Ligase IV syndrome can present with microcephaly and radial ray anomalies similar to Fanconi anaemia plus fatal kidney malformations

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ARTICLE INFO
Keywords:
LIG4
Cystic dysplastic kidneys
Microcephaly
Fanconi anaemia
Radial ray defects
Acrorenal syndrome

ABSTRACT
Ligase IV (LIG4) syndrome is a rare disorder of DNA damage repair caused by biallelic, pathogenic variants in LIG4. This is a phenotypically heterogeneous condition with clinical presentation varying from lymphoreticular malignancies in developmentally normal individuals to significant microcephaly, primordial dwarfism, radiation hypersensitivity, severe combined immunodeficiency and early mortality. Renal defects have only rarely been described as part of the ligase IV disease spectrum.

We identified a consanguineous family where three siblings presenting with antenatal growth retardation, microcephaly, severe renal anomalies and skeletal abnormalities, including radial ray defects. Autozygosity mapping and exome sequencing identified a novel homozygous frameshift variant in LIG4, c.597_600delTCAG, p.(Gln200LysfsTer33), which segregated in the family.

LIG4 is encoded by a single exon and so this frameshift variant is predicted to result in a protein truncated by 678 amino acids. This is the shortest predicted LIG4 protein product reported and correlates with the most severe clinical phenotype described to date. We note the clinical overlap with Fanconi anaemia and suggest that LIG4 syndrome is considered in the differential diagnosis of this severe developmental disorder.

1. Introduction

Ligase IV syndrome (MIM 606593) (Altmann and Gennery, 2016a) is a rare, phenotypically heterogeneous disorder caused by biallelic truncating variants in LIG4 (O’Driscoll et al., 2001). The clinical features range from increased sensitivity to ionising radiation in a developmentally normal child (Riballo E Doherty et al., 2001) to microcephaly, mild immunodeficiency, developmental delay, and pancytopenia (O’Driscoll et al., 2001; Unal et al., 2009), short stature (Buck et al., 2006; Enders et al., 2006; Murray et al., 2014), severe combined immunodeficiency (SCID) (Buck et al., 2006; Enders et al., 2006), extreme growth failure and syndactyly (Murray et al., 2014).

As LIG4 is encoded by only one exon, and variants that introduce a premature stop codon will result in transcripts that escape nonsense-mediated decay and so produce a truncated protein. The severity of clinical phenotype in ligase IV syndrome has been correlated with the position of the truncating mutation and the size of the resultant truncated protein (Murray et al., 2014). Biallelic truncating mutations at the distal (3’) end of the gene result in a milder phenotype (Murray et al., 2014). Distal mutations in trans with a more proximal truncating mutation are associated with a severe growth phenotype, chronic or progressive cytopenia and immune dysfunction. A homozygous 5’ mutation, which removes the entire enzymatic domain, has been reported to cause the most severe phenotype reported to date (Murray et al., 2014).
1.1. Clinical report

Here, we report a consanguineous British Pakistani family with three affected fetuses each with a severe phenotype resulting in termination of pregnancy (Fig. 1). Key phenotypic features included cystic dysplastic kidneys, oligohydramnios or anhydramnios, microcephaly and intrauterine growth retardation, hypoplastic thumbs and radial ray deformities.

The parents were healthy first cousins and have both had normal renal tract ultrasound scans. The mother had a spontaneous abortion at 8 weeks into her first pregnancy (II-1). She had a termination of her second pregnancy (II-2) at 24 weeks gestation for intrauterine growth retardation, cystic kidneys and anhydramnios, all as visualised on ultrasonography. Post mortem was declined. Her third pregnancy (II-3) resulted in the birth of a normal female child.

During her fourth pregnancy (II-4), ultrasound scan at 15+ weeks gestation showed severe oligohydramnios and the kidneys were not visualised. A termination of pregnancy was carried out at 16 + 3 weeks’ gestation. The post-mortem examination identified symmetrical growth retardation with all growth parameters > 2SD below mean, corresponding to a fetus of 14–15 weeks gestation. Examination of the upper limbs showed bilateral proximally inserted hypoplastic thumbs. The left leg was internally rotated with talipes of the left foot. The right kidney weighed 0.07 g and left kidney weighed 0.08 g, each at the 10th

Fig. 1. Pedigree of the family affected by LIG4 syndrome, indicating both affected (shaded symbols) and unaffected (unshaded symbols) individuals.

