Mikromacierz kliniczna jako narzędzie do identyfikacji wrodzonych wad rozwojowych układu moczowego o uwarunkowaniu genetycznym

Materna-Kiryluk A., Zaniew M., Drożdż D., Krzemień G., Miklaszewska M., Mizerska-Wasiak M., Sikora P., Szczepańska M, Szmigielska A., Tkaczyk M, Adamczyk P., Borszewska-Kornacka M, Jarmoliński T., Korecka K., Pawluch R, Tomczyk D., Zachwieja K., Adamska Z, Baranowska-Jaźwiecka A, Bieniaś B., Cichańska L., Habura I., Jander A, , Karczewski M., Kołbuc M., Krakowska A., Machura E, Moczulska A., Pawlaczyk K., Rychwalska M., Siniewicz-Luzeńczyk K., Szadkowska A., Sanna-Cherchi S.,Gharavi A., Latos-Bieleńska A.

COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK

UNIWERSYTET MEDYCZNY IM. KAROLA MARCINKOWSKIEGO W POZNANIU

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Centra Genetyki Medycznej

PRWWR
POLSKI REJESTR WRODZONYCH WAD ROZWOJOWYCH

- Katedra i Zakład Genetyki Medycznej UM w Poznaniu
- Centrum Genetyki Medycznej Genesis
- Polski Rejestr Wrodzonych Wad Rozwojowych i
- liczna grupa lekarzy głównie nefrologów z wielu ośrodków w Polsce współpracuje od 2007r z Oddziałem Nefrologii Uniwersytetu w Nowym Jorku

Badania nad genetycznym uwarunkowaniem wad układu moczowego.

Badania mikromacierzy - narzędzie do analizy genomu o wysokiej rozdzielczości i wykrywania submikroskopowych rearanżacji genomowych określanych jako warianty lub zmienności kopii

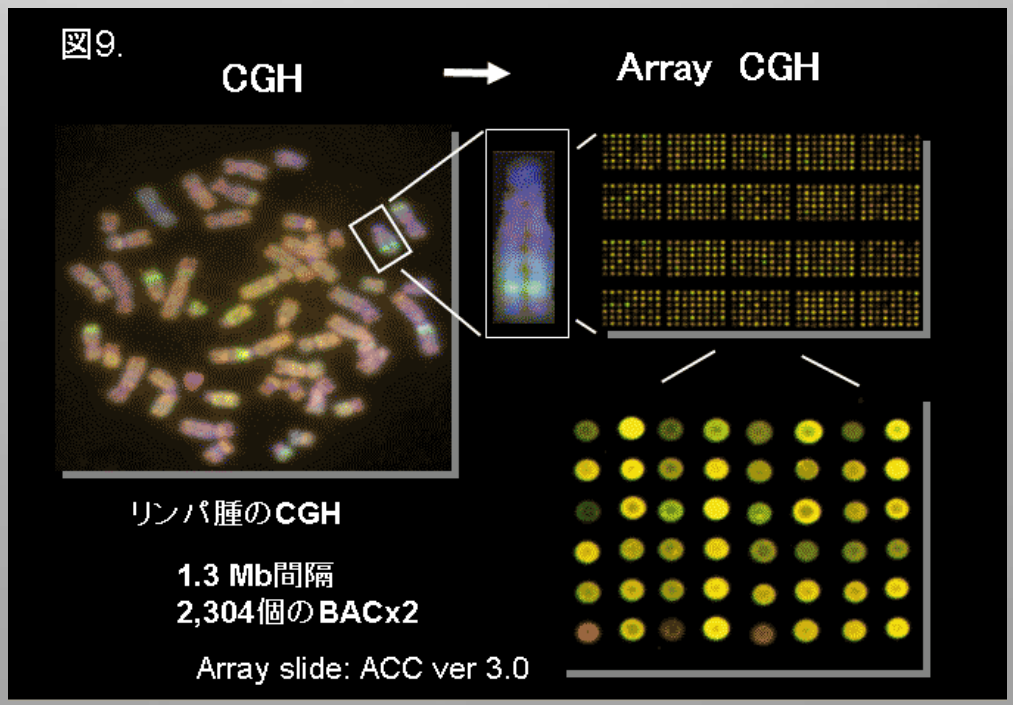
(CNVs ang. *copy number variations*).

Nie są one wykrywane w klasycznych badaniach cytogenetycznych.

Mikromacierz DNA (ang. *DNA microarray*)

Mikromacierze DNA (chipy DNA) - zbiór sond molekularnych z sekwencjami jednoniciowych cząsteczek DNA, które mają zdolność specyficznego wiązania komplementarnych sekwencji badanego DNA.

Florescencja sondy zależy od ilości cząsteczek związanego badanego DNA, umożliwiając odczyt delecji i duplikacji.



