NFIA haploinsufficiency is associated with a CNS malformation syndrome and urinary tract defects

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Abstract

Complex central nervous system (CNS) malformations frequently coexist with other developmental abnormalities, but whether the associated defects share a common genetic basis is often unclear. We describe five individuals who share phenotypically related CNS malformations and in some cases urinary tract defects, and also haploinsufficiency for the NFIA transcription factor gene due to chromosomal translocation or deletion. Two individuals have balanced translocations that disrupt NFIA. A third individual and two half-siblings in an unrelated family have interstitial microdeletions that include NFIA. All five individuals exhibit similar CNS malformations consisting of a thin, hypoplastic, or absent corpus callosum, and hydrocephalus or ventriculomegaly. The majority of these individuals also exhibit Chiari type I malformation, tethered spinal cord, and urinary tract defects that include vesicoureteral reflux. Other genes are also broken or deleted in all five individuals, and may contribute to the phenotype. However, the only common genetic defect is NFIA haploinsufficiency. In addition, previous analyses of Nfia(-/-) [...]
NFIA Haploinsufficiency Is Associated with a CNS Malformation Syndrome and Urinary Tract Defects

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Complex central nervous system (CNS) malformations frequently coexist with other developmental abnormalities, but whether the associated defects share a common genetic basis is often unclear. We describe five individuals who share phenotypically related CNS malformations and in some cases urinary tract defects, and also haploinsufficiency for the NFIA transcription factor gene due to chromosomal translocation or deletion. Two individuals have balanced translocations that disrupt NFIA and renal defects. PLoS Genet 3(5): e80. doi:10.1371/journal.pgen.0030080

Introduction

Complex human developmental phenotypes represent an especially difficult problem in human genetics. In many cases, congenital birth defects are believed to result from the combined effect of many genes, often with an environmental contribution, and frequently culminate in perinatal demise. Thus, for many cases, extended families do not exist, and approaches to disease gene identification based on linkage analysis are not possible. In addition, many developmental disorders are genetically heterogeneous, making the ascertainment of single contributory genes difficult.

The analysis of human balanced chromosome rearrangements offers a potential approach to this problem. Although


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Abbreviations: ACC, agenesis of the corpus callosum; aCGH, array comparative genomic hybridization; BAC, bacterial artificial chromosome; CNS, central nervous system; FISH, fluorescence in situ hybridization; OMIM, Online Mendelian Inheritance in Man; UPJ, ureteropelvic junction; UVJ, ureterovesical junction; VCUG, voiding cystourethrogram; VUR, vesicoureteral reflux

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Central nervous system (CNS) and urinary tract abnormalities are common human malformations, but their variability and genetic complexity make it difficult to identify the responsible genes. Analysis of human chromosomal abnormalities associated with such disorders offers one approach to this problem. In five individuals described herein, a novel human syndrome that involves both CNS and urinary tract defects is associated with chromosomal disruption or deletion of NFIA, encoding a member of the Nuclear Factor I (NFI) family of transcription factors. This syndrome includes brain abnormalities (abnormal corpus callosum, hydrocephalus, ventriculomegaly, and Chiari type I malformation), spinal abnormalities (tethered spinal cord), and urinary tract abnormalities (vesicoureteral reflux). NFia disruption in mice was already known to cause hydrocephalus and abnormal corpus callosum, and is now shown to exhibit renal defects and disturbed ureteral development. Other genes besides NFIA are also disrupted or deleted and may contribute to the observed phenotype. However, loss of one copy of NFIA is the only genetic defect common to all five patients. The authors thus provide evidence that genetic loss of NFIA contributes to a distinct CNS malformation syndrome with urinary tract defects of variable penetrance.

unforeseen rearrangements and position effects may supersede [1,2], and a background rate of birth defects exists, human translocations provide powerful tools to identify genes that are essential to human development. Translocations may result in haploinsufficiency, the generation of fusion transcripts, or position effects, or act in combination with a second loss-of-function allele. The Developmental Genome Anatomy Project, DGAP (http://dgap.harvard.edu), has as its specific goal the ascertainment, recruitment, and analysis of individuals with chromosomal rearrangements and developmental disorders. A conspicuous class of such disorders is that involving the formation of the CNS and visceral organs.

Within the CNS, the corpus callosum is the largest interconnecting white matter tract in the brain, and it connects the association fibers of both hemispheres. Agenesis of the corpus callosum (ACC) is among the most common brain malformations in humans, with an incidence of 1 per 4,000 live births [3–5] and a prevalence as high as 3%–5% in individuals with neurodevelopmental disabilities [6,7]. In human embryos, the corpus callosum begins to develop at 11–12 wk gestation when the first fibers cross the midline to form the genu in the region of the commissural plate, and subsequent development proceeds from anterior to posterior, with formation of the anterior body, posterior body, and splenium, followed by a progressive enlargement that reflects the rapid expansion of the cerebral hemispheres [8]. Abnormalities of the corpus callosum can occur through a number of mechanisms, including defects in the genesis or survival of neuronal cells whose axons form the corpus callosum, and defects in axonal outgrowth, pathfinding, and midline crossing [9]. The etiology of ACC is thus heterogeneous and multifactorial, and both autosomal recessive and X-linked recessive mechanisms have been described [9] (see also Online Mendelian Inheritance in Man [OMIM, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM]). ACC is associated with certain chromosomal rearrangements [9] and occurs as a component of other genetic syndromes [10] and metabolic conditions [11], but its genetic heterogeneity and phenotypic pleiotropy have limited identification of the responsible genes to only a few of the more than 20 distinct loci that are associated with ACC, including one on the short arm of Chromosome 1 [12].