Fig. 2. Skeletal survey of Fetus II-5. There are bilateral radial defects of the upper limbs. On the right, the thumb is hypoplastic. The radius and ulna are present. On the left, the radius is absent, the ulna is hypoplastic and bowed and the left thumb is absent.
centile (Archie et al., 2006). Histologically the kidneys had cystic dilatation of the tubules surrounded by undifferentiated mesenchyme-like cells, hallmarks of renal dysplasia (Woolf et al., 2004). The urinary bladder and ureters appeared normal on direct examination. External examination of the genitalia was indeterminate but there was histological evidence of primitive ovarian tissue. Genetic investigations revealed a normal female karyotype and a normal chromosomal microarray (OGT ISCA 8 × 60 K oligo array). Chromosome breakage studies were not undertaken. Fetal X ray showed 11 pairs of ribs, but no other evidence of a skeletal dysplasia.

In the fifth pregnancy (II-5) early ultrasound scans again identified oligohydramnios and abnormal kidneys. On subsequent scans at 17 and 18 weeks the kidneys were not visible. Termination of pregnancy was carried out at 18 + 4 weeks gestation. The postmortem examination identified a male fetus with symmetrical intrauterine growth retardation, with all external growth parameters measuring <5th centile for gestation. The ears were low set and eyelids fused. Upper limb examination showed a small left forearm with the hand connected to the elbow on the left. The left thumb was absent and the right thumb was hypoplastic. There was bilateral positional talipes of the lower limbs, with the lower limb smaller on the left than the right and left 2–3 toe syndactyly. External male genitalia were seen but testes were absent. A skeletal survey confirmed the absent left thumb and radius, hypoplastic and bowed left ulna, hypoplastic right thumb and a hypoplastic sacrum (Fig. 2). On direct inspection both kidneys appeared to be absent with a thin fibrous wall was present. Cytogenetic breakage studies were not undertaken. Fetal X ray showed 11 pairs of ribs, but no other evidence of a skeletal dysplasia.

| Table 1 | Comparison of clinical features in individuals with LIG4 syndrome and Fanconi anaemia. |
|---------|--------------------------------------------------------------------------------------|
|         | LIG4 syndrome (Murray et al., 2014) (Altmann and Gennery, 2016a) | Fanconi Anaemia (Mehta et al., 1993–2020) (Tischkowitz and Hodgson, 2004) (Dokal, 2000) |
| IUGR    | Common                                                                           | Common |
| Short stature | Common                                                                       | Variable |
| Microcephaly | Common                                                                         | Common |
| Upper limb thumb/radial ray defects | Rare (this report)                                                               | 35% |
| Craniofacial malformation | Common, beak-like nose, prominent mid-face, receding forehead and micrognathia | (Altmann and Gennery, 2016a) Rare |
| Skeletal malformation | Not reported                                                                   | 70% |
| Renal malformation | Rare                                                                           | 20–34% |
| Skin pigmentation (café au lait macules) | Reported                                                                      | 40–64% |
| Intellectual and/or developmental delay | Variable                                                                      | (O’Driscoll et al., 2001) 10–16% |
| Ophthalmological defect (microphthalmia) | Not reported                                                                 | 20–38% |
| Cardiac defect | Not reported                                                                   | 6–13% |
| Gastrointestinal tract defect | Not reported                                                                  | 5–16% |
| Hearing loss | Not reported                                                                   | 10% |
| Haematological defect (pancytopenia) | Common                                                                         | 90% |
| Immunological defect | Common, prone to infection                                                     | Reported |
| Acute myeloid leukaemia | Not reported                                                                  | Common (500 fold increased risk) |
| Solid tumour | Not reported                                                                   | Common, especially head and neck squamous cell carcinoma |
| Chromosome instability | Not reported                                                                  | Yes |
| Cellular radiosensitivity | Yes                                                                           | Reported |