Copy-Number Disorders Are a Common Cause of Congenital Kidney Malformations

Simone Sanna-Cherchi,[1,2] Krzysztof Kiryluk,[1] Katelyn E. Burgess,[1] Monica Bodria,[3] Matthew G. Sampson,[5] Dexter Hadley,[4] Shannon N. Nees,[1] Miguel Verbitsky,[1] Brittany J. Perry,[1] Roel Sterken,[1] Vladimir J. Lozanovski,[6] Anna Materna-Kiryluk,[7] Cristina Barlassina,[8,9] Akshata Kini,[4] Valentina Corbani,[10] Alba Carrea,[3] Danio Somenzi,[11] Corrado Murtas,[3] Nadica Ristoska-Bojkovska,[6] Claudia Izzi,[12] Beatrice Bianco,[11] Marcin Zaniew,[13] Hana Flogelova,[14] Patricia L. Weng,[1] Nilgun Kacak,[1] Stefania Giberti,[11] Maddalena Gigante,[15] Adela Arapovic,[16] Kristina Drnasin,[17] Gianluca Caridi,[3] Simona Curioni,[8] Franca Allegri,[18] Anita Ammenti,[19] Stefania Ferretti,[20] Vinicio Goj,[21] Luca Bernardo,[21] Vaidehi Jobanputra,[22] Wendy K. Chung,[23] Richard P. Lifton,[24] Stephan Sanders,[24] Matthew State,[24] Lorraine N. Clark,[25] Marijan Saraga,[16,26] Sandosh Padmanabhan,[27] Anna F. Dominiczak,[27] Tatiana Foroud,[28] Loreto Gesualdo,[15] Zoran Gucev,[6] Landino Allegri,[11] Anna Latos-Bieleńska,[7] Daniele Cusi,[8] Francesco Scolari,[12] Velibor Tasic,[6] Hakon Hakonarson,[4,5] Gian Marco Ghiggeri,[3] and Ali G. Gharavi[1,*]

We examined the burden of large, rare, copy-number variants (CNVs) in 192 individuals with renal hypodysplasia (RHD) and replicated findings in 330 RHD cases from two independent cohorts. CNV distribution was significantly skewed toward larger gene-disrupting events in RHD cases compared to 4,733 ethnicity-matched controls ($p = 4.8 \times 10^{-11}$). This excess was attributable to known and novel (i.e., not present in any database or in the literature) genomic disorders. All together, 55/522 (10.5%) RHD cases harbored 34 distinct known genomic disorders, which were detected in only 0.2% of 13,839 population controls ($p = 1.2 \times 10^{-58}$). Another 32 (6.1%) RHD cases harbored large gene-disrupting CNVs that were absent from or extremely rare in the 13,839 population controls, identifying 38 potential novel or rare genomic disorders for this trait. Deletions at the *HNF1B* locus and the DiGeorge/velocardiofacial locus were most frequent. However, the majority of disorders were detected in a single individual. Genomic disorders were detected in 22.5% of individuals with multiple malformations and 14.5% of individuals with isolated urinary-tract defects; 14 individuals harbored two or more diagnostic or rare CNVs. Strikingly, the majority of the known CNV disorders detected in the RHD cohort have previous associations with developmental delay or neuropsychiatric diseases. Up to 16.6% of individuals with kidney malformations had a molecular diagnosis attributable to a copy-number disorder, suggesting kidney malformations as a sentinel manifestation of pathogenic genomic imbalances. A search for pathogenic CNVs should be considered in this population for the diagnosis of their specific genomic disorders and for the evaluation of the potential for developmental delay.

Introduction

Congenital malformations of the kidney and urinary tract are present in 3–7 out of 1,000 births,[1,2] accounting for 23% of birth defects.[3] These malformations account for 40%–50% of pediatric and 7% of adult end-stage renal disease worldwide.[4–6] Among these malformations, renal aplasia, agenesis, hypoplasia, and dysplasia (referred to hereafter as renal hypodysplasia [RHD]) represent severe forms of disease with a profound impact on long-term renal survival.[6] Currently, the diagnosis is based on prenatal or postnatal imaging studies demonstrating absent or small kidneys with or without additional urinary-tract defects. The etiology of the majority of cases remains unknown. However, multiple lines of evidence suggest a strong genetic contribution to the pathogenesis of these birth defects. For example, many cytogenetic abnormalities and genetic syndromes are associated with

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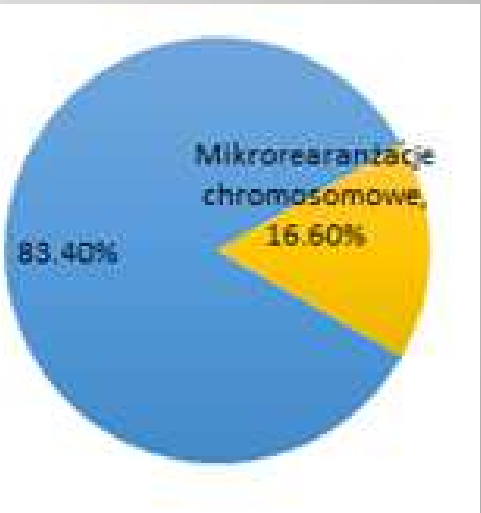