In addition to other CNS defects with which ACC is frequently associated, ACC may occur in conjunction with visceral organ malformations. For example, ACC and its associated brain and spinal cord lesions have been linked to vesicoureteral reflux (VUR), cystic kidney disease, renal agenesis and insufficiency, and neurogenic lower urinary tract dysfunction, a condition that includes neurogenic bladder and VUR [13–16]. Alternatively, single gene mutations may produce developmental defects in both the CNS and urinary tract. We show here that a novel human syndrome involving both CNS and urinary tract defects is associated with disruption or deletion of the NFIA gene at 1p31.3, which encodes a member of the Nuclear Factor I (NFI) family of transcription factors [17]. Disruption of NFia in mice results in perinatal lethality, hydrocephalus, and ACC [18], and a recent study shows that NFIA controls the transition from neurogenesis to gliogenesis in the developing spinal cord [19]. Similar studies indicate that two other Nfi family members, Nfia and Nfrc, are essential for lung, brain and tooth development [20,21]. However, the role that NFI transcription factors play in human disease has been unknown. Our results establish that NFIA haploinsufficiency is a likely contributor to a range of CNS defects, including ACC, hydrocephalus, ventriculomegaly, Chiari type I malformation, and tethered spinal cord, and that renal defects can also result from a disturbance in ureteral development.

**Results**

**CNS and Urinary Tract Defects in Individuals with 1p31 Rearrangements**

We investigated five individuals enrolled in DGAP (i.e., DGAP104, 689, 174, 205–1, and 205–1s), each with a similar spectrum of CNS defects, and in three cases, of urinary tract defects. All five individuals had chromosomal rearrangements that variously involved 1p31. DGAP104 is a 6-y-old female diagnosed at birth with congenital hydrocephalus, a thin corpus callosum, Chiari I malformation, tethered spinal cord, and a low vertebral deformity (Figure 1A, 1B, and 1K). She was also found to have congenital bilateral dysplastic kidneys, and subsequently developed bilateral VUR, pyelonephritis, a ureterovesical junction diverticulum, and hydronephrosis, and required ureteral reimplantation surgery at age 2 y (Figure 1L; Tables 1 and S1). Chromosome analysis in the neonatal period revealed an apparent de novo balanced chromosome translocation between 1p31 and 20q13.

A second individual, DGAP089, is an 8-y-old male whose clinical profile was described previously (Table 1, S1) [22]. He has both an interstitial deletion on 2q and a balanced translocation involving 1p and 2q [22]. As an infant, he had poor fetal movement, and a CT scan revealed a vertical orientation of the ventricles consistent with a primary defect of the corpus callosum. At age 2 y, a CT scan revealed ventriculomegaly, and at age 6.5 y a brain MRI showed a hypoplastic corpus callosum, nonprogressive ventriculomegaly, and a gray matter heterotopia (Figure 1C and 1D; Tables 1 and S1). Renal ultrasound revealed no major abnormalities,
but a definitive evaluation for urinary reflux (i.e., a voiding cystourethrogram, or VCUG) was not performed.

A third individual, DGAP174, who exhibited complete ACC and enlarged ventricles by second trimester ultrasound, had both a de novo translocation of 1p31.1 and 3q25.1 and an interstitial deletion of 1p31. A postnatal brain CT scan confirmed these findings and also revealed ventriculomegaly and a tethered spinal cord. A brain MRI at age 3 y revealed a Chiari type I malformation, a downward displacement of the tip of the cerebellar tonsils below the foramen magnum, was found in DGAP104 (arrowhead in A), DGAP174 (arrowhead in E), and DGAP205-1s (arrowhead in I). Congenital ventriculomegaly is present in DGAP089 (D), DGAP174 (F), and DGAP205-1s (J), and hydrocephalus was found in DGAP104 (B) and DGAP205–1 (H). An occipital shunt (arrow in B) was placed in DGAP104 to relieve severe hydrocephalus. (K) DGAP104 MRI shows a tethered spinal cord, with the extremity of the conus medullaris (arrow) at the level of the L4 vertebral body. Arrowhead shows a fishhook deformity of the lower sacral and coccygeal vertebrae. (L) VCUG of DGAP104 depicts left vesicoureteral reflux with retrograde tracking of dye through the ureter into the renal pelvis (arrow) and a right diverticulum at the ureterovesical junction (arrowhead). (M) Spine MRI of DGAP205–1 shows a tethered spinal cord with conus lying at the L3/L4 level (arrow). (N) VCUG of DGAP205–1 shows left vesicoureteral reflux (arrow). (O) Spine MRI of DGAP205–1s depicts a tethered spinal cord with conus lying at L5 (arrow).

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Cytogenetic and Genetic Analyses of the Chromosomal Rearrangements

To determine whether a common genetic defect underlay the phenotypes of these individuals, we analyzed the 1p31 region in all five patients and found that the NFIA gene was either disrupted or deleted in each case. DGAP104 has a de novo balanced translocation, 46,XX,t(1;20)(p31.3;q13.31)dn (Figure 2A, 2B). By metaphase fluorescence in situ hybridization (FISH), we identified a bacterial artificial chromosome (BAC) clone (RP4-802A10) that mapped to 1p31.3 and hybridized to the breakpoints of the der(1) and der(20) chromosomes (Figure 2C). The translocation breakpoint at 1p31.3 disrupts intron 2 of NFIA, which is composed of 11
exons and spans ~374 kb of genomic DNA (Figure 2I). This result was confirmed by Southern blot analysis (Figure S1).

In addition to disruption of NFIA at 1p31.3 in DGAP104, C20orf32 was also disrupted by the 2q13.31 breakpoint. A contribution of C20orf32 disruption to the spinal and kidney phenotypes in DGAP104 is unlikely, however, because C20orf32 expression was not detected in the developing spinal cord or kidney by in situ hybridization (Figure S2). By array comparative genomic hybridization (aCGH), we excluded any additional chromosome abnormalities in DGAP104 at ~1 Mb resolution.

For DGAP089, sequential FISH led to identification of a 1p31.3 breakpoint-spanning BAC, RP5-902P15, which hybridized to Chromosome 1 and to both der(1) and der(2) chromosomes (Figure 2D). Subsequent FISH and Southern blot analyses (Figure S3) refined the breakpoint to ~9.9 kb between exons 7 and 8 of NFIA (Figure 2I). Similar analyses of the 2q breakpoint revealed a split signal for BAC RP11-745P9, thus localizing the breakpoint to ~138 kb in 2q22.1, which contains no annotated genes. However, metaphase FISH followed by aCGH at ~1 Mb resolution revealed a ~12-Mb interstitial deletion in 2q proximal to the 2q translocation breakpoint, for which the karyotype is 46,XY,t(1;2)(p31.3;q22.1),del(2)(q14.3;q21)dn. The 39 genes within this deletion interval may thus also contribute to the DGAP089 phenotype [22].

