Clinical genetic diagnostics in Danish autosomal dominant polycystic kidney disease patients reveal possible founder variants

Nielsen, Marlene L.; Lildballe, Dorte L.; Rasmussen, Maria; Bojesen, Anders; Birn, Henrik; Sunde, Lone

Published in:
European Journal of Medical Genetics

DOI:
10.1016/j.ejmg.2021.104183

Publication date:
2021

Document version:
Final published version

Document license:
CC BY-NC-ND

Citation for published version (APA):

Go to publication entry in University of Southern Denmark's Research Portal

Terms of use
This work is brought to you by the University of Southern Denmark. Unless otherwise specified it has been shared according to the terms for self-archiving. If no other license is stated, these terms apply:

- You may download this work for personal use only.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying this open access version

If you believe that this document breaches copyright please contact us providing details and we will investigate your claim. Please direct all enquiries to puresupport@bib.sdu.dk

Download date: 04. Jun. 2021
Clinical genetic diagnostics in Danish autosomal dominant polycystic kidney disease patients reveal possible founder variants

Marlene L. Nielsen, Dorte L. Lildballe, Maria Rasmussen, Anders Bojesen, Henrik Birn, Lone Sunde

1. Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic kidney disease (Torres and Harris, 2009). The disease leads to cyst formation and growth, massive kidney enlargement and, ultimately, end-stage renal disease (Rossetti et al., 2007).

Most cases of ADPKD appear to be caused by heterozygosity for a pathogenic variant in PKD1 or PKD2: according to the literature, variants in PKD1 are identified in ~85% of ADPKD families with a known pathogenic variant, while variants in PKD2 are identified in ~15% of these families (Rossetti et al., 2007; Harris and Torres, 2014; Cornec-Le Gall et al., 2014; Paul and Vanden Heuvel, 2014). Within each of these genes, several thousands of unique variants are reported to be pathogenic or likely pathogenic (Figgermann et al., 2006). Recently, heterozygosity for variants in GANAB and DNAJB11 were reported to cause ADPKD in 0.3% and 0.1% of families, respectively (Porath et al., 2016; Cornec-Le Gall et al., 2018). Inter- and intra-familiar variability of the phenotype is observed for all types of variants, however, patients with truncating variants in PKD1 (large rearrangements, frameshifts, indels and variants affecting canonical splice sites) tend to have the most severe phenotype (Hwang et al., 2016).

Despite the large number of ADPKD patients undergoing genetic testing, it is often difficult to determine whether a variant in PKD1 or PKD2 is pathogenic, mostly because many variants are missense
variants, many are only identified in one family and some patients carry multiple variants (Carrera et al., 2016). Compiling findings in multiple studies of ADPKD families can be helpful in the classification of variants.

In order to further characterize variants associated with ADPKD, we report the distribution of pathogenic or possibly pathogenic variants in Danish patients referred for screening of PKD1, PKD2, and GANAB during a five-year period. In addition, we report the observed novel variants associated with ADPKD and the occurrence of recurrent variants.

2. Material and methods

2.1. Patients

This is a retrospective study including samples (N = 147) sent to the Department of Clinical Genetics, Aarhus University Hospital (AUH), Denmark, for screening of genes associated with cystic kidney disease from April 2014 to April 2019. This public laboratory was the first to offer the analysis in Denmark; therefore, most Danish patients analysed in this 5-year period were included in the present study. Screening was requested either from a department of clinical genetics or from a department of nephrology.

Eighty-one patients (55%) were females. The median age at the time of laboratory analysis was 47 years (range: 0–76 years); 135 patients (92%) were 18 years or older at the time of analysis.

Data including personal identifiers, the clinical indication for performing the analysis as stated on the requisition form, type of tissue, method(s) and results were retrieved from the laboratory database of the Department of Clinical Genetics, AUH. In Denmark, quality control projects like the present, are exempted from approval by institutional review boards. As this study took place in a department of clinical genetics, we did not have access to systematic and valid information about the phenotype, e.g. the severity of the disease. However, we did have access to pedigrees constructed at the departments of clinical genetics during the genetic work-up.