ORIGINAL ARTICLE

Mutations in *DSTYK* and Dominant Urinary Tract Malformations

S. Sanna-Cherchi, R.V. Sampogna, N. Papeta, K.E. Burgess, S.N. Nees, B.J. Perry, M. Choi, M. Bodria, Y. Liu, P.L. Weng, V.J. Lozanovski, M. Verbitsky, F. Lugani, R. Sterken, N. Paragas, G. Caridi, A. Carrea, M. Dagnino, A. Materna-Kiryluk, G. Santamaria, C. Murtas, N. Ristoska-Bojkovska, C. Izzi, N. Kacak, B. Bianco, S. Giberti, M. Gigante, G. Piaggio, L. Gesualdo, D. Kosuljandic Vukic, K. Vukojevic, M. Saraga-Babic, M. Saraga, Z. Gucev, L. Allegri, A. Latos-Bielenska, D. Casu, M. State, F. Scolari, R. Ravazzolo, K. Kiryluk, Q. Al-Awqati, V.D. D'Agati, I.A. Drummond, V. Tasic, R.P. Lifton, G.M. Ghiggeri, and A.G. Gharavi

ABSTRACT

BACKGROUND

Congenital abnormalities of the kidney and the urinary tract are the most common cause of pediatric kidney failure. These disorders are highly heterogeneous, and the etiologic factors are poorly understood.

METHODS

We performed genomewide linkage analysis and whole-exome sequencing in a family with an autosomal dominant form of congenital abnormalities of the kidney or urinary tract (seven affected family members). We also performed a sequence analysis in 311 unrelated patients, as well as histologic and functional studies.

RESULTS

Linkage analysis identified five regions of the genome that were shared among all affected family members. Exome sequencing identified a single, rare, deleterious variant within these linkage intervals, a heterozygous splice-site mutation in the dual serine–threonine and tyrosine protein kinase gene (*DSTYK*). This variant, which resulted in aberrant splicing of messenger RNA, was present in all affected family members. Additional, independent *DSTYK* mutations, including nonsense and splice-site mutations, were detected in 7 of 311 unrelated patients. DSTYK is highly expressed in the maturing epithelia of all major organs, localizing to cell membranes. Knockdown in zebrafish resulted in developmental defects in multiple organs, which suggested loss of fibroblast growth factor (FGF) signaling. Consistent with this finding is the observation that DSTYK colocalizes with FGF receptors in the ureteric bud and metanephric mesenchyme. *DSTYK* knockdown in human embryonic kidney cells inhibited FGF-stimulated phosphorylation of extracellular-signal-regulated kinase (ERK), the principal signal downstream of receptor tyrosine kinases.

CONCLUSIONS

We detected independent *DSTYK* mutations in 2.3% of patients with congenital abnormalities of the kidney or urinary tract, a finding that suggests that DSTYK is a major determinant of human urinary tract development, downstream of FGF signaling. (Funded by the National Institutes of Health and others.)

The authors' full names, degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Gharavi at the Division of Nephrology, Columbia University College of Physicians and Surgeons, 1150 St. Nicholas Ave., Russ Berrie Pavilion 413, New York, NY 10032, or at ag2239@columbia.edu.

Drs. Sampogna and Papeta and Ms. Burgess contributed equally to this article.

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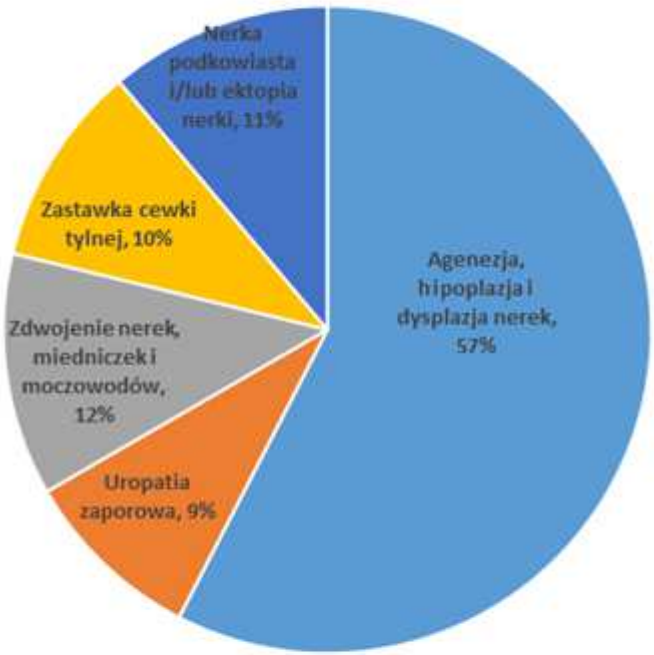
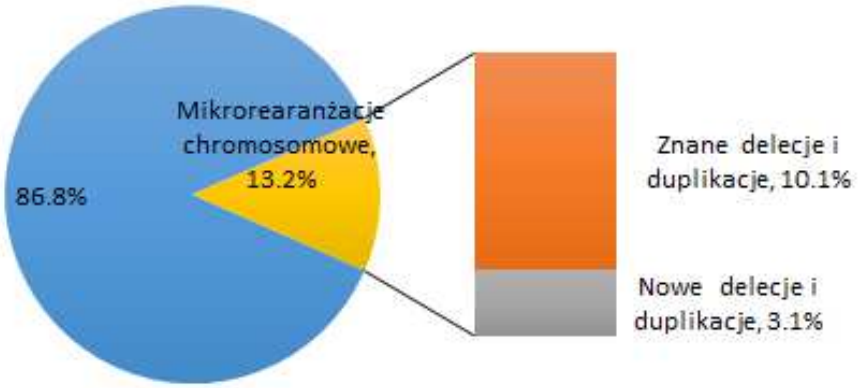
DOI: 10.1056/NEJMoa1214479