To establish further whether disruption of NFIA is primarily associated with the congenital CNS anomalies observed in DGAP104 and DGAP089, we next investigated DGAP174, which has both a t(1;3)(p31.1;q25.1)dn and an interstitial deletion, del(1)(p13.3p32.1)dn. The 1p31.1 and 3q25.1 translocation breakpoints were refined to 150 and 180 kb, respectively. The other potential relevant gene in these intervals is NEGR1, which is disrupted by the 1p31.1 breakpoint. In rat, NEGR1 protein is expressed only after E16, with peak expression occurring postnatally, after corpus callosum formation [24]. However, by RT-PCR, NEGR1 transcript is expressed in human cerebral cortex, hippocampus, corpus callosum, and cerebellum (unpublished data); hence, a contribution to the DGAP174 CNS phenotype is possible. On the other hand, the chromosome deletion in DGAP174, which was delimited by FISH and aCGH to 2.2 Mb at 1p31.3–1p32.1 (Figures 2E and S4), also results in the complete deletion of NFIA and of eight additional genes (Figure 2I).

Lastly, we performed metaphase FISH analyses on chromosomes isolated from the two half-siblings DGAP205–1 and DGAP205–1s and their mother DGAP205–2 (Figure 2F–2I). The 1p31.3–1p32.1 region, encompassing ~12 Mb and containing the entire NFIA gene and ~47 additional genes, is deleted in both DGAP205–1 and DGAP205–2 (Figure 2G–2I). The mother, DGAP205–2, is phenotypically normal but has an apparent balanced rearrangement in which 1p31.3p32.3 is inserted into Chromosome 4 with no loss of genetic material, and her karyotype is therefore designated 46,XX,ins(4;1)(q35;p31.3p32.3) (Figure 2H) [23].

**NFIA Haploinsufficiency Is Common to All Five Cases**

All five individuals share strikingly similar CNS phenotypes, including abnormalities of the corpus callosum, hydrocephalus, and ventriculomegaly. All also share disruption or deletion of NFIA, and in each case, the nonrearranged or nondeleted NFIA allele was subjected to DNA sequencing and no mutations were identified (unpublished data). Therefore, all five cases have NFIA haploinsufficiency in common. NFIA is highly expressed in multiple regions of the human brain, including the embryonic and adult corpus callosum (Figure S5) [25], and ACC and hydrocephalus were observed in Nfia<sup>−/−</sup> mutant mice [18].

In each DGAP case, one or more additional genes were also directly affected as a consequence of either the translocation or deletion. These additional genes may contribute to or modify the nature of the phenotype attributable to NFIA disruption or deletion. However, the identities of these genes differ among the five DGAP cases, except for 205–1 and 205–1s, who share the same 12-Mb deletion; and 205–1, 205–1s, and 174, who share a common 2.2-Mb deletion region (Figure 2I). C20orf32 is disrupted in addition to NFIA in DGAP104, whereas in DGAP089 disruption of NEGR1 and deletion of 39 genes in del(2)(q14.3;q21) occurred. Besides the disruption or deletion of NFIA, none of the other of these genetic aberrations is shared by more than three cases. Therefore,
Figure 2. NFIA is Deleted or Disrupted in All Five Individuals with 1p31.3 Rearrangements

(A and B) Partial karyogram (A) and ideogram (B) of DGAP104 show the chromosome translocation t(1;20)(p31.3;q13.31)dn.

(C) FISH analysis of DGAP104; BAC RP4-802A10 (red signals) hybridizes to the normal Chromosome 1 and der(1) and der(20) chromosomes, thus spanning the 1p31.3 breakpoint.

(D) FISH analysis of DGAP089 depicts BAC RP5-902P15 (red signals) hybridization to the normal Chromosome 1 and der(1) and der(2) chromosomes, thus spanning the 1p31.3 breakpoint.

(E) FISH analysis reveals that BAC RP5-902P15 (overlapping with NFIA, orange color) is deleted from the der(1) in DGAP174. The nondeleted BAC RP11-134C1 (green) is present on both the normal and derivative Chromosome 1.

(F) DGAP089 pedigree with NFIA deletion (arrow indicates the DGAP205–1 proband). DGAP205–1 and half-sister DGAP205-1s have an unbalanced interstitial microdeletion, del(1)(p31.3p32.3), while their phenotypically normal mother DGAP205–2 has a balanced chromosome rearrangement due to an insertion of 1p31.3-p32.3 into Chromosome 4, ins(4;1)(q35;p31.3p32.3). del-NFIA, deletion of NFIA; ins-NFIA, insertion of NFIA.

(G and H) FISH analyses show that BAC RP5-902P15 (overlapping with NFIA, orange color) is absent from the der(1) in DGAP205–1 (G) and der(1) in the mother DGAP205–2 (H), but present in the der(4) in the latter (H). The nondeleted BAC RP4-654H19 (green) is present on both the normal and derivative Chromosome 1.

(I) NFIA exon–intron structure is shown in the upper part with select exons numbered, and the relevant BAC contig below. Locations of the 1p31.3 translocation breakpoints in DGAP104 and DGAP089 are indicated by red dotted vertical lines. The lower part of (I) depicts 1p31.3–1p32.3 genomic regions with the cytogenetic bands on the short arm of Chromosome 1. TEL represents telomeric orientation, and CEN represents centromeric orientation. Known genes in this region are represented by dots and gene names. FISH-verified BAC clones are represented by horizontal bars. The full names of the BAC clones are listed in Materials and Methods. A 2.2-Mb genomic region deleted in DGAP174 and a ~12-Mb genomic region deleted in DGAP205–1 and 205–1s are shown. Deleted BAC clones tested by FISH are designated in red and nondeleted clones in blue. BAC 802A10 overlaps with NFIA and is deleted.

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hydrocephalus, and urinary tract defects (Table S2). Sequence analysis was also performed on a group of 96 individuals that included both syndromic and nonsyndromic ACC, and on another group of 39 individuals with nonsyndromic tethered cord syndrome. Although several known SNPs were detected, no intragenic mutations were identified. Thus, intragenic mutation in NFIA is not a frequent cause of the CNS defects described here.