2. Discussion

We report a severe phenotype of LIG4 syndrome due to a biallelic truncating mutation p.(Gln200LysfsTer33) in LIG4. This variant is the most proximal pathogenic variant reported to date (Murray et al., 2014). Previous studies indicated that truncating mutations close to the N-terminus result in a near complete loss of ligase 4 enzyme function and correlate with a more severe phenotype (Murray et al., 2014). The data presented here is consistent with this hypothesis. We speculate that variants that occur more proximally than the one reported here will also be incompatible with life, as is seen with complete knockout of lig4 in mice (Barnes et al., 1998).

Microcephaly and severe intrauterine growth retardation (IUGR)
have both been previously reported in severe cases of LIG4 syndrome (Murray et al., 2014). However, severe renal anomalies, as seen in these affected fetuses, are not a common feature of LIG4 syndrome. In a case series of eleven children with LIG4 syndrome, renal anomalies including atrophic or dysplastic kidneys were reported in only two cases, although renal imaging was not reported for all cases (Murray et al., 2014). One male with biallelic variants in LIG4 presented with dysplastic kidneys, bilateral vescourethral reflux and urethral valves (IJspeert et al., 2013), whereas the other was a female with an ‘atrophic’ kidney (Murray et al., 2014). The sibling of the latter case, who also had LIG4 syndrome, did not have documented renal involvement. In contrast to the severe renal involvement affecting all of the fetuses in the family described here, the milder renal tract anomalies did not appear to segregate in the other families with the specific LIG4 variants. Renal imaging would be appropriate in all individuals with LIG4 syndrome to establish the relationships between LIG4 variants and renal tract disease.

Of note the position of the variant within LIG4 did not correlate with the occurrence of milder kidney or lower urinary tract involvement. It will be informative to document renal involvement in other cases with proximal truncating LIG4 variants. In an individual with biallelic variants in LRIG2, the causal gene for urofacial syndrome (MIM 615112), and a homozygous LIG4 missense variant, the affected child had recurrent episodes of urosepsis, secondary to severe vescicoureteral reflux, leading to left kidney hypoplasia and scarring (Fadda et al., 2016). In this case the lower urinary tract disease and kidney disease was attributed to co-occurrence of urofacial syndrome which is characterised by abnormal bladder voiding and vescicoureteric reflux.

Murine lig4 variant models have not been reported to have renal or urinary tract involvement (Rucci et al., 2010), but there is no specific description of whether the kidneys were examined. Further homozygous null mutants die in midgestation at a time before the metanephric kidney initiates (Barnes et al., 1998; Frank et al., 1998). Of note, the GUDMAP mouse gene expression database (Gudmap.org/Genito) shows that LIG4 is highly expressed in the ureteric bud and metanephric mesenchyme. Indeed, it has been postulated that aberrant induction between these respective precursors of collecting ducts and nephrons underlies the pathogenesis of renal dysplasia (Woolf et al., 2004).

Radial ray anomalies have not been reported in individuals with LIG4 syndrome. Previous case reports show other skeletal abnormalities, including hypoplastic ribs, fusion of carpal bones or abnormal vertebrae (Murray et al., 2014). The radial ray defects in combination with UIGR, microcephaly and renal defects suggest an overlapping phenotype between LIG4 syndrome and Fanconi anaemia (FA, MIM 227650), a clinically and genetically heterogeneous autosomal recessive disorder which like LIG4 syndrome impacts on the DNA damage repair pathway, and can present with multiple congenital anomalies, including lethal antenatal presentation (Table 1). Approximately half of all patients with FA have renal involvement, including dysplastic, pelvic, malrotated kidneys, crossed fused ectopia, horseshoe, and multicystic kidneys (Sathyarayana et al., 2018).

