen of the 20 probands we studied had been diagnosed following abnormal fetal ultrasonography. This finding triggered further investigation of other asymptomatic family members, often revealing additional cases. None of the above disease-causing mutations was suspected on clinical grounds prior to the current study, and affected patients were not clinically distinguished from other RHD patients without mutations. This highlights several important clinical implications. First, syndromic RHD can initially be presented as isolated RHD. Following the current study, mutation-carrying subjects were further evaluated for subtle clinical signs, revealing mild renal coloboma in two sibs with *PAX2* mutation and hyperuricemia in two sibs with *HNF1B* mutation. Second, familial RHD can be mistakenly considered sporadic when the familial nature of the malformation is overlooked due to lack of thorough family evaluation. For example, both families with *HNF1B* mutations included adult subjects with CKD who were not considered by their physician to have congenital lesions. Thus the pediatric congenital renal malformation within these families was not recognized as related to the adult's kidney phenotype, leading to under-recognition of the familial genetic syndrome. Diagnosis of congenital RHD cases during adulthood may be complicated.[5] Many patients are asymptomatic during childhood and present late in adulthood with CKD and bilateral small kidneys, a common pathway for numerous other CKD etiologies that cannot easily be distinguished. On the other hand, meticulous clinical evaluation of all family members may reveal new and presumably "unaffected" subjects. Following our study, two new affected subjects were identified. Importantly, in one case (family 2, subject #5) this recognition, which revealed an *HNF1B* mutation, excluded the subject from donating a kidney to his severely affected sib with ESRD. He was initially considered a candidate since his US imaging showed mild renal size asymmetry, considered within normal range.

SUPPLEMENTARY TABLES

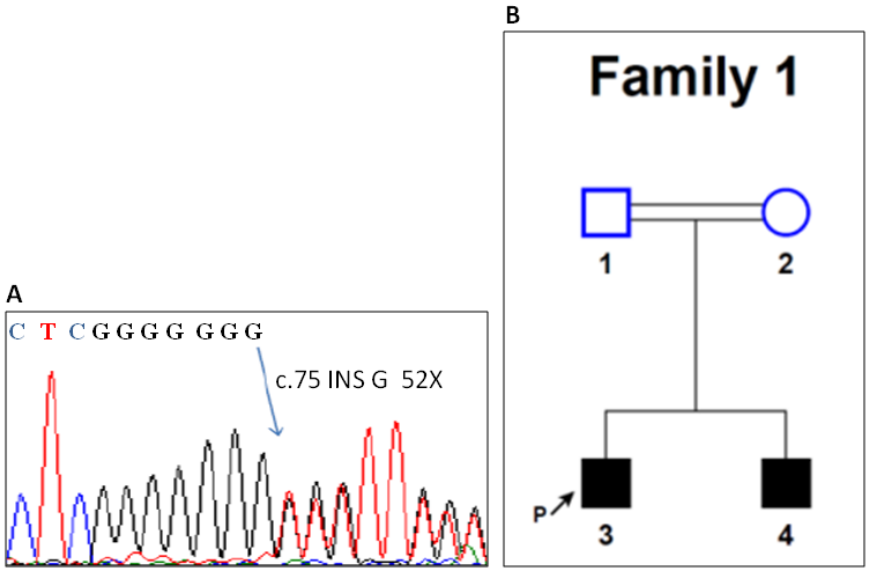
Family Number	Age	Sex	Renal Phenotype	Affected Family Members (age)	Renal Phenotype of Affected Family Members
1	4	m	Bilateral hypodysplasia	1.Brother (3)	1. Bilateral hypodysplasia
2	2	f	Bilateral Hypodysplasia	1.Brother (4) 2.Father (32) 3.Unt (34) 4.Unt (36) 5.Grandmother (61)	1.Right aplasia 2.Left hypodysplasia 3.Bilateral hypodysplasia 4. Normal renal US, MODY5. 5.Bilateral hypodysplasia
3	21	m	Bilateral hypodysplasia	1.Mother (50) 2.Sister (17)	1.Bilateral hypodysplasia 2.Bilateral hypodysplasia
4	14	m	Left hypodysplasia	1.Brother (8)	1.Left hypodysplasia
5	6	f	Left hypodysplasia	1.Sister (5)	1.Right hypodysplasia
6	4	m	Left hypodysplasia	1.Father (36) 2.Uncle (28)	1. Left hypodysplasia 2. Left hypodysplasia
7	3	f	Bilateral hypodysplasia	1.Brother (1.5) 2.Mother (33)	1.Bilateral hypodysplasia 2.Right hypodysplasia
8	3	f	Left hypodysplasia	1.Mother (33) 2.Gradfaher (dead)	1.Left aplasia 2.ESRD
9	11	m	Right hpodysplasia	1.Sister (13)	1.Right hypodysplasia
10	19	m	Bilateral hypodysplasia	1.Uncle (40) 2.Grandfather (died)	1.Bilateral hypodysplasia 2.CKD
11	15	f	Right hypodysplasia	1.Sister (13) 2.Brother (12)	1.Left hypodysplasia 2.Lefy hypodysplasia
12	8	f	Left hypodysplasia	1.Sister (13) 2.Cousin (8)	1.Left aplasia 2.Right Hypodysplasia
13	13	f	Right hypodysplasia	1.Brother (12)	1.Left hypodysplasia
14	4	f	Right hypodysplasia	1.Grandfather (62) 2.Great grandmother (80)	1.Right aplasia 2.Right Hypodysplasia
15	10	f	Right hypodysplasia	1.Brother (4)	1. Left hypodysplasia
16	2	m	Right hypodysplasia	1.Sister (9) 2.Sister(15) 3.Uncle (35)	1.Right hypodysplasia 2.Bilateral hypodysplasia 3.Bilateral hypodysplasia
17	2	f	Left aplasia	1.Sister (2)	1.Lest hypodysplasia
18	8	M	Left aplasia	1.Brother (10)	1.Left aplasia
19	1	F	Bilateral hypodysplasia	1.Brother (11)	1.Bilateral hypodysplasia
20	1	F	Bilateral hypodysplasia	1.Sib (TOP)	1. Bilateral hypodysplasia