**Further Analysis of Mouse Nfia Mutants Reveals Additional Phenotypes**

To gain further evidence for the assignment of NFIA as the gene responsible for the CNS and urinary tract defects observed in the five individuals, we re-investigated the previously described Nfia−/− knockout mouse [18]. Because syringomyelia, a persistent patency of the central canal within the spinal cord, is often associated with Chiari type I malformation [26] and was observed in DGAP205-1s but not previously noted in Nfia−/− mice [18], we first examined Nfia expression in the developing spinal cord (Figure 3A–3D). From E11.5–13.5, the expression of Nfia in the spinal cord moves dynamically from rostral to caudal as development proceeds, coincident with the timing of central canal closure (Figure 3A, 3B). Moreover, Nfia expression resides in the basal ventricular zone surrounding the central canal (Figure 3C, 3D). Four of six (66%) Nfia−/− newborns exhibited syringomyelia that was manifest as an enlarged central canal and principally confined to the lumbar region (Figure 3E, 3F). Syringomyelia has been proposed to result from progressive, mechanical overload and dissection of the rostral spinal cord by elevated cerebrospinal fluid hydrostatic pressure such as that associated with hydrocephalus [27]. Indeed, all five individuals with NFIA haploinsufficiency exhibited hydrocephalus or ventriculomegaly. However, mouse Nfia expression in the spinal cord surrounds the central canal and defective canal closure in Nfia−/− mutants precedes the development of hydrocephalus. This suggests that syringomyelia may result from a developmental defect in the dynamic process of canal closure.

**Nfia Expression and Abnormal Kidney Development in Nfia Mutants**

DGAP104, 205–1 and 205–1s share similar urinary tract phenotypes. Although the CNS defects in all five individuals are consistent with findings in mouse Nfia mutants [18], it remained possible that the urinary tract defects might result from the genetic lesion at 20q13.31 (in DGAP104), from haploinsufficiency for other genes in the 1p31.3-p32.3 deletion interval (in DGAP205–1 and DGAP205-1s), or from incidental mutations in other loci. Previously, it was noted that Nfia homozygotes died shortly after birth [18]. Based upon prior experience with mouse mutants with renal agenesis and dysplasia [28–32], we hypothesized that some part of this perinatal lethality might be explained by defects in renal development.

To determine whether the urinary tract phenotypes in DGAP104, 205–1, and 205–1s were linked to NFIA disruption, we investigated Nfia expression in the developing murine kidney and renal morphology in Nfia−/− mice. By in situ hybridization, Nfia was abundantly expressed in the developing nephric duct and metanephros from E9.5 to E16.5, correlating with the stages of ureteric bud outgrowth, the most parsimonious explanation for the observed CNS phenotypes is NFIA haploinsufficiency, which is the only common genetic defect shared by all five individuals.

To test whether intragenic mutations in NFIA are associated with abnormal callosal development and other CNS phenotypes, we sequenced the 11 exons and intron-exon boundaries of NFIA in 84 patients with various combinations of syndromic CNS phenotypes, including abnormal corpus callosum, tethered spinal cord, Chiari I malformation,
metanephric induction, and rapid nephron morphogenesis; expression began to be downregulated at E17.5 (Figure 4A–4I). At E9.5, Nfia expression appeared in the nephric duct and persisted at E10.5 and 11.5 in the developing ureter, becoming more restricted to the distal ureter by E12.5 (Figure 4A and 4B). Nfia was expressed in both ureteric bud epithelium and the surrounding mesenchyme at these time points (Figure 4E and 4F). By E16.5, Nfia expression became restricted to stromal mesenchyme, and was downregulated a day later (Figure 4C, 4D, 4G, and 4H).

We next examined the kidney morphology of Nfia+/−/C0 newborn mice. Remarkably, 13 of 19 (68%) Nfia+/−/C0 newborns displayed agenesis, dysplastic, cystic, or duplex kidneys (Figure 5A–5K). A small number of Nfia+/− mutants exhibited a partial duplex kidney phenotype. This was apparent from both the elongated kidney morphology with a central constriction, and from histological evidence of an ectopic nephrogenic zone that demarcated discrete rostral and caudal nephric poles (Figure 5D, 5H, and 5I).

The presence of kidney defects was not confined to Nfia+/− mutants. Four of 18 (22%) of Nfia+/− newborn mice also expressed hydronephrosis (Figure 5B). The possibility that these Nfia+/− newborns might be Nfia−/− mice that were misgenotyped was excluded because the Nfia−/− affecteds were offspring of an Nfia−/− × Nfia+− cross. The sensitivity of mouse kidney development to Nfia gene dosage supports the conclusion that kidney defects can occur in humans carrying disruption or loss of a single NFIA allele. Therefore, disruption or deletion of NFIA in DGAP104, 205–1, and 205–1s likely explains the kidney phenotypes observed in these individuals.

We next sought to characterize further the renal defects in Nfia−/− embryos by marker experiments. Markers analyzed included Wt1 (glomerular podocytes), DBA (distal tubule and collecting duct), LTL (proximal tubule), and E-cadherin (cell adhesion and aggregation). No consistent changes from wild type that would reflect a deficiency of a particular cell type were observed (unpublished data). These results suggest that the renal defects in Nfia mutants are not lineage- or segment-specific.

**Nfia Kidney Defects Involve Defective Ureteral Development**

The relative preservation of tubular and glomerular markers in Nfia mutant kidneys, the diverse spectrum of renal abnormalities, and the presence of hydronephrosis suggested that the renal parenchymal changes might be secondary to ureteral reflux or obstruction. To test this hypothesis, we crossed a Hoxb7 promoter-directed GFP reporter allele into the Nfia−/− and Nfia+/− backgrounds. The Hoxb7-GFP transgene is specifically expressed in ureteral epithelium as early as E10.5, and thereafter in the ureteric

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**Figure 4. Nfia Expression in the Developing Mouse Urinary Tract**

(A–D) Whole mount in situ hybridization shows Nfia expression in the developing nephric duct (arrow in A), ureter (arrow in B), and metanephros between E9.5 (not shown) and E16.5, becoming downregulated at E17.5. Reticular pattern of Nfia expression is in the kidney from E14.5–16.5 (C). (E–H) Section in situ hybridizations shows Nfia expression in the ureteral epithelium (arrow in F) and surrounding mesenchyme (arrowhead in F) at E11.5 (F is the enlarged view of boxed region in E), and in stromal mesenchyme (arrows in G and H) at E16.5–17.5. (I) RT-PCR of the developing mouse kidney showing that Nfia is abundantly expressed up to E16.5 and begins to be downregulated at E17.5. β-actin is used as RNA loading control.