Dzieci z wadami układu moczowego (CAKUT) z polskiej populacji objęte badaniami metodą mikromacierzy



Agenezja, hipoplazja i dysplazja nerek	357
Uropatia zaporowa	54
Zdwojenie nerek, miedniczek i moczowodów	75
Zastawka cewki tylnej	64
Nerka podkowiasta i/lub ektopia nerki	72
Ogólna liczba	631

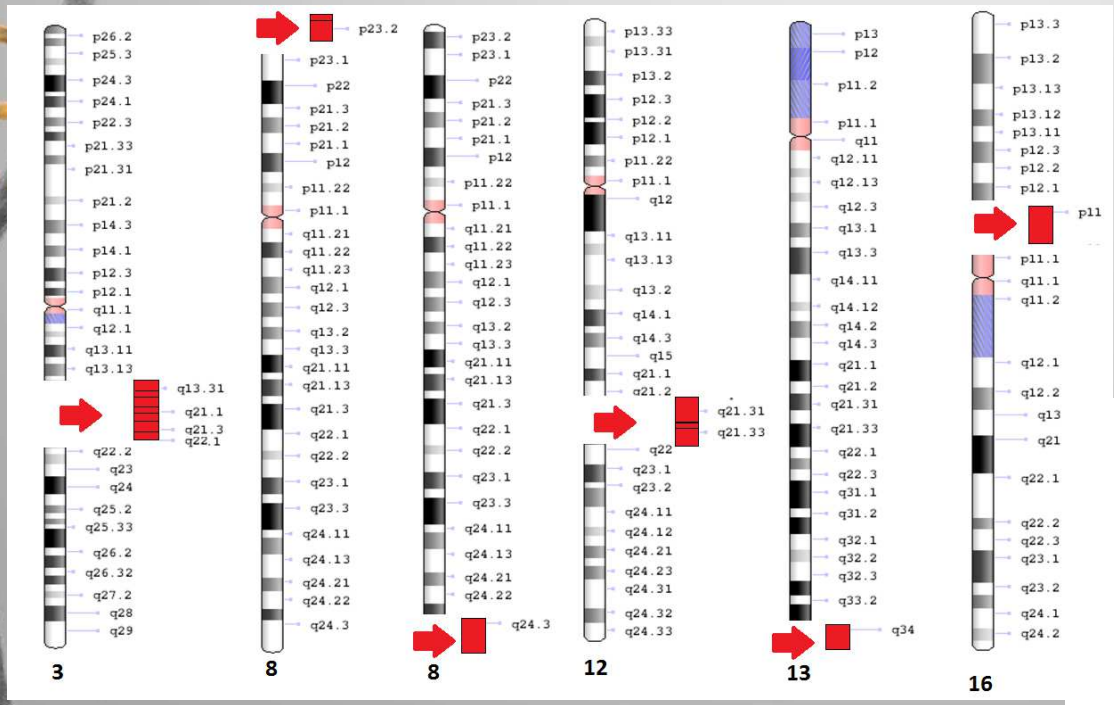
Agenezja, hipoplazja, dysplazja nerek



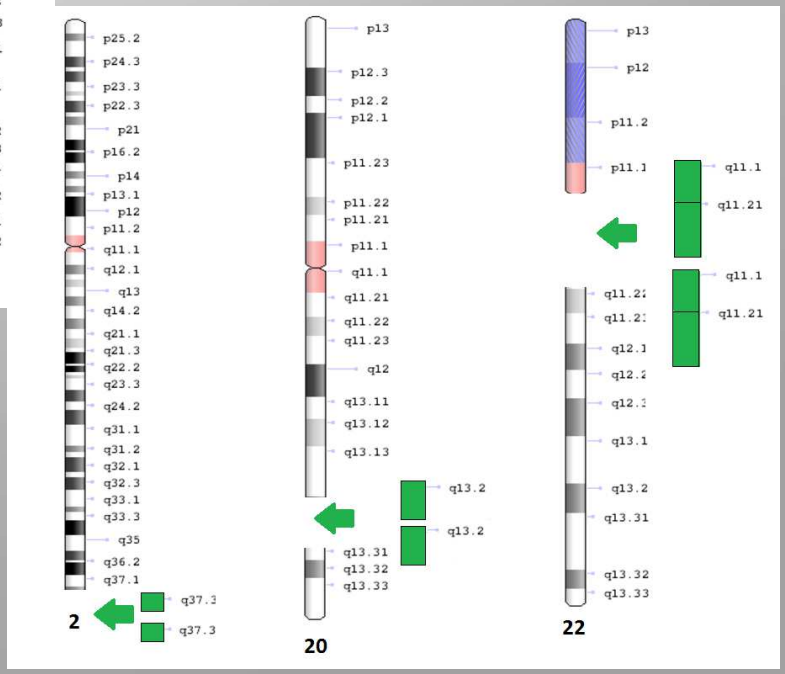
Znane delecje i duplikacje	36
Nowe delecje i duplikacje	11
Ogólna liczba	**357**

Nowe potencjalnie patogenne mikrorearanżacje chromosomowe wykryte metodą miromacierzy u 10 pacjentów z agenezją i dysplazją nerek

