Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

*Birth Defects Res*. Author manuscript; available in PMC 2021 April 29.

Published in final edited form as:

*Birth Defects Res*. 2019 August 01; 111(13): 932–937. doi:10.1002/bdr2.1522.

# The role of genetic variation in *DGKK* on moderate and severe hypospadias

**Melissa A. Richard**[1], **Pagna Sok**[1], **Stephen Canon**[2,3], **Austin L. Brown**[1], **Erin C. Peckham-Gregory**[1], **Wendy N. Nembhard**[4], **Suzan L. Carmichael**[5], **Erik A. Ehli**[6], **Noah A. Kallsen**[6], **Shanna A. Peyton**[6], **Gareth E. Davies**[6], **Ashay Patel**[2,3], **Ismael Zamilpa**[2,3], **Richard A. Wyatt**[7], **Charlotte A. Hobbs**[8], **Michael E. Scheurer**[1], **Philip J. Lupo**[1]

[1]Department of Pediatrics, Baylor College of Medicine, Houston, Texas

[2]Arkansas Children's Hospital, Little Rock, Arkansas

[3]Department of Urology, University of Arkansas for Medical Sciences, Little Rock, Arkansas

[4]Department of Epidemiology, Fay W. Boozman College of Public Health, University of Arkansas for Medical Sciences and Arkansas Children's Research Institute, Little Rock, Arkansas

[5]Department of Pediatrics, Stanford University School of Medicine, Stanford, California

[6]Avera Institute for Human Genetics, Sioux Falls, South Dakota

[7]School of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas

[8]Division of Birth Defects Research, Department of Pediatrics, College of Medicine, University of Arkansas for Medical Sciences, Arkansas Children's Research Institute, Little Rock, Arkansas

## Abstract

**Background:** Recent genome-wide association studies of hypospadias have implicated the role of genetic variants in or near the diacylglycerol kinase kappa (*DGKK*) gene. However, these variants are largely identified among samples of mild and moderate hypospadias cases. Therefore, we evaluated previously identified *DGKK* variants among second- and third-degree hypospadias cases and controls recruited in Arkansas, a state characterized by a high birth prevalence of hypospadias.

**Methods:** Second- and third-degree hypospadias non-Hispanic white cases (n=36 and n=9, respectively) and controls (n=45) were recruited at Arkansas Children's Hospital. Preputial tissue was collected on cases and controls between 2013 and 2017. Cases and controls were genotyped using the Illumina Infinium Global Screening Array. We used logistic regression models to assess

**Corresponding author**: Philip J. Lupo, PhD, Section of Hematology-Oncology, Department of Pediatrics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, Phone: 713-798-2960, philip.lupo@bcm.edu.

Author contributions:

All authors contributed to the 1) conception, design, acquisition of data, analysis and interpretation of data; 2) drafting and critical revision of manuscript; 3) final approval of manuscript; and 4) are accountable for all aspects of work.

**Conflicts of Interest**: none

**Data availability statement**: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

the association of genotyped and imputed genetic variants mapped to the *DGKK* region with second- and third-degree hypospadias.

**Results:** All families self-reported as non-Hispanic white and genetic principal component analyses did not demonstrate evidence of population stratification. Five *DGKK* variants previously reported as associated with hypospadias were identified in the genotype data. None of the variants were associated with second- or third-degree hypospadias (range of odds ratios (OR) = 0.7 to 0.9, all P>0.05).

**Conclusions:** In our analyses, genetic variation in *DGKK* does not play a role in the development of moderate and severe hypospadias. Our findings provide support to the etiologic heterogeneity of hypospadias by all classifications of severity.

### Keywords

hypospadias; urology; genetics; epidemiology

## INTRODUCTION

Hypospadias is one of the most common genitourinary malformations and occurs when the urethral opening develops ventrally rather than at the tip of the glans (Carmichael, Shaw, & Lammer, 2012). The severity of hypospadias is defined by the location of the urethral meatus on or proximal to the penis. Broadly, hypospadias can be classified either as first-degree (urethral opening on the glans penis), second-degree (urethral opening between the mid-penile and distal penile areas), or third-degree (urethral opening located on the perineum to proximal penile area). Within this classification, hypospadias severity is stratified in ascending categories based on the severity of this disease. However, studies of hypospadias may use different classifications to describe severity and often fail to characterize additional features of hypospadias such as the presence of chordee (Chen et al., 2014).