NB, newborn.
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bud and its derivatives, and eventually in renal tubular epithelium [33].

Remarkably, when assayed by GFP expression, four of 20 (20%) \( Nfia^{+/+} \) and two of six (33%) \( Nfia^{-/-} \) newborns exhibited clear abnormalities of ureteral development (Figure 6A–6F). These abnormalities fell into three classes. First, we observed a partial duplication of the ureter (Figure 6C), which correlates with the duplex kidney phenotype. A second class

Figure 5. Spectrum of Kidney Phenotypes in \( Nfia^{+/+} \) Newborn Mice
Whole mounts of dissected urinary tracts from newborn mice of the indicated genotype (A–F). Normal wild-type newborn kidney and renal histology (A and G); (B) rare \( Nfia^{+/+} \) showing hydronephrosis (arrow); (C–F and H–K) \( Nfia^{-/-} \) mutants showing bilateral renal agenesis (arrow in C), an elongated, partial duplex kidney with the abnormal cortical zone that demarcates the two poles shown with an arrow (D, H, and I), severely dysplastic kidney with irregular renal surface (arrow in E) and disorganized renal parenchyma (E and J), and nodular \( Nfia \) homozygous newborn kidney (F) with renal tubule cystic dilatation (K).
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Figure 6. \( Nfia \) is Required for Normal Ureteral Development
A \( Hoxb7-GFP \) transgenic reporter in the \( Nfia^{+/+} \) and \( Nfia^{-/-} \) backgrounds reveals ureteral defects in newborn mice. (A–D) Wild-type and \( Nfia^{+/+} \) newborns with megaureter (with extent of dilation indicated by two-headed arrow) (A, C) and partial ureteral duplication (arrow) in the mutant (C). Higher power views of boxed areas in (A) and (C), showing distortion of the mutant ureter at the UVJ (B and D); note that the vas deferens in the \( Nfia^{+/+} \) male (C) is also GFP-positive.

(E and F) Abnormalities in \( Nfia^{-/-} \) newborns include proximal flexion of the ureter (E) and dysplastic kidney (F).
k, kidney; t, testis; ur, ureter; UVJ, ureterovesical junction; v, vas deferens
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Discussion

NFIA Haploinsufficiency as a Pathogenetic Mechanism

The five individuals studied here share NFIA haploinsufficiency caused by translocation (DGAP089 and 104) or deletion (DGAP174, 205–1, and 205–1s). All five also share abnormalities of the corpus callosum, and partly share other CNS phenotypes, including ventriculomegaly, congenital hydrocephalus, Chiari type I malformation, and tethered spinal cord. All five individuals also have developmental delay and three exhibited seizure disorders. Three of the five also have urinary tract defects, including VUR. Prior work established that Nfia loss of function in the mouse results in ACC and abnormal development of the hippocampal commissure, two major axonal tracts that connect the cerebral hemispheres, and an associated hydrocephalus that develops in rare postnatal survivors [18,34]. We also found that the mouse Nfia mutant recapitulates the VUR phenotype in these humans. Therefore, although other affected genes may contribute to the overall phenotype, these cases suggest that NFIA haploinsufficiency can account for the observed CNS and renal defects.

It is important to acknowledge that a contribution to the developmental phenotypes identified here from additional genes that reside within various deletion intervals, or that also suffer disruption by breakpoints, is not excluded. In all five cases, additional genes besides NFIA are also disrupted or deleted, so that in no single case is a defect in NFIA the only genetic abnormality. While DGAP104 only inactivates Nfia and C20orf32 and the latter is an unlikely contributory factor, the most extreme cases are DGAP089 and the DGAP205 half-siblings, which contain deletions that involve 39 and 47 genes, respectively. Because these two deletions involve different chromosomes, none of the deleted genes are shared. However, in both cases, many more genes are affected than just NFIA, and some may participate in the observed phenotypes. For example, two cases described in the literature report 2q deletions that overlap with the del(2)(q14.3q21) in DGAP089, and these also involve ACC [35,36]. Therefore, in the absence of intragenic mutations in NFIA, the definitive argument that NFIA is the gene responsible for the CNS and renal phenotypes in these five patients cannot be made.

The inability to identify intragenic mutations in NFIA in cases involving ACC, hydrocephalus, tethered cord syndrome, and urinary tract defects could suggest that the phenotype of heterozygous intragenic loss-of-function NFIA mutations might differ from that described here. Indeed, as noted, it is quite plausible that in any individual DGAP case, the
observed phenotype represents the additive effect of NFIA haplinsufficiency plus other loci that are deleted or disrupted. On the other hand, at least 20 discrete loci have been implicated in ACC alone, so that the failure to detect intragenic mutations in NFIA is not surprising. Ultimately, formal definition of the NFIA hemizygous loss-of-function phenotype would be strengthened by identification of intragenic loss-of-function NFIA mutations.

Lastly, it is well recognized that both chromosomal translocations and deletions may engender position effects that alter gene expression at considerable distances from the site of a chromosomal aberration (reviewed in [1,2]). For example, Shh expression in the limb bud mesenchyme is controlled by a regulatory region located ~1 Mb upstream within the unrelated Lmbr1 gene [37]. Position effects on neighboring genes for mouse knockouts have been described [38]. The mouse Nfia mutant results from a small exon 2 deletion, yet still accurately recapitulates many features of the human phenotype. Therefore, one would have to posit the existence of a conserved regulatory element within the exon 2 deletion region that would act on genes 3' to Nfia, which exhibit conservation of synteny between mouse and human. However, the genes immediately neighboring NFIA are not known to play a role in CNS or kidney development. These include Clor178 (GenID 127795) and TM2D1 (beta amyloid binding protein, GenID 83941), which reside approximately 1 Mb 5' and 200 kb 3' of NFIA, respectively. Additional genes that reside at larger distances from NFIA might be affected by a position effect, but none are obvious candidates. Taking these factors into consideration, we conclude that a true position effect is unlikely to explain the observed phenotypes.