Supplementary Table 1 – Renal Phenotype of 20 Probands with Isolated Familial RHD. RHD – Renal Hypodysplasia. MODY 5 – Maturity Onset Diabetes of the Young. ESRD – End Stage Renal Failure. CKD – Chronic Kidney Disease. TOP – Termination of Pregnancy.

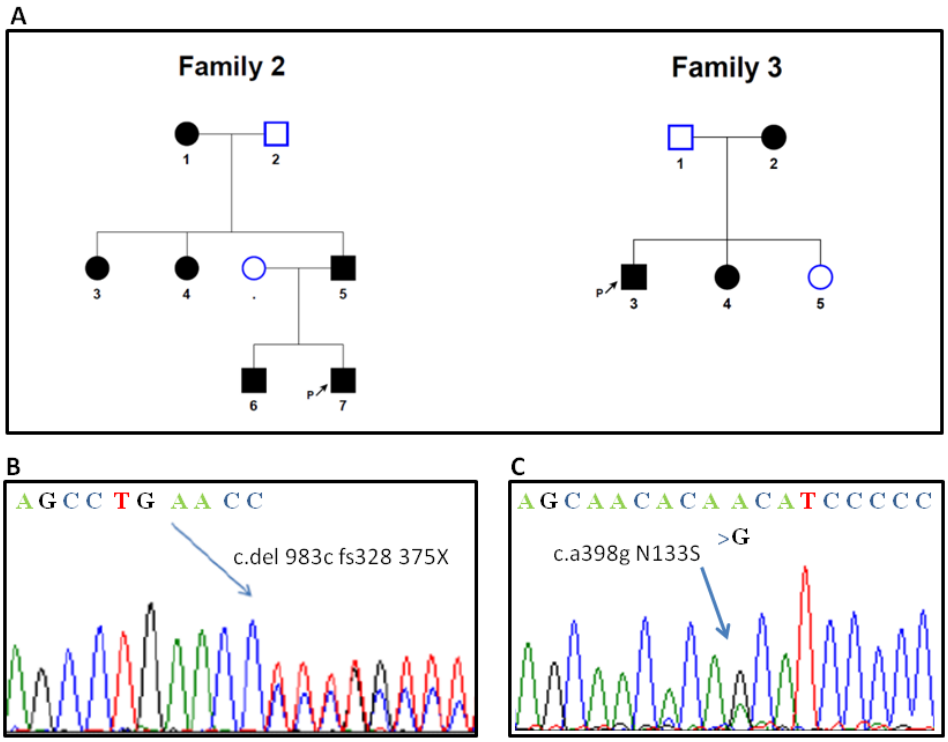
SUPPLEMENTARY FIGURES



Supplementary Figure 1 – Pedigree Structures of the Twenty Families with Familial Renal Hypodysplasia Studied. Arrows identify the index cases (probands). DNA was available from all individuals except for Subjects #1and #2 in Family 10 and Subject #1 in Family14. Proband family numbers correspond to Table 1.



Supplementary Figure 2 – Pedigree of Family 1. Pedigree of Family 1 (Panel B) demonstrating a *PAX2* c.75InsG mutation (Panel A).This mutation cosegregated with the presence of bilateral renal hypoplasia in the family. Each of the sibs carried a mutant allele while unaffected parents did not, suggesting the presence of germline mosaicism. *PAX2* mutations, responsible for the RCS, were also reported among patients with isolated RHD with only subtle extra-renal manifestations.[6] The condition in Family 1 suggestive of germline mosaicism has been previously reported in two families with *PAX2* mutations.[6-7] Squares indicate male family members and circles female family members; black filled squares indicate that the patients are affected. Double lines between parents indicate that the parents are related.



Supplementary Figure 3 – Pedigrees of Family 2 and Family 3. The pedigrees of Family 2 and Family 3 show six and three affected members, respectively (Panel A). Squares indicate male family members and circles female family members; filled squares and circles indicate that the patients are affected. The *HNF1B* mutations, c.del 983C (Panel B) and c.a398g (Panel C) cosegregated with the presence of HNF1B-related phenotype in both families. Family 2 clearly demonstrates the broad clinical spectrum associated with *HNF1B* mutations, as Patient #3 is the only affected individual with MODY type 5 and has normal renal ultrasound. In Family 3 all affected members were found to have additional hyper-uricemia which is characteristic of *HNF1B* mutations. *HNF1B* mutations, which are responsible for RCAD, have been recognized to result in a wide clinical spectrum that includes highly variable renal phenotype, genital tract abnormalities, abnormal liver enzymes, hyperuricemia and hypomagnesimemia.[2-4]

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