We scrutinised the clinical information given on the requisition form. If it said that the patient was suspected of ADPKD or that the patient had cystic kidneys, we classified the patient as suspected to suffer from ADPKD. 118 patients fulfilled one of these criteria. For 27 patients there were clinical information on the requisition form, however neither ADPKD nor cystic kidneys were stated. For 2 patients no information regarding phenotype was given.

3. Molecular analysis

3.1. DNA extraction

DNA had been extracted from peripheral blood, tissue samples and amnion cells.

3.2. Sequencing

The genes PKD1 (NM_001009944.2), PKD2 (NM_000297.3) and GANAB (NM_198335.5) had been sequenced using a next generation sequencing (NGS) panel targeting exons. 1 μg DNA was used for construction of a library for Illumina paired-end sequencing using the KAPA HTP Library Preparation Kit according to the manufacturer’s instructions (KAPA Biosystems Inc., Wilmington, MA, USA). The libraries were enriched for regions of interest using a customized targeting probe set (SeqCap EZ Choice, Roche Nimblegen, Inc., Madison, WI, USA) and sequenced on a MiSeq or NexSeq Sequencer (Illumina, San Diego, CA, USA). The sequencing reads were aligned to the human genome (GRCh37), and variants were called in coding exons and flanking regions ± 10 bp, using Biomedical Genomics Workbench v.2 (CLC bio-Qiagen, Aarhus, Denmark). All regions not covered 30x were sequenced using direct Sanger sequencing; primer sequences and other PCR details are available upon request.

Relatives had been analysed for the family-specific variant(s) by direct Sanger sequencing using BigDye® Terminator v.1.1 Cycle Sequencing Kit, as described by the manufacturer (Applied Biosystems, Life Technology) and analysed using ABI 3500x Genetic Analyzer (Applied Biosystems, CA, USA). Further details are available upon request.

3.3. Multiplex ligation-dependent probe amplification assay

All samples in which no pathogenic or possibly pathogenic variant (as defined below), was detected by sequencing, had been analysed for deletion/duplication of one or more exons by multiplex ligation-dependent probe amplification (MLPA) using probe sets P251 and P352 following the manufacturer’s instructions (MRC-Holland, Amsterdam, Holland).

3.4. Variant filtration and classification

Variants had been called in RefSeq for each gene, and the following variants had been excluded: synonymous variants except for those located ±2 bp of exon-intron borders; variants registered in a public database (ExAC, gnomAD, and/or whole-exome sequencing of 2000 Danish individuals (Lohmueller et al., 2013)) with a minor allele frequency (MAF) > 5% and variants detected in >10% of the patients registered in the in-house frequency database of the laboratory.

In the present study, the variants, that had been reported by the clinical laboratory as clinically relevant were classified as follows: Variants were deemed to be known if listed in the Autosomal Dominant Polycystic Kidney Disease: Mutation Database (PKDB) (Eggermann et al., 2006) and/or published in the Human Genome Mutation Database (HGMD, Qiagen) (Stenson et al., 2003). Novel variants were classified using VarSome (Kopanos et al., 2019) exclusively. The software classifies variants according to the American College of Medical Genetics & Genomics (ACMG) guidelines (Richards et al., 2015) developed for interpretation of sequence variants. Known variants were further classified based on information in the PKDB. Both novel and known variants were classified into two groups: pathogenic (P) or possibly pathogenic (PP), (Table 1). Only variants classified as pathogenic and definitely pathogenic by ACMG and PKDB, respectively, were classified as P. Among the remaining variants those that were either classified as pathogenic, likely pathogenic or variant of uncertain significance by ACMG, and/or definitely pathogenic, highly likely pathogenic, likely pathogenic, likely hypomorphic or variant of uncertain significance by PKDB were classified as PP. Using this algorithm, the group of variants classified as PP is quite heterogeneous.

Segregation analysis was possible in 17 families with a PP variant; in two of these families, more than one informative meiosis was available. Included were only family members diagnosed with multiple bilateral kidney cysts and family members who were unaffected at the age of 40 years or above. For phenotypic classification of family members, we used clinical data from the requisition forms and pedigrees drawn at departments of clinical genetics.