Nature of the CNS Phenotype

Formation of the corpus callosum causes inversion of the cingulate gyri, which gives the medial surface of the brain its characteristic pattern. In ACC, the cingulum remains everted at sites of agenesis, and the sulci of the medial brain extend into the third ventricle. The findings in DGAP174 of an everted cingulate gyrus and longitudinal bundles of Probst are consistent with primary dysgenesis of the corpus callosum. Three midline populations contribute to formation of the corpus callosum: the glial sling, the glial wedge, and glia within the indusium griseum and its precallosal extension, of the corpus callosum. Three midline populations contribute to formation of the corpus callosum: the glial sling, the glial wedge, and glia within the indusium griseum and its precallosal extension.

The function of NFIA in formation of these neuronal populations is not within the class of genes that regulate axonal midline crossing. The prototypical regulatory gene in this class is roundabout or robo, which was originally identified in Drosophila. Roundabout encodes a transmembrane receptor expressed by migratory axons after they cross the CNS midline. Robo binds the extracellular ligand, Slit, which is expressed by midline glia and functions as a chemorepulsive cue that prevents axons from midline recrossing. This function extends to mammals, as mice lacking Robo1 or Robo2 exhibit CNS phenotypes that include abnormal midline commissural axonal guidance, and Robo1 mutants in particular exhibit callosal dysgenesis [42,43]. Similarly, Slit ligands also play a role in callosal development. Slit2 mutants display a small corpus callosum with a reduced number of traversing axons [44–46], while Slit2 glial expression during callosal development in Nfia mutants is reduced [34].

Interestingly, Nfia, Robo2, and Slit2 mouse mutants share not only axonal midline crossing defects, but also phenotypically related renal and ureteral defects. Mice deficient for Robo2 or Slit2 exhibit duplex kidney and megaureter phenotypes [32,47] that in some ways resemble those in Nfia mutants. Our recent study also implicated ROBO2 signaling in the pathogenesis of a subset of human VUR [32]. These related phenotypes raise the possibility that Nfia and SLIT–ROBO signaling are functionally linked in both CNS and ureteral development.

Relationship between Kidney and Ureter Phenotypes

Based on Nfia expression in the developing kidney and the presence of kidney hypoplasia in DGAP104, we identified several distinct kidney phenotypes, including renal dysplasia and hydronephrosis in Nfia mutant mice. Hydronephrosis usually results from an obstruction in the flow of urine at the level of the UPJ or UVJ. This results in an obstructive uropathy in which back pressure from the accumulation of urine in the ureter and renal pelvis results in destruction and distortion of the renal parenchyma. The presence of hydronephrosis in Nfia−/− and Nfia+/− mutants therefore suggests that the observed renal defects reflect a primary disturbance in ureteral development.

A striking finding in Nfia mutant embryos and newborns is the presence of clear ureteral abnormalities: megureter, abnormal ureteral folding, abnormalities at the UVJ and UPJ, and in a small number of cases, partial duplication of the ureter. These findings are consistent with the strong expression of Nfia in the developing nephric duct and ureter at E9.5–13.5. The ureteral duplication phenotype, distinct from normal patterns of ureteric bud branching [48], very likely explains the finding of a partial duplex kidney. Because ureteric bud contact with uninduced metanephric mesenchyme triggers the inductive cascade [49], contact by two separate ureteral branches should produce a partial duplex kidney. In addition to abnormal ureteral development, the VUR in DGAP104 and in the DGAP205 half-siblings may also develop as a consequence of the tethered spinal cord defect [50,51].

In DGAP089 and 174, renal ultrasound revealed no major abnormalities and urinary reflux was not observed. However, subtle anatomic defects in the ureter or kidney are often subclinical, and may exist below the limit of detection. In addition, these results are consistent with those in Nfia+/− and Nfia−/− mutants, where the penetrance of overt kidney or ureteral defects was only 22% and 68%, respectively. One explanation for the incomplete penetrance of ureteral or kidney defects in Nfia mutants could be functional redundancy with Nbhl. In mice, Nbhl is strongly expressed in the developing nephric duct, kidney, and ureter at E10.5–11.5, where its expression overlaps with that of Nfia (unpublished data). The other two Nuclear Factor I family members, Nfie and Nfia, are expressed only at lower levels in the developing ureter and kidney. In addition, Nbhl−/− mice exhibit callosal agenesis and forebrain defects similar to those seen in Nfia−/− mice [21]. Thus, partial redundancy may exist between Nfia and Nbhl in both CNS and urinary tract development. Genetic combinations of mutant alleles for Nfia and Nbhl will be
required to address this question definitively, and to further disclose the roles of Nh factors in development.

In sum, our results suggest that NFIA haploinsufficiency in humans results in a thin, hydropic or absent corpus callosum, and define the spectrum of defects attributable to NFIA loss of function to include additional CNS and urinary tract defects that were not previously apparent in the Nfia mouse mutant. These results illustrate the powerful synergy that occurs when corresponding human and mouse disorders are investigated in parallel.

Materials and Methods

DGAP individuals studied. DGAP104. DGAP104 is the product of in vitro fertilization via intracytoplasmic sperm injection, whose parents of European descent were unrelated with no reported medical problems. Amniocentesis demonstrated a 46,XX,t(1;12)(p32.3;q13.31)dn, but ultrasound revealed no organ malformations at 20 wk of pregnancy. Because of placenta previa and persistent vaginal bleeding, DGAP104 was delivered at 31 wk via elective cesarean section and weighed 1,980 g with Apgar scores of 5/10. She was diagnosed with prematurity, hydrocephaly, Chiari I malformation, tethered spinal cord, congenital hydrenephrosis, left hypoplastic kidney, bilateral inguinal hernia, hyaline membrane disease grade 2, and gastroschepalag reflux. Imaging studies indicated a thin posterior corpus callosum and an open aqueduct with progressive ventricular enlargement. She was delayed in reaching developmental milestones; at 2 y, she exhibited major motor delay with inhibited movement and was wheelchair bound. Speech was also delayed, and limited to a few words. At 6 y 7 mo of age, DGAP104 received a performance IQ score of 42, verbal IQ score of 68, and global IQ score of 52 on the Weschler Preschool and Primary Scale of Intelligence-Revised test (WPPSI-R).