Delecje

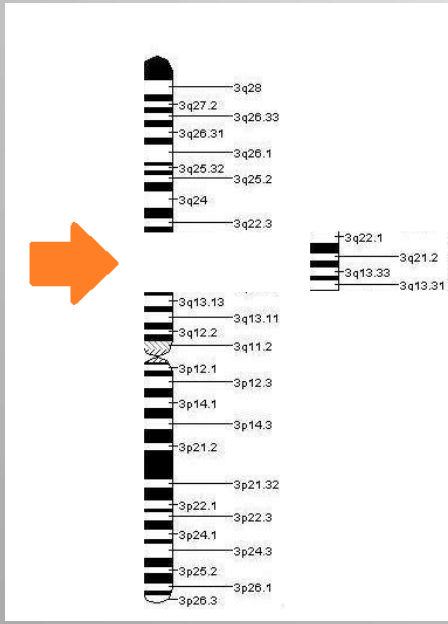


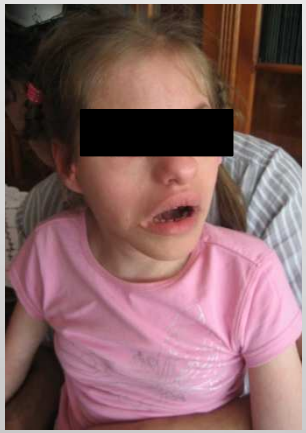
Duplikacje

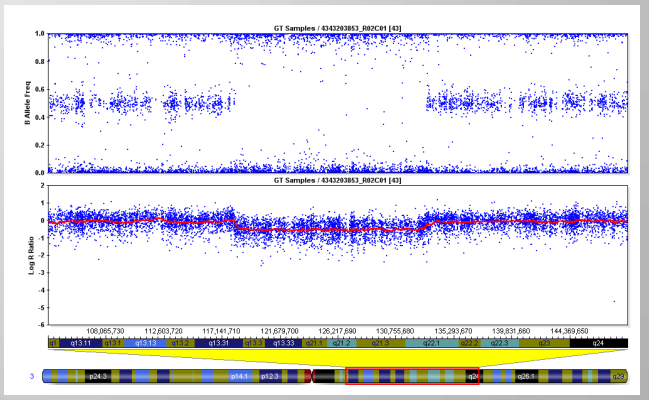


Nowy zespół - delecja chromosomu 3q13.31-22.1 (15 Mb)

(nie opisany wcześniej w literaturze)







101 known genes encompassed by the 3q13.31-22.1 deletion:

IGSF11 UPK1B B4GALT4 CDGAP TMEM39A KTELC1 C3orf1 CD80 ADPRH PLA1A POPDC2 COX17 C3orf15 NR1I2 GSK3B GPR156 FSTL1 NDUFB4 HGD RABL3 GTF2E1 STXBP5L POLQ ARGFX FBXO40 HCLS1 GOLGB1 IQCB1 EAF2 SLC15A2 ILDR1 CD86 ***CASR*** *CSTA CCDC58 FAM162A WDR5B KPNA1 PARP9 DTX3L PARP15 PARP14 HSPBAP1 DIRC2 SEMA5B PDIA5 SEC22A ADCY5 MYLK ROPN1 KALRN UMPS ITGB5 MUC13 SLC12A8 ZNF148 SNX4 OSBPL11 ALG1L ROPN1B SLC41A3 ALDH1L1 KLF15 ZXDC UROC1 CHST13 C3orf22 TXNRD3IT1 TXNRD3 CHCHD6 PLXNA1 TPRA1 MCM2 PODXL2 ABTB1 MGLL KBTBD12 SEC61A1 RUVBL1 EEFSEC DNAJB8 GATA2 C3orf27 RPN1 RAB7A ACAD9 GP9 RAB43 ISY1 CNBP COPG C3orf37 H1FX MBD4 IFT122 RHO H1FOO PLXND1 TRH COL29A1 PIK3R4 ATP2C1 ASTE1 NEK11 MRPL3 CPNE4*

Pediatr Nephrol (2014) 29:257–267
DOI 10.1007/s00467-013-2625-2

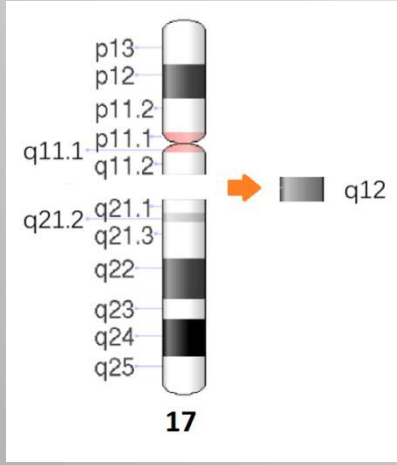
ORIGINAL ARTICLE

The emerging role of genomics in the diagnosis and workup of congenital urinary tract defects: a novel deletion syndrome on chromosome 3q13.31-22.1

Anna Materna-Kiryluk · Krzysztof Kiryluk · Katelyn E. Burgess · Arkadiusz Bieleninik · Simone Sanna-Cherchi · Ali G. Gharavi · Anna Latos-Bielenska

Delecja chromosomu 17q12 (utrata genu *HNF1B)*
Renal Cysts and Diabetes (RCAD) Syndrome

Delecja 17q12 (1.5 Mb)