Renal anomalies and limb defects are characteristic of a clinically heterogeneous group of disorders termed acrorenal syndrome (Kroes et al., 2004). In a series of 197 cases with acrorenal syndrome, eight had radial ray defects and renal anomalies without a syndromal diagnosis (Murray et al., 2014). Our report suggests that LIG4 syndrome should be a differential diagnosis to consider in individuals with acrorenal syndrome.

This report expands the phenotypic spectrum LIG4 syndrome due to premature truncating mutations to include antenatal severe renal phenotype and radial ray skeletal anomalies. The finding in this family facilitates the option for prenatal testing in future pregnancies, and cascade genetic testing in the wider family if married consanguineously to determine their reproductive risks. We recommend that LIG4 is added to the extensive list of genes to be tested when a diagnosis of Fanconi anaemia is considered as part of the differential diagnosis.

CRediT authorship contribution statement

Rajesh Madhu: Methodology, Formal analysis, Investigation, Writing - original draft. Glenda M. Beaman: Methodology, Formal analysis, Investigation, Writing - review & editing. Kate E. Chandler: Resources, Writing - review & editing. James O’Sullivan: Methodology, Formal analysis, Investigation, Writing - review & editing. Jill E. Urquhart: Methodology, Formal analysis, Investigation, Writing - review & editing. Naz Khan: Resources, Writing - review & editing. Elizabeth Martindale: Resources, Writing - review & editing. Tracy A. Briggs: Resources, Writing - review & editing. Jill Clayton-Smith: Resources, Writing - review & editing. Jenny Higgs: Resources, Writing - review & editing. Gauri Batra: Resources, Writing - review & editing. Bronwyn Kerr: Resources, Writing - review & editing. Adrian S. Woolf: Conceptualization, Methodology, Writing - original draft, Supervision, Project administration, Funding acquisition. William G. Newman: Conceptualization, Methodology, Writing - original draft, Supervision, Project administration, Funding acquisition.

Acknowledgments

We thank the family for their participation. We acknowledge the support of Kidney Research UK Paed_RP.002.20190925 (ASW and WGN) Medical Research Council MR./L002744/1 (ASW); Newlife Foundation 15-15/03 and 15-16/06 (ASW and WGN). WGN is supported by the Manchester NIHR BRC IS-BRC-1215-20007.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2020.103974.

References

Altmann, T., Gennery, A.R., 2016a. DNA ligase IV syndrome; a review. Orphanet J. Rare Dis. 11, 137.
Arkic, J.G., Collins, J.S., Lebel, R.R., 2006. Quantitative standards for fetal and neonatal autopsiy. Am. J. Clin. Pathol. 126, 256–265.
Barnes, D., et al., 1998. Targeted disruption of the gene encoding DNA ligase IV leads to lethality in embryonic mice. Curr. Biol. 8, 1395–1398.
Buck, D., Moshou, D., de Chassey, R.A., Mas, Y., le Deist, F., Cavazzana-Calvo, M., Fischer, A., Casanova, J.L., Lieber, M.R., de Villartay, J.P., 2006. Severe combined immune-deficiency and microcephaly in siblings with hypomorphic mutations in DNA ligase IV. Eur. J. Immunol. 36, 224–235.
Carr, I.M., Flintoff, K.J., Taylor, G.R., Markham, A.F., Bonhtron, D.T., 2006. Interactive visual analysis of SNP data for rapid autozygosity mapping in consanguineous families. Hum. Mutat. 27, 1041–1046.
Cissold, R.L., Hamilton, A.J., Hattersley, A.T., Ellard, S., Bingham, C., 2015. HNF1B-associated renal and extra-renal disease-an expanding clinical spectrum. Nat. Rev. Nephrol. 11, 102–112.
Deeb, A., Robertson, A., MacColl, G., Bouloux, P.M., Gibson, M., Winyard, P.J., Woolf, A.S., Moghal, N.E., Cheetham, T.D., 2001. Multicytic dysplastic kidney and Kalniman’s syndrome: a new association? Nephrol. Dial. Transplant. 16, 1170–1175.
Dokal, I., 2000. The genetics of Fanconi’s anaemia. Baillieres Best Pract. Res. Clin. Haematol. 13, 407–425.
Enders, A., Fisch, P., Schwarz, K., Duffner, U., Pannicke, U., Nikolopoulos, E., Peters, A., Orlowska-Volk, M., Schindler, D., Friedrich, W., Selle, R., Niemeyer, C., Ehf, S., 2006. A severe form of human combined immunodeficiency due to mutations in DNA ligase IV. J. Immunol. 176, 5060–5066.
Fadda, A., Butt, F., Tomei, S., Deola, S., Lo, B., Robay, A., Al-Shakaki, A., Al-Hajjiri, N., Crystal, R., Kambouris, M., Wang, E., Marincola, F.M., Falkho, K.A., Cagno, C., 2016. Two hits in one: whole genome sequencing unveils LIG4 syndrome and urofacial syndrome in a case report of a child with complex phenotype. BMC Med. Genet. 17, 84.
Frank, K.M., Sekiguchi, J.M., Seidl, K.J., Swat, W., Rathbun, G.A., Cheng, H.L., Davidson, L., Kangalos, L., Alt, F.W., 1998. Late embryonic lethality and impaired V(D)J recombination in mice lacking DNA ligase IV. Nature 396, 173–177.
Gudmap.org, GUDMAP genitourinary development molecular anatomy Project. [online] Available at: https://www.gudmap.org/chaise/record/#2/Common:Gene/RID=Q-7004.
IJspeert, H., Warris, A., van der Flier, M., Reisli, I., Keles, S., Chishimba, S., van Dongen, J.J., van Gent, D.C., van der Burg, M., 2013. Clinical spectrum of LIG4 deficiency is broadened with severe dysmmaturity, primordial dwarfism, and neurological
abnormalities. Hum. Mutat. 34, 1611–1614.