Recurrent variants were accessed in the HGMD and the Danish Cystic Kidney Disease Registry developed by us, at the Department of Clinical Genetics, AUH, (Danish Data Protection Agency reference number: 1-16-02-364-13). This registry holds data on the phenotype and genotype of 97 families in which a minimum of one family member suffers from a cystic kidney disease and has received counselling at the Department of Clinical Genetics, AUH, or Aalborg University Hospital, and/or had PKD1 and PKD2 analysed prior to April 2014. The analysis had been performed by commercial laboratories in Europe at the request of Department of Clinical Genetics, AUH.

The description at protein level in all tables is given without any experimental evidence.
No variants were observed in Table 2. All variants can be found in PKDB. 94 unique P or PP variants were observed: 74 in patients with cystic kidneys, for whom a specific suspicion of ADPKD was indicated using Fisher's exact test (p<0.05).

### 4. Results

During a five-year period, 147 patients were screened for pathogenic variants in PKD1, PKD2 and GANAB. Among 118 patients suspected to have ADPKD, a P or PP variant was identified in 103, producing a diagnostic yield of 87%. No P or PP variants were identified in the 29 patients with cystic kidneys, for whom a specific suspicion of ADPKD was not indicated on the requisition form.

In total, 112 P or PP variants were identified of which 94% (105/112) were identified using NGS and 6% (7/112) using MLPA. A total of 94 unique P or PP variants were observed: 74 in PKD1 and 20 in PKD2. No variants were observed in GANAB. The distribution of P and PP variants according to gene, is given in Table 2. For detailed information of P and PP variants, see Supplementary Table 1 and Supplementary Table 2. All variants can be found in PKDB.

The majority of variants were missense (37%), followed by nonsense variants (27%), frameshift variants (18%), large rearrangements (7%), variants affecting canonical splice sites (6%), in-frame insertions/deletions of >5 amino acids (3%) and variants causing alternative splicing (1%), (Table 3).

For the 103 patients in whom P or PP variants were identified, pedigrees were assessed. None of these patients appeared to be related. In 63 patients, one variant in PKD1 was identified, five patients had two variants in PKD1, 31 patients had one variant in PKD2, one patient had two variants in PKD2 and three patients had one variant in PKD1 and one variant in PKD2 (Supplementary Table 3). In five of the nine patients with both variants, one variant was classified as P and the other as PP. For one variant, segregation analysis was informative: One patient carried two PP variants in PKD1: c.4562G>T and c.9829C>T. However, segregation analysis indicated that c.9829C>T alone is insufficient to cause ADPKD as it was identified in an unaffected relative. According to the PKDB, the variant c.9829C>T is classified as likely hypomorphic and might contribute to disease when observed in combination with other variants.

Ten recurrent variants were observed in a minimum of 2 and up to 7 families. A total of 27 families harboured a recurrent variant. In one of these families, two recurrent variants were observed. Recurrent variants were identified both in PKD1 (N = 4) and PKD2 (N = 6). Eight of these variants were known, and two were novel, (Table 4). In the family harbouring two recurrent variants, one of these was c.8293C>T in PKD1. The frequency of this variant in the background population is 0.8565% according to GnomAD, and the variant is classified as benign according to VarSome and as likely hypomorphic according to the PKDB and the literature (Rossetti et al., 2009).

### 5. Discussion

This study included 147 patients, of whom 118 were specifically suspected to suffer from ADPKD according to information given by the doctor requesting the gene analysis. A pathogenic or possibly pathogenic variant was identified in 103 of these 118 patients, whereas no such variants were observed among 29 patients for whom analysis of PKD1, PKD2, and GANAB was requested without the requisition form indicating a suspicion of ADPKD. Hence, the diagnostic yield was 70% (103/147) among all patients, and 87% (103/118) among patients specifically indicated to be suspected of ADPKD. The diagnostic yield was 80% in an Italian cohort in which 21.8% of the patients had an uncertain clinical diagnosis (Carrera et al., 2016). Yields in other studies of Caucasians range from 89.1 to 92.4% (Rossetti et al., 2007; Hwang et al., 2016; Audrezet et al., 2012; Heyer et al., 2016). Barring one (Hwang et al., 2016), these studies included patients diagnosed using Pei’s criteria (Ravine et al., 1994). The yield observed in the present study is relatively high taking into