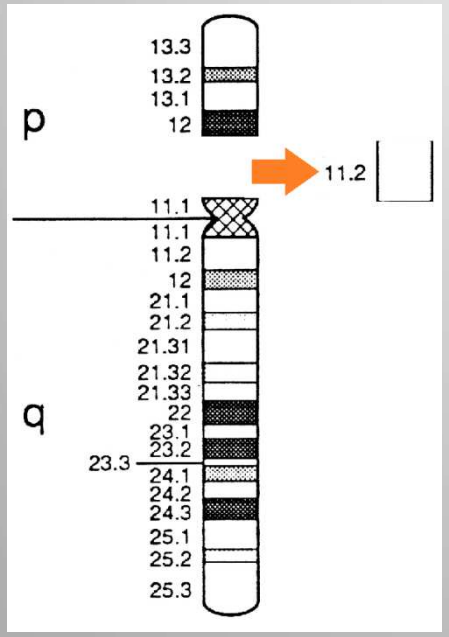


6 pacjentów -
znaczenie prognostyczne
wykrytej mutacji

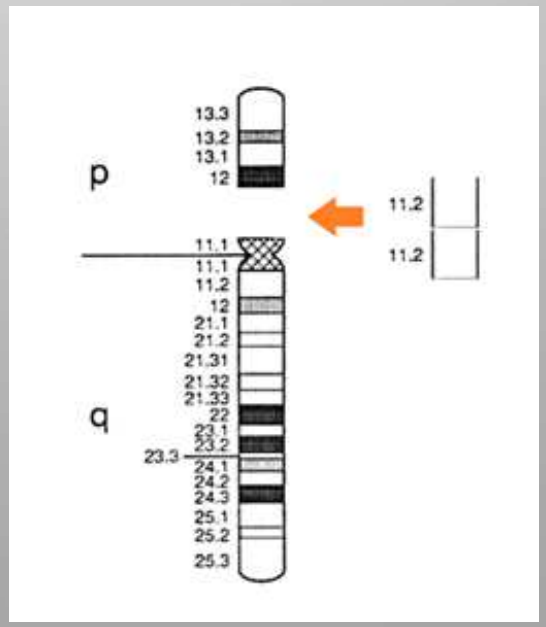
Wielonarządowa manifestacja:

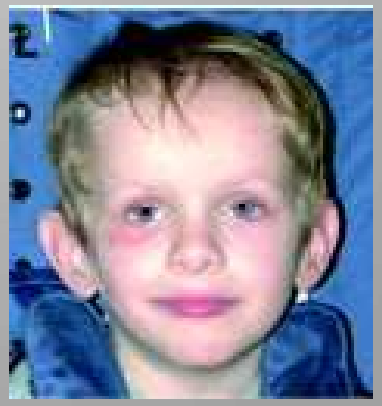
- wady morfologiczne nerek (dysplazja torbielowata) z postępującą niewydolnością nerek
- hipoplazję i/lub niewydolność trzustki, wczesna cukrzyca typu MODY
- zaburzenia gospodarki magnezowej i potasowej
- zaburzenia funkcji wątroby
- różnorodne wady układu płciowego

Delecja chromosomu 17p11.2 (3.5 Mb)
Zespół Smith-Magenis – pacjent z agenezją nerki i OPM



Duplikacja chromosomu 17p11.2 (3.4 Mb)
Zespół Potocki-Lupski – pacjent z agenezją nerki i opóźnieniem mowy



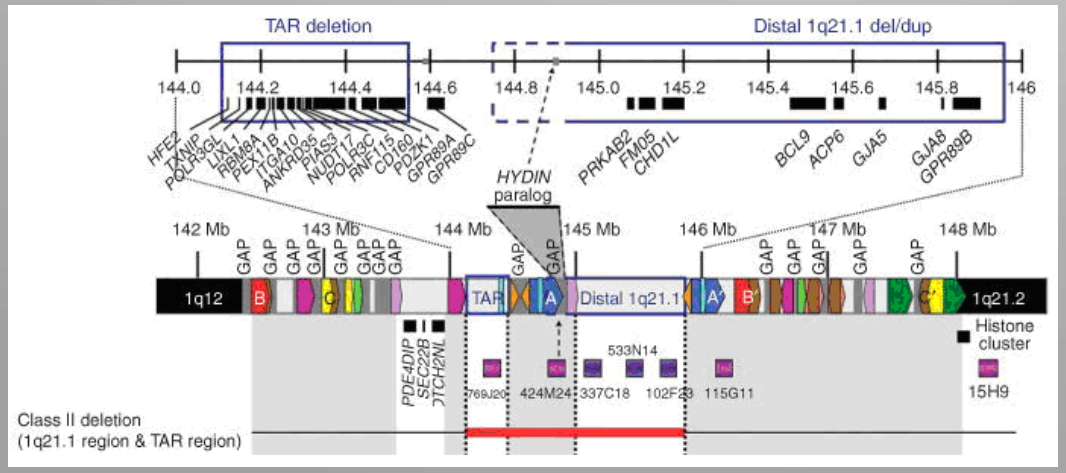


Delecja chromosomu 1q21.1

– 6 pacjentów w tym u 1 pacjenta zespół TAR

Obniżona penetracja i zmienna ekspresja
Zróżnicowany obraz kliniczny, różny stopień niepełnosprawności intelektualnej