Jadeja, S., Smyth, I.Pitera JE., Taylor, M., van Haelst, M., Bentley, E., McGregor, L., Hopkins, J., Chalepakis, G., Philip, N., Perez-Aytes, A., Watt, F.M., Darling, S.M., Jackson, I., Woolf, A.S., Scambler, P.J., 2005. Identification of a new gene mutated in Fraser syndrome and mouse myelencephalic blebs. Nat. Genet. 37, 520–525.

Kroes, H.Y., Olney, R.S., Rosano, A., Liu, Y., Castillo, E.E., Cocchi, G., De Vigan, C., Martinez-Frias, M.L., Mastroiacovo, P., Merlo, P., Mutchinick, O., Ritvanen, A., Stoll, C., van Essen, A.J., Cobben, J.M., Cornel, M.C., 2004. Renal defects and limb deficiencies in 197 infants: is it possible to define the "acrorenal syndrome"? Am. J. Med. Genet. 129A, 149–155.

McGregor, L., Makela, V., Darling, S.M., Vrontou, S., Chalepakis, G., Roberts, C., Smart, N., Rutland, P., Prescott, N., Hopkins, J., Bentley, E., Shaw, A., Roberts, E., Mueller, R., Jadeja, S., Philip, N., Nelson, J., Francannet, C., Perez-Aytes, A., Megarbane, A., Kerr, B., Wainwright, B., Woolf, A.S., Winter, R.M., Scambler, P.J., 2003. Fraser syndrome and mouse blebbled phenotype caused by mutations in FRAS1/Fras1 encoding a putative extracellular matrix protein. Nat. Genet. 34, 203–208.

Mehta, P.A., Tolar, J., 1993–2020. Fanconi anaemia. In: Adam, M.P., Ardinger, H.H., Pagon, R.A., Wallace, S.E., Bean, L.J.H., Stephens, K., Amemiya, A. (Eds.), GeneReviews® [Internet]. University of Washington, Seattle, Seattle (WA) 2002 Feb 14 [updated 2018 Mar 8].