The etiology of hypospadias is complex and remains incompletely understood, but genetic heritability seems to play a role. Genome-wide association studies (GWAS) and candidate gene approaches have identified variants associated with hypospadias risk. In particular, several variants in or near the *DGKK* gene have shown strong associations with hypospadias risk (Carmichael, Ma, et al., 2013; Geller et al., 2014; Hozyasz et al., 2018; van der Zanden et al., 2011), as have *DGKK* haplotypes (Carmichael, Mohammed, et al., 2013). However, *DGKK* associations are primarily identified in samples where mild and moderate hypospadias are grouped together and there is evidence of differential effects of *DGKK* variants for severe hypospadias (Carmichael, Mohammed, et al., 2013; van der Zanden et al., 2011; Xie et al., 2018).

In this study we recruited both cases and controls from Arkansas, a region where incidence of hypospadias of all severities is increasing (Canon, Mosley, Chipollini, Purifoy, & Hobbs, 2012). A large proportion (70%) of the mothers giving birth in this region are non-Hispanic white, among whom the rates of boys born with first-degree hypospadias are substantially higher than for mothers of other races or ethnicities (Canon et al., 2012). However, birth

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

rates for second- and third-degree hypospadias are equivalent among non-Hispanic white and African American mothers in Arkansas (Canon et al., 2012), which may additionally support etiologic heterogeneity of hypospadias by severity through shared environmental or genetic risk factors. We therefore investigated the associations of genetic variants within the *DGKK* region among a sample selected for moderate to severe hypospadias, which we have classified as second-degree and third-degree hypospadias, respectively, in a region characterized by an increasing prevalence of this genital malformation.

## METHODS

### Subjects

All subjects were recruited at Arkansas Children's Hospital from 2013 to 2017. Cases included affected males undergoing hypospadias-corrective surgery at 10.1 months of age, on average. Hypospadias classification was performed by one of the three pediatric urologists at the institution. Second-degree hypospadias was defined as a urethral opening in the mid penile to subcoronal area and third-degree hypospadias was classified based on a urethral opening in the perineum to proximal penile area. Unaffected male infants undergoing circumcision at a mean age of 10.7 months were recruited as controls. Preputial specimens for both hypospadias patients and controls were collected at the time of surgery and preserved in liquid nitrogen within 30 minutes from the time of harvest in the operating room with subsequent preservation in the Arkansas Center for Birth Defects Research and Prevention DNA Bank. Surgical tissue samples were stored at $-80^{O}$C. DNA was extracted from a 1–2 mm section of the frozen tissue shredded with a scalpel prior to processing for DNA isolation using the PerkinElmer Prepito Tissue10 kit according to the manufacturer's protocol. DNA concentration was determined by use of the Qubit fluorometer and the Qubit dsDNA BR Assay kit (Life Technologies).

### Genotyping and quality control

Genotyping was performed at the Avera Institute for Human Genetics on the Infinium Global Screening Array (Illumina, San Diego, CA). Genotypes were called using GenomeStudio 2.0 (Guo et al., 2014) and quality control was performed using PLINK 1.9 (Chang et al., 2015; Purcell et al., 2007). Variants were filtered for cluster separation, call rate >95%, minor allele frequency >5%, and Hardy Weinberg Equilibrium p-value $<10^{-7}$. Samples were filtered to have call rate >95% and heterozygosity within 4 standard deviations of the sample mean. One control subject was excluded based on imputed sex mismatch, i.e., likely having a sex chromosomal abnormality. The pseudoautosomal region on the X chromosome was parsed using PLINK. Variants were imputed to the 1000 Genome Project Phase 3 Version 5 cosmopolitan reference panel using the Michigan Imputation Server (Das et al., 2016) and filtered for imputation quality >0.3.