Zespół TAR (2.2 Mb)
-hipoplazja i ektopia nerki prawej
-zarośnięcie cewki moczowej z przetoką do pochwy
-obustronny brak kości promieniowej
-potworniak okolicy krzyżowej
-trombocytopenia



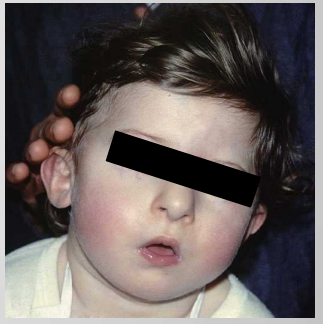
- 4 pacjentów z hipoplazją lub agenezją nerki (1.3 Mb)
- 1 pacjent z agenezją nerki i zdwojeniem drugiej nerki oraz cechami dysmorfii (1.3 Mb)

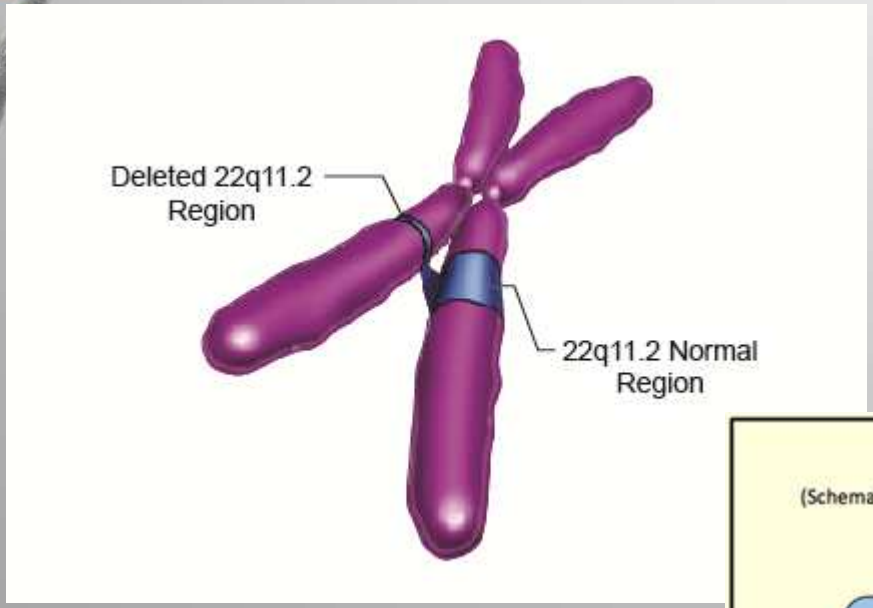
Uwaga rodziny ryzyka genetycznego

Delecja chromosomu 22q11.2 – u 4 pacjentów

Zespół DiGeorge

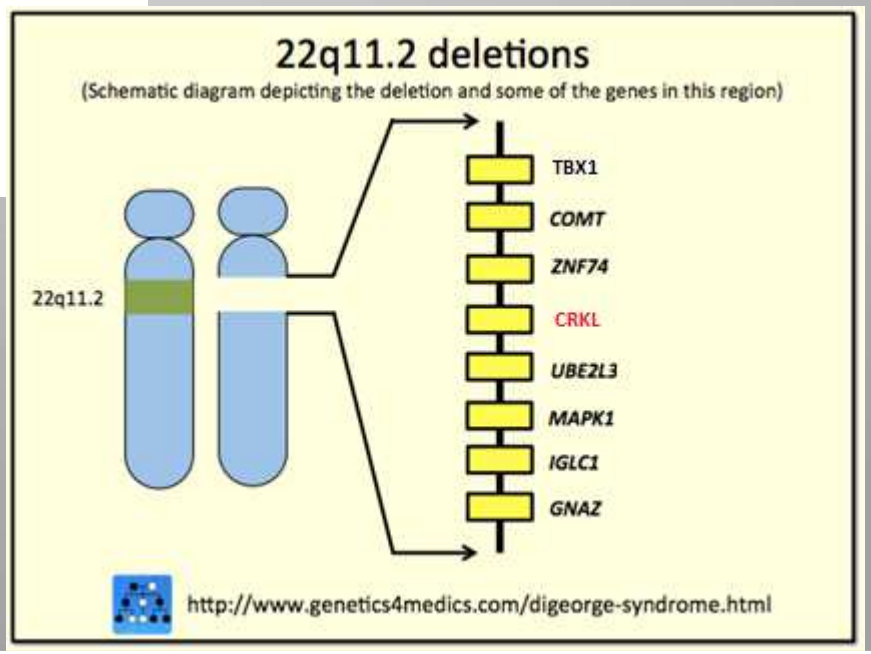
Zespół podniebienno-sercowo-twarzowy





- wady serca 75%
- **wady nerek 30%**
- wady podniebienia
- hipokalcemia
- hipoplazja grasicy