Murray, J., Bicknell, L.S., Yigit, G., Duker, A.L., van Kogelenberg, M., Haghyegh, S., Wieczorek, D., Kayserili, H., Albert, M.H., Wise, C.A., Brandon, J., Kleefstra, T., Warrir, A., van der Flier, M., Bamforth, J.S., Doonan, K., Ades, L., Ma, A., Field, M., Johnson, D., Shackley, F., Firth, H., Woods, C.G., Nünzberg, P., Gatti, R.A., Hurles, M., Bober, M.B., Wollnik, B., Jackson, A.P., 2014. Extreme growth failure is a common presentation of ligaase IV deficiency. Hum. Mutat. 35, 76–85.

O’Driscoll, M., Cerosaletti, K.M., Girard, P.M., Dai, Y., Stumm, M., Kysela, B., Hirsch, B., Gennery, A., Palmer, S.E., Seidel, J., Gatti, R.A., Varon, R., Oettinger, M.A., Neitzel, H., Jego, P.A., Concannon, P., 2001. DNA ligaase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. Mol. Cell 8, 1175–1185.

Ribiello E Doherty, A.J., Dai, T., Stiff, T., Oettinger, M.A., Jego, P.A., Kysela, B., 2001. Cellular and biochemical impact of a mutation in DNA ligase IV conferring clinical radiosensitivity. J. Biol. Chem. 276, 31124–31132.

Rucci, F., Notarangelo, L.D., Fazioli, A., Patrizi, L., Hickernell, T., Paganini, T., Couklely, K.M., Detre, C., Keserzi, M., Walter, J.E., Feldman, L., Cheng, H.L., Poliani, P.L., Wang, J.H., Balter, B.B., Recher, M., Andersson, E.M., Zhu, S., Giliani, S., Terhorst, C., Alt, F.W., Yan, C.T., 2010. Homozygous DNA ligase IV R278H mutation in mice leads to leaky SCID and represents a model for human LIG4 syndrome. Proc. Natl. Acad. Sci. U. S. A. 107 3024–9.

Sathyarayananaya, V., Lee, B., Wright, N.B., Santos, R., Bonney, D., Wynn, R., Patel, L., Chandler, K., Cheesman, E., Schindler, D., Webb, N.J.A., Meyer, S., 2018. Patterns and frequency of renal abnormalities in Fanconi anaemia: implications for long-term management. Pediatr. Nephrol. 33, 1547–1551.

Tischkowitz, M.D., Hodgson, S.V., 2003. Fanconi anaemia. J. Med. Genet. 40, 1–10.

Unal, S., Cerosaletti, K., Uckan-Cetinkaya, D., Cetin, M., Gumruk, F., 2009. A novel mutation in a family with DNA ligase IV deficiency syndrome. Pediatr. Blood Canc. 53, 482–484.

Woolf, A.S., Price, K.L., Scambler, P.J., Winyard, P.J., 2004. Evolving concepts in human renal dysplasia. J. Am. Soc. Nephrol. 15, 998–1007.

Karczewski, K.J., Francioli, L.C., Tiao, G., Cummings, B.B., Alföldi, J., Wang, Q., Collins, R.L., Laricchia, K.M., Ganna, A., Birnbaum, D.P., Gauthier, L.D., Brand, H., Solomonson, M., Watts, NA, Rhodes, D, Singer-Berk, M, England, EM, Seaby, EG, Kosmicki, JA, Walters, RK, Tashman, K, Farjoun, Y, Banks, E, Poterba, T, Wang, A, Seed, C, Whiffin, N, Chong, JX, Samocha, KE, Pierce-Hoffman, E, Zappala, Z, O'Donnell-Luria, AH, Minikel, EV, Weir, B.L, Okaty, J, Weitz, K, Civikli, KL, Kollmers, J, Van Doren, M, Donnelly, S, Fumagalli, S, Deschacht, J, Gentry, J, Gupta, N, Jeandet, T, Kaplan, D, Llanwarne, C, Munshi, R, Novod, S, Pedrillo, N, Roazen, D, Ruano-Rubio, V, Salzmann, A, Schleicher, M, Soto, J, Tabb, K, Tolonen, C, Wode, G, Talkowski, M, Genome Aggregation Database Consortium, Neale, BM, Daly, MJ, MacArthur, DG, 2020. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 581, 434–443.