### Statistical analysis

We analyzed imputed SNPs with minor allele frequency >5% within 100 kilobases up- or downstream of the *DGKK* gene (chrX: 50,082,184–50,282,184; build 37) for association with hypospadias using SNPTEST v2.5.4 (Marchini & Howie, 2010). Specifically, we used the Newton-Raphson algorithm for logistic regression and an additive mode of inheritance.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

We assessed population stratification in our sample by estimating principal components of genetic ancestry using PLINK. LocusZoom (Pruim et al., 2010) was used to visualize results within *DGKK*. Based on our sample size and the frequency of *DGKK* minor alleles (0.3), we had 90% power to detect ORs of 2.5, which are consistent with previously reported associations for these variants (Geller et al., 2014; van der Zanden et al., 2011).

## RESULTS

Analyses included 45 boys with second- or third-degree hypospadias and 45 boys without hypospadias (Table). All families self-reported as non-Hispanic white and genetic principal component analyses did not demonstrate evidence of population stratification. All 45 hypospadias cases were classified either as second- or third-degree; however, the presence of chordee and location of the urethral opening varied. Among the nine cases with third-degree hypospadias, all had chordee, and the urethral opening was penoscrotal in seven cases, perineal in one case, and penile proximal in one case. Among the 12 cases of second-degree hypospadias with chordee, the urethral opening was penile distal in six cases and penile midshaft in an additional six cases. Among the 24 cases of second-degree hypospadias without chordee, the urethral opening was penile distal in 18 cases and penile midshaft in six cases.

Among five genetic variants previously associated with hypospadias, we did not identify evidence of association with second- and third-degree hypospadias, combined, in our sample (Table, OR=0.8–0.9, all confidence intervals include the null value). Analyses examining only second-degree hypospadias cases further showed no evidence of association of *DGKK* variants (Table, OR=0.7–0.8, all confidence intervals include the null value). We additionally mapped 166 variants to within 100 kilobases of the *DGKK* gene. No variant within this region demonstrated a nominally significant association with second- and third-degree hypospadias combined (Figure, all P>0.05).

## CONCLUSIONS

Within a sample of second- and third-degree hypospadias cases, we did not identify genetic associations within the *DGKK* region. The five *DGKK* variants previously associated with hypospadias are in strong linkage disequilibrium in the 1000 Genomes reference population, and we did not identify association of any other index variants in the region. Prior studies show strong association of *DGKK* variants with mild to moderate hypospadias, which have been reported as homogeneous and grouped together for risk estimation (Carmichael, Mohammed, et al., 2013; Geller et al., 2014; Ma et al., 2015). However, separate risk estimates for mild and moderate cases have not been published. In contrast, several studies have reported lack of association of *DGKK* variants with severe hypospadias (Carmichael, Mohammed, et al., 2013; Ma et al., 2015; Xie et al., 2018), whereas others have suggested associations of select SNPs or haplotypes with severe cases (Carmichael, Mohammed, et al., 2013; van der Zanden et al., 2011). We identified similar and non-significant measures of association for all *DGKK* variants both when considering only moderate hypospadias and when moderate cases are grouped with severe cases. Our findings are consistent with previous studies that have not identified associations of individual *DGKK* variants among

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

severe hypospadias cases from populations where linkage patterns could differ (Carmichael, Mohammed, et al., 2013; Ma et al., 2015; van der Zanden et al., 2011; Xie et al., 2018), but are inconsistent with previous studies that suggest associations with moderate cases. Of note, most associations of *DGKK* variants with hypospadias are based on a larger proportion of moderate than mild cases, but the strongest estimates for *DGKK* association with hypospadias were reported by a study that had the highest proportion of mild forms of hypospadias (van der Zanden et al., 2011). The association of *DGKK* variants with mild hypospadias has yet to be confirmed in our study population, but strong effects of *DGKK* should have been detectable among our sample of moderate cases. Taken in the context of previous findings, our analyses suggest that genetic effect estimates should be reported stratified by hypospadias severity.