region chromosomu z genami kandydującymi



ORIGINAL ARTICLE

Genetic Drivers of Kidney Defects in the DiGeorge Syndrome

E. Lopez-Rivera, Y.P. Liu, M. Verbitsky, B.R. Anderson, V.P. Capone, E.A. Otto, Z. Yan, A. Mitrotti, J. Martino, N.J. Steers, D.A. Fasel, K. Vukojevic, R. Deng, S.E. Racedo, Q. Liu, M. Werth, R. Westland, A. Vivante, G.S. Makar, M. Bodria, M.G. Sampson, C.E. Gillies, V. Vega-Warner, M. Maiorana, D.S. Petrey, B. Honig, V.J. Lozanovski, R. Salomon, L. Heidet, W. Carpentier, D. Gaillard, A. Carrea, L. Gesualdo, D. Cusi, C. Izzi, F. Scolari, J.A.E. van Wijk, A. Arapovic, M. Saraga-Babic, M. Saraga, N. Kunac, A. Samii, D.M. McDonald-McGinn, T.B. Crowley, E.H. Zackai, D. Drozdz, M. Miklaszewska, M. Tkaczyk, P. Sikora, M. Szczepanska, M. Mizerska-Wasiak, G. Krzemien, A. Szmigielska, M. Zaniew, J.M. Darlow, P. Puri, D. Barton, E. Casolari, S.L. Furth, B.A. Warady, Z. Gucev, H. Hakonarson, H. Flogelova, V. Tasic, A. Latos-Bielenska, A. Materna-Kiryluk, L. Allegri, C.S. Wong, I.A. Drummond, V. D'Agati, A. Imamoto, J.M. Barasch, F. Hildebrandt, K. Kiryluk, R.P. Lifton, B.E. Morrow, C. Jeanpierre, V.E. Papaioannou, G.M. Ghiggeri, A.G. Gharavi, N. Katsanis, and S. Sanna-Cherchi

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Drs. Lopez-Rivera, Liu, Verbitsky, Anderson, and Capone contributed equally to this article.

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DOI: 10.1056/NEJMoa1609009

ABSTRACT

BACKGROUND

The DiGeorge syndrome, the most common of the microdeletion syndromes, affects multiple organs, including the heart, the nervous system, and the kidney. It is caused by deletions on chromosome 22q11.2; the genetic driver of the kidney defects is unknown.

METHODS

We conducted a genomewide search for structural variants in two cohorts: 2080 patients with congenital kidney and urinary tract anomalies and 22,094 controls. We performed exome and targeted resequencing in samples obtained from 586 additional patients with congenital kidney anomalies. We also carried out functional studies using zebrafish and mice.

RESULTS

We identified heterozygous deletions of 22q11.2 in 1.1% of the patients with congenital kidney anomalies and in 0.01% of population controls (odds ratio, 81.5; $P=4.5\times10^{-14}$). We localized the main drivers of renal disease in the DiGeorge syndrome to a 370-kb region containing nine genes. In zebrafish embryos, an induced loss of function in *snap29*, *aifm3*, and *crkl* resulted in renal defects; the loss of *crkl* alone was sufficient to induce defects. Five of 586 patients with congenital urinary anomalies had newly identified, heterozygous protein-altering variants, including a premature termination codon, in *CRKL*. The inactivation of *Crkl* in the mouse model induced developmental defects similar to those observed in patients with congenital urinary anomalies.

CONCLUSIONS

We identified a recurrent 370-kb deletion at the 22q11.2 locus as a driver of kidney defects in the DiGeorge syndrome and in sporadic congenital kidney and urinary tract anomalies. Of the nine genes at this locus, *SNAP29*, *AIFM3*, and *CRKL* appear to be critical to the phenotype, with haploinsufficiency of *CRKL* emerging as the main genetic driver. (Funded by the National Institutes of Health and others.)

Badania molekularne zmienności CNVs metodą mikromacierzy:

- istotne narzędzie w diagnostyce dzieci z wrodzonymi wadami rozwojowymi
- wartość prognostyczna
- ważne dla poradnictwa genetycznego
- umożliwiają określenie regionów chromosomowych zawierających geny kandydujące i przyczyniają się do zidentyfikowania nowych genów odgrywających istotną rolę w rozwoju układu moczowego u ludzi.

POLYGENES

Polish Kidney Genetics Network (POLYGENES) is a collaborative network of Polish nephrologists, pediatricians, and geneticists that aims to advance genetics studies of Polish patients affected by rare inherited forms of kidney disease. The POLYGENES network represents a collaborative effort between the Division of Nephrology at Columbia University in New York and the Polish Registry of Congenital Malformations (PRCM) in the Department of Genetics at Poznań University of Medical Sciences, Poland and The Center of Medical Genetics GENESIS, Poznań, Poland. The network includes a large number of clinical field centers across Poland.

COLUMBIA UNIVERSITY

GHARAVI LAB

DZIĘKUJĘ ZA UWAGĘ

Mikromacierz CGH (aCGH)

Znakuje się fluorochromami DNA **pacjenta** (zielona fluorescencja i DNA **kontrolny** (czerwona fluorescencja), a następnie oba DNA się łączy i nanosi na mikromacierz, gdzie konkurują o hybrydyzację do sond molekularnych mikromacierzy. Przewaga fluorescencji zielonej wskazuje na **duplikację**, przewaga fluorescencji czerwonej – na **delecję** odpowiadającego sondzie molekularnej fragmentu DNA u pacjenta.
Pomiar fluorescencji jest prowadzony przez skaner, a uzyskany obraz jest analizowany z zastosowaniem specjalnych programów komputerowych.