To relieve hydrocephalus, which caused progressive macrocephaly, seven neurosurgical operations were performed over 3 y, including a third ventriculostomystomy, a ventriculoperitoneal shunt with a flow-regulated valve, and several revisions because of hydrodrainage or blockage of the shunt. At 7 d of age, abdominal ultrasonography revealed bilateral hypoplastic kidneys and bilateral dilatation of the renal pelvis. At 1 y, ultrasound showed left and right kidney lengths of 45 mm and 51 mm, respectively (mean length for age, 52 mm), and both kidneys lacked corticomedullary differentiation. At 2 y, the left kidney length was 49 mm and the right 58 mm (mean length for age, 55 mm), but both lacked discernable corticomedullary differentiation. At 5 y, the left kidney was 53 mm and the right 61 mm (mean length for age, 66 mm). DGAP104 first exhibited left grade II VUR by VCUG. VUR increased to grade III with a right vescoureteral junction diverticulum, and pyelonephritis developed that required ureteral reimplantation surgery (Cohen operation).

Urea and creatinine levels were normal at 5 y of age. At 7 y, and was 4 ft tall and weighed 72.5 lb. DGAP104-1s received a performance IQ score of 68, and global IQ score of 52 on the Weschler Preschool and Primary Scale of Intelligence-Revised test (WPPSI-R).

DGAP174. DGAP174 was born to a 20-y-old mother at 37 wk by cesarean section and weighed 2,770 g (95th percentile) with length 49.5 cm (25th percentile) and occipitofrontal circumference 37.5 cm (75th percentile). Pregnancy was uncomplicated by teratogenic exposures or maternal illness. A second trimester prenatal ultrasound showed agenesis of the corpus callosum and enlarged ventricles. A CT scan of the brain did not reveal any posterior fossa abnormalities, and prenatal findings, and revealed ventriculomegaly with parallel lateral ventricles representing longitudinal bundles of Probst, and a tethered spinal cord. A small ventricular septal defect was also noted at birth. Chromosome analysis of peripheral blood lymphocytes revealed 46,XY,t(13p)22q21.1dn. The neonate was discharged and seen again at 13 d of age in the genetics clinic. He was found to have metopic stenosis and bitemporal narrowing that was surgically corrected at 20 mo of age. Neurological exam revealed normal tone and reflexes. At 8 mo of age, he was noted to be gaining weight rapidly, unrelated to any change in eating pattern. At 12 mo of age, he was noted to have hypotonia and showed severe delay in gross motor development, macrocephaly without hydrocephalus, and height, weight, and length above the 95th percentile for his age. Additionally, a dimple on the posterior aspect of the right helix, creases behind each earlobe, and esotropia secondary to telecanthus and epicanthal folds were noted. A left inguinal hernia was detected at 24 mo. At 24 mo, classes were prescribed to correct hypertonia and strabismus, and all milestones were on track except for speech. At 36 mo, expressive language was still delayed. Due to progressive delay of the patient, he was seen at age 27 mo for a formal developmental assessment.

DGAP089. DGAP089 is a male with an interstitial deletion on 2q and a balanced rearrangement involving 1p and 2q. Additional clinical data are summarized in Table S1 and described in more detail elsewhere [22]. Seizure onset occurred at 19 mo, with treatment until age 6 y. DGAP089 was diagnosed with autism spectrum disorder and is currently on Ritalin. He was noted to have right hypertropia and strabismus, and all milestones were on track except for speech. At 10 y of age, he was noted to have normal speech and was reading at an age equivalent of 35 mo. At 7 y of age, DGAP089 underwent balance developmental assessment. His IQ score was 68 on a Leiter International Performance Scale (LIPS) test, corresponding to an age equivalent of 36 mo. He received a standard score of 60 in the Peabody Picture Vocabulary Test (Revised, Form M, PPVT-R) corresponding to an age equivalent of 35 mo. His standard score for the Developmental Test of Visual-Motor Integration (VMI) was 67, which also corresponded to an age equivalent of 35 mo. At 6 y of age, DGAP089 underwent a successful Chiari decompression and repair of the tethered spinal cord. At that time, he was diagnosed with attention deficit and hyperactivity disorder and is currently on Ritalin. He was noted to have right hemihyptropia and strabismus, for which he referred to an orthopedics clinic. At age 8 y and 4 mo (January 2007), DGAP174 was functioning at the kindergarten level, and received occupational and physical therapy for speech.

DGAP205–1, 205–1s, and 205–2. DGAP205–1 and DGAP205-1s are two half-siblings with an interstitial microdeletion, del(1)(p31.3p32.3), that was inherited as an unbalanced segregant resulting from a balanced rearrangement in their mother, DGAP205–2. Both half-siblings had congenital CNS and urinary tract defects while their mother was phenotypically normal. DGAP205–1 had the Bayley Scales of Infant Development test (BSID) at 4.5 y of age and received scores corresponding to an age equivalent of 15 mo of mental development, 20 mo for cognition, and 17 mo for fine motor. At age 5 y, DGAP205–1s was functioning at an age equivalent of 15 mo of age. At age 10 y he was functioning at an age equivalent of ~7 y, and was 4 ft tall and weighed ~65 lb. DGAP205–1 has limited verbal skills and uses a combination of words and signs for communication. He has not had any formal testing at 10 y of age, but can only read and spell three-letter words. DGAP205-1s received the Bayleys Scales of Infant Development test (BSID) at 2 y of age, and the Stanford-Binet test at 9 y. Both tests demonstrated moderate global cognitive impairment (scores not available). At 9 y of age, DGAP205–1s was functioning at the kindergarten level. Other clinical data for the affected sibs are summarized in Table S1, and described in more detail elsewhere [23].