Previous population-based studies have primarily been comprised of boys with mild or moderate hypospadias born to non-Hispanic white mothers as these are the most common demographics for hypospadias; however, other studies have examined hypospadias in more diverse populations, namely the Han Chinese and residents of California. Recruitment in Arkansas enabled us to include moderate to severe cases of hypospadias, but limited our sample size and power, particularly for haplotype analyses. However, the previously-identified associations between *DGKK* variants and hypospadias in population-based samples of mild and moderate cases are quite strong (OR>2.5); therefore, if *DGKK* variants are associated with moderate and severe hypospadias, we had power to detect such a strong signal. Future studies should consider the etiologic heterogeneity of hypospadias by all severities; in particular, genetic susceptibility to the most severe forms of hypospadias remains to be characterized.

## Funding:

ABI/Arkansas Children's Research Institute

## REFERENCES

Canon S, Mosley B, Chipollini J, Purifoy JA, & Hobbs C (2012). Epidemiological assessment of hypospadias by degree of severity. J Urol, 188(6), 2362–2366. doi:10.1016/j.juro.2012.08.007 [PubMed: 23088968]

Carmichael SL, Ma C, Choudhry S, Lammer EJ, Witte JS, & Shaw GM (2013). Hypospadias and genes related to genital tubercle and early urethral development. J Urol, 190(5), 1884–1892. doi:10.1016/j.juro.2013.05.061 [PubMed: 23727413]

Carmichael SL, Mohammed N, Ma C, Iovannisci D, Choudhry S, Baskin LS, … Lammer EJ (2013). Diacylglycerol kinase K variants impact hypospadias in a California study population. J Urol, 189(1), 305–311. doi:10.1016/j.juro.2012.09.002 [PubMed: 23177175]

Carmichael SL, Shaw GM, & Lammer EJ (2012). Environmental and genetic contributors to hypospadias: a review of the epidemiologic evidence. Birth Defects Res A Clin Mol Teratol, 94(7), 499–510. doi:10.1002/bdra.23021 [PubMed: 22678668]

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, & Lee JJ (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience, 4, 7. doi:10.1186/s13742-015-0047-8 [PubMed: 25722852]

Chen MJ, Macias CG, Gunn SK, Dietrich JE, Roth DR, Schlomer BJ, & Karaviti LP (2014). Intrauterine growth restriction and hypospadias: is there a connection? International Journal of Pediatric Endocrinology.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Das S, Forer L, Schonherr S, Sidore C, Locke AE, Kwong A, … Fuchsberger C (2016). Next-generation genotype imputation service and methods. Nat Genet, 48(10), 1284–1287. doi:10.1038/ng.3656 [PubMed: 27571263]

Geller F, Feenstra B, Carstensen L, Pers TH, van Rooij IA, Korberg IB, … Melbye M (2014). Genome-wide association analyses identify variants in developmental genes associated with hypospadias. Nat Genet, 46(9), 957–963. doi:10.1038/ng.3063 [PubMed: 25108383]

Guo Y, He J, Zhao S, Wu H, Zhong X, Sheng Q, … Long J (2014). Illumina human exome genotyping array clustering and quality control. Nat Protoc, 9(11), 2643–2662. doi:10.1038/nprot.2014.174 [PubMed: 25321409]

Hozyasz KK, Mostowska A, Kowal A, Mydlak D, Tsibulski A, & Jagodzinski PP (2018). Further Evidence of the Association of the Diacylglycerol Kinase Kappa (DGKK) Gene With Hypospadias. Pediatric Urology, 15(5), 272–276.

Ma Q, Tang Y, Lin H, Xu M, Xu G, Fang X, … Geng H (2015). Diacylglycerol kinase kappa (DGKK) variants and hypospadias in Han Chinese: association and meta-analysis. BJU Int, 116(4), 634–640. doi:10.1111/bju.12965 [PubMed: 25327554]

Marchini J, & Howie B (2010). Genotype imputation for genome-wide association studies. Nat Rev Genet, 11(7), 499–511. doi:10.1038/nrg2796 [PubMed: 20517342]

Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, … Willer CJ (2010). LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics, 26(18), 2336–2337. doi:10.1093/bioinformatics/btq419 [PubMed: 20634204]

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, … Sham PC (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet, 81(3), 559–575. doi:10.1086/519795 [PubMed: 17701901]

van der Zanden LF, van Rooij IA, Feitz WF, Knight J, Donders AR, Renkema KY, … Knoers NV (2011). Common variants in DGKK are strongly associated with risk of hypospadias. Nat Genet, 43(1), 48–50. doi:10.1038/ng.721 [PubMed: 21113153]

Xie H, Lin XL, Zhang S, Yu L, Li XX, Huang YC, … Chen F (2018). Association between diacylglycerol kinase kappa variants and hypospadias susceptibility in a Han Chinese population. Asian J Androl, 20(1), 85–89. doi:10.4103/aja.aja_13_17 [PubMed: 28597849]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

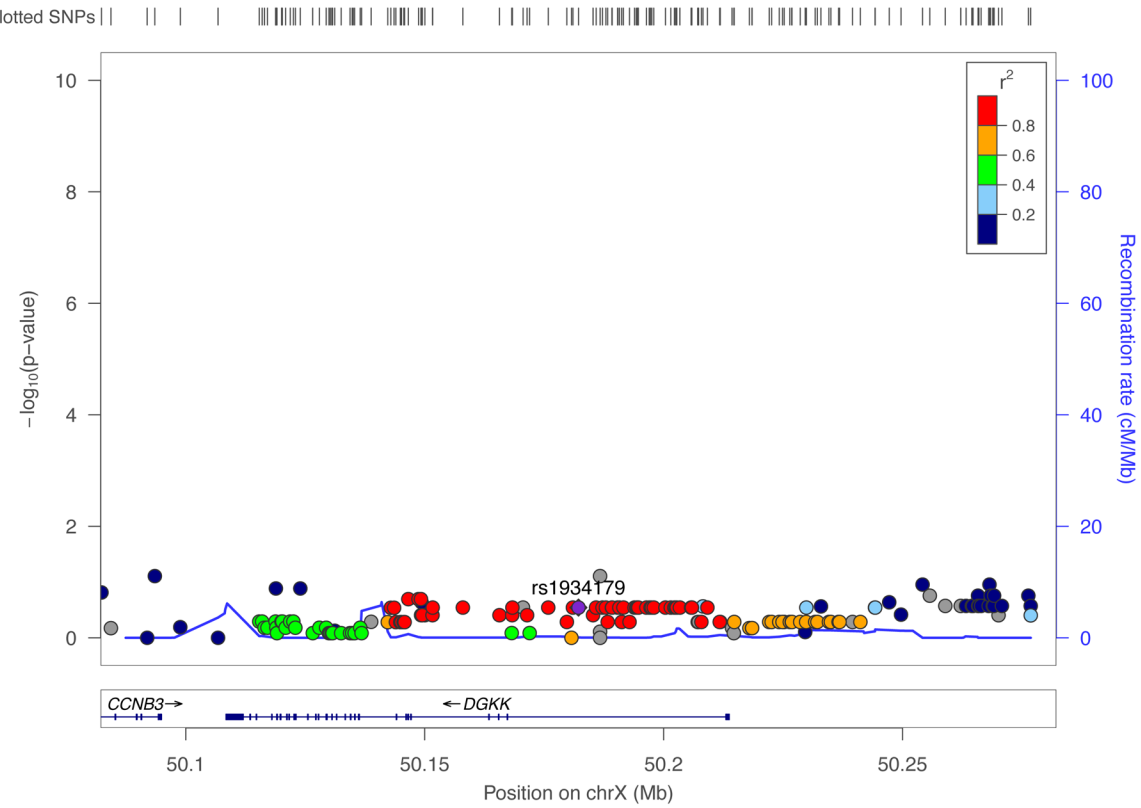


**Figure.**

Regional association plot of the *DGKK* locus for association with second- or third-degree hypospadias. Linkage disequilibrium is shown according to the 1000 Genomes European reference panel.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