FISH, aCGH, and mutation screening. Metaphase FISH was performed according to standard methods. BAC clones were obtained from BACPAC Resources (http://bACPAC.chori.org). Labeled as FISH probes, and hybridized to metaphase chromosomes prepared from lymphoblastoid cell lines established from all five individuals. The full BAC names provided in Figure 2I are: RP4-654H19, RP5-156K19, RP5-156K19, RP5-11078M7, RP5-534K7, RP11-80K19, RP11-1301O15, and RP11-89K2. aCGH experiments were performed with the Spectral Genomics 2600 BAC array by the Cytogenetics Core Facility of the Dana-Farber/ Harvard Cancer Center for DGAP089 and DGAP174, and by Spectral Genomics for DGAP104. NFIA mutation screening employed PCR amplification of the exon 11–16 boundaries, followed by purification and bidirectional DNA sequencing. NFIA cDNA sequence AB037860 (http://www.ncbi.nlm.nih.gov/ entrez/viewer.fcgi?db=nucleotide&val=7245275) was used to calculate
nucleotide positions. MLPA analysis of the NFIA coding region was performed in a subset of syndromic and nonsyndromic ACC samples, and no copy number changes were identified.

RT-PCR analysis. RT-PCR analyses were performed by routine protocols. RT-PCR primers used to amplify the Nfia 354 bp cDNA were 5'-CGTGGCACCACCATCACGAG-3' and mNfia-F: 5'-CAAGCCTCCAACCACATCAAC-3'. RT-PCR primers used to amplify the C20orf32 436 bp cDNA were mC20orf32-F: 5'-GGGCACTCTACGACAACCAT-3' and mC20orf32-R: 5'-CTGGGAAAGCAGACAGAGG-3'.

Southern and northern blot analysis. Southern blotting was performed by standard methods. Probes were labeled using the MegaPrime labeling kit (Amersham/GE Healthcare, http://www.amershamhealth-us.com). Genomic DNA from the DGAP089 cell line and from a karyotypically normal control were digested with DraII, PstI, and SspI and hybridized with a probe containing a 700-bp probe amplified from DGAP104 and genomic DNA from a karyotypically normal male control were hybridized with a 623-bp probe that was amplified from RT-PCR products amplified using PCR primers used to amplify the Nfia mutant allele. A 218-bp probe was amplified from the Nfia wild-type allele.

Analysis of Nfia mutant mice. The generation and analysis of brain defects in Nfia knockout mice in a C57BL/6 background has been previously described [18]. Nfia+/- mice analyzed in Figure S6 were C57BL/6X129S6 F1 hybrid animals that have longer postnatal survival than C57BL/6 inbred mice. The Nfia+/- allele was genotyped by PCR amplification using the mutant allele specific forward primer Nfia-inl-F2 (5'-CGTGGCACCACCATCACGAG-3'), the reverse primer Nfia-inl-R2 (5'-GGGCACTCTACGACAACCAT-3'), and an internal probe containing poly A+ RNA from multiple regions of human brain (Human Brain V blot, Clontech, http://www.clontech.com) was hybridized with a probe corresponding to exons 2-6 of NFIA following a standard protocol.

In situ hybridization and immunohistochemistry. Tissue in situ hybridization of whole mount and cryosections was performed according to standard protocols using cRNA probes complementary to the 5'-UTRs of Nfia and C20orf32. For WT1 immunostaining, kidney sections were sectioned at 4 µm and stained with anti-WT1 antibody (Santa Cruz Biotechnology, sc-369). GFP fluorescence illumination of the mouse urinary tract was evaluated using a Nikon SMZ-1500 epi-fluorescence stereomicroscope (http://www.nikonusa.com).

Ethics. All human studies were performed under informed consent protocols approved by the Human Research Committee of Partners HealthCare System, Boston. Mouse protocols were approved by the Institutional Animal Care and Use Committee at Harvard Medical School or at Boston University Medical Center.

Supporting Information

Figure S1. Southern Blot Analysis of 1p31.3 Breakpoint in DGAP104

NFIA is disrupted in DGAP104 and the breakpoint lies within intron 2 of NFIA, as shown by DGAP104 DNA digested with Bmp1, BspHI, DraII, EcoRI, and XhoI.

Table S1. Clinical Findings in Five Individuals with Chromosome Abnormalities Involving 1p31

Table S2. Phenotypes of 84 Patients with Callosal and other CNS Malformations and Urinary Tract Defects Subjected to NFIA Intragenic Mutation Screening

Accession Numbers

The GenID numbers for the Entrez Gene (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene) discussed in this paper are AK3L2 (387851), AL66 (29929), ANGPTL3 (27392), ANKH3 (163782), ATG4C (84938), BNSD (7890), Clofil141 (400757), Clofil146 (199920), CTA (731), C8B (732), C20orf32 (50079), C20orf32 (329664), CAGHD1 (57685), CYP2J2 (1573), DAB1 (1600), DNAJC6 (50829), DOCK7 (85440), FTO (55277), FLJ45337 (400754), FOXD3 (27092), HOOK1 (51361), IL23R (14923), INADL (19097), INSL5 (10092), ITGB3BP3 (23421), JAK1 (3716), JUN (3725), KIAA1799 (84455), LITLD1 (65496), LEPR (39535), LEPROT (47141), MIER1 (57708), NEGR1 (257194), NFIA (4774), Nfia (18027), OMAI (113509), PCSK9 (25578), PDE4B (5142), PCMI (5236), PPAP2B (8613), PRKAA2 (5563), RAVER2 (52925), ROR1 (1919), ScGpI (84251), SLCL5D1 (23169), TACSTD2 (4070), TCEX1D1 (200132), TM2D1 (89341), UBE2U (148581), USP1 (7398), and WDR78 (79819).

The disease identifiers for the OMIM (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM) genetic disorders discussed in this paper are as follows: Abnormalities Involving 1p31.3 (225690), hydrocephalus and ventriculomegaly (OMIM 235660), Chiari malformation type I (OMIM 118420), and vescoureteral reflux (OMIM 193060 and 610878).
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Author contributions. WL, FQR, YF, CCM, JFG and RLM conceived and designed the experiments. WL, FQR, YF, FSA, DJD, QX, QD and RLM performed the experiments. WL, FQR, YF, FSA, DJD, DJH, HF, CK, AH, AHL, BJQ, CCM, JFG and RLM analyzed the data. WL, CGC, ALS, EHS, AA, BR, PP, AGB, CAM, RG, KD, CCM, and RLM contributed reagents/materials/analysis tools. WL, FQR, JFG, and RLM wrote the paper.

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References