**Table.**

Odds ratios and confidence intervals for *DGKK* variants previously associated with hypospadias are shown by severity of hypospadias among cases from Arkansas and in the context of previous reports of the association of these variants with hypospadias. All variants are in high linkage disequilibrium in the 1000 Genomes CEU reference panel ($r^2$: 0.66–1.00)

| | | | Mild/Moderate | | | Moderate only | | | Moderate/Severe | | | Severe only | | | All Severities[‡] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SNP** | **Study population** | **Risk allele[†]** | **n** | **OR** | **95% CI** | **n** | **OR** | **95% CI** | **n** | **OR** | **95% CI** | **n** | **OR** | **95% CI** | **n** | **OR** | **95% CI** |
| rs4554617 | Han Chinese[§] | G | 58/113 | 2.1 | (1.4–3.2) | | NR | | | NR | | 128 | 1.1 | (0.6–1.8) | 322 | 1.6 | (1.2–2.3) |
| | Polish[¶] | C | 110/66 | 1.6 | (1.1–2.4) | | NR | | | | | | | | | | |
| | European[††] | C | | NR | | | NR | | | NR | | | NR | | 2,978 | 2.5 | NR[†††] |
| | Arkansas | C | | | | 36 | 0.7 | (0.5–1.2) | 36/9 | 0.8 | (0.5–1.2) | | NR | | | | |
| rs7063116 | Han Chinese[§] | A | 58/113 | 2.1 | (1.4–3.3) | | NR | | | NR | | 128 | 1.2 | (0.7–2.1) | 322 | 1.8 | (1.2–2.6) |
| | Han Chinese[‡‡] | A | 125/126 | 1.2 | (0.7–1.9) | | NR | | | NR | | 186 | 1.2 | (0.7–2.1) | 466 | 1.3 | (0.8–1.9) |
| | Polish[¶] | A | 110/66 | 1.4 | (0.9–2.0) | | NR | | | | | | | | | | |
| | California[§§] | A | 91/336 | 1.3 | (1.0–1.7) | | NR | | | NR | | 221 | 0.9 | (0.7–1.3) | 665 | 1.2 | (1.0–1.5) |
| | Dutch[¶¶] | A | 436 | 2.3 | (1.7–3.0) | | NR | | | NR | | 87 | 0.9 | (0.5–1.5) | 523 | 2.3 | (1.7–3.0) |
| | Swedish[¶¶] | A | 266 | 2.2 | (1.6–3.0) | | NR | | | NR | | 62 | 1.7 | (1.0–2.9) | 328 | 2.2 | (1.6–3.0) |
| | Arkansas | A | | | | 36 | 0.8 | (0.5–1.2) | 36/9 | 0.9 | (0.6–1.3) | | NR | | | | |
| rs11091748 | Polish[¶] | G | 110/66 | 1.9 | (1.3–2.8) | | NR | | | | | | | | | | |
| | Arkansas | G | | | | 36 | 0.7 | (0.5–1.2) | 36/9 | 0.8 | (0.5–1.2) | | NR | | | | |
| rs12171755 | Han Chinese[§] | A | 58/113 | 1.6 | (1.2–2.3) | | NR | | | NR | | 128 | 0.9 | (0.6–1.4) | 322 | 1.3 | (1.0–1.7) |
| | Polish[¶] | T | 110/66 | 1.8 | (1.2–2.7) | | NR | | | | | | | | | | |
| | California[§§] | T | 91/336 | 1.1 | (0.9–1.4) | | NR | | | NR | | 221 | 0.8 | (0.5–1.1) | 665 | 1.0 | (0.8–1.3) |
| | Arkansas | T | | | | 36 | 0.8 | (0.5–1.2) | 36/9 | 0.9 | (0.6–1.3) | | NR | | | | |
| rs1934179 | Han Chinese[§] | T | 58/113 | 1.7 | (1.2–2.3) | | NR | | | NR | | 128 | 0.9 | (0.6–1.4) | 322 | 1.3 | (1.0–1.7) |

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



| SNP | Study population | Risk allele[†] | Mild/Moderate | | | Moderate only | | | Moderate/Severe | | | Severe only | | | All Severities[‡] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | n | OR | 95% CI | n | OR | 95% CI | n | OR | 95% CI | n | OR | 95% CI | n | OR | 95% CI |
| | Han Chinese[‡‡] | A | 125/126 | 1.4 | (1.0–2.0) | | NR | | | NR | | 186 | 1.2 | (0.8–1.7) | 466 | 1.3 | (1.0–1.8) |
| | Polish[¶] | T | 110/66 | 1.6 | (1.1–2.4) | | NR | | | | | | | | | | |
| | California[§§] | T | 91/336 | 1.8 | (1.4–2.3) | | NR | | | NR | | 221 | 0.9 | (0.7–1.3) | 665 | 1.4 | (1.2–1.8) |
| | Dutch[¶¶] | A | 436 | 2.5 | (1.9–3.2) | | NR | | | NR | | 87 | 1.3 | (0.8–2.0) | 523 | 2.5 | (1.9–3.2) |
| | Swedish[¶¶] | A | 266 | 2.5 | (1.8–3.4) | | NR | | | NR | | 62 | 1.9 | (1.1–3.3) | 328 | 2.5 | (1.8–3.4) |
| | Arkansas | A | | | | 36 | 0.7 | (0.5–1.2) | 36/9 | 0.8 | (0.5–1.2) | | NR | | | | |

Abbreviations: CI, confidence interval; NR, not reported (data available); OR, odds ratio; SNP, single nucleotide polymorphism.

[†]Risk alleles remain as reported by the study; rs4554617 alleles are G/T on the reverse/bottom strand and C/A on the forward/top strand; rs12171755 alleles are C/T on the forward/bottom strand; rs1934179 alleles are C/T on the forward/bottom strand and A/G on the reverse/top strand.

[‡]All Severities includes unknown and not otherwise specified cases of hypospadias.

[§]Xie, H., Lin, X. L., Zhang, S., Yu, L., Li, X. X., Huang, Y. C., … Chen, F. (2018). Association between diacylglycerol kinase kappa variants and hypospadias susceptibility in a Han Chinese population. Asian J Androl, 20(1), 85–89. doi:10.4103/aja.aja_13_17

[¶]Hozyasz, K. K., Mostowska, A., Kowal, A., Mydlak, D., Tsibulski, A., & Jagodzinski, P. P. (2018). Further Evidence of the Association of the Diacylglycerol Kinase Kappa (DGKK) Gene With Hypospadias. *Pediatric Urology, 15*(5), 272–276.

[††]Geller, F., Feenstra, B., Carstensen, L., Pers, T. H., van Rooij, I. A., Korberg, I. B., … Melbye, M. (2014). Genome-wide association analyses identify variants in developmental genes associated with hypospadias. *Nat Genet, 46*(9), 957–963. doi:10.1038/ng.3063

[‡‡]Ma, Q., Tang, Y., Lin, H., Xu, M., Xu, G., Fang, X., … Geng, H. (2015). Diacylglycerol kinase kappa (DGKK) variants and hypospadias in Han Chinese: association and meta-analysis. *BJU Int, 116*(4), 634–640. doi:10.1111/bju.12965

[§§]Carmichael, S. L., Mohammed, N., Ma, C., Iovannisci, D., Choudhry, S., Baskin, L. S., …Lammer, E. J. (2013). Diacylglycerol kinase K variants impact hypospadias in a California study population. *J Urol, 189*(1), 305–311. doi:10.1016/j.juro.2012.09.002

[¶¶]van der Zanden, L. F., van Rooij, I. A., Feitz, W. F., Knight, J., Donders, A. R., Renkema, K. Y., … Knoers, N. V. (2011). Common variants in DGKK are strongly associated with risk of hypospadias. *Nat Genet, 43*(1), 48–50. doi:10.1038/ng.721

[†††]95% CI not reported in Geller et al 2014; P-value is 1.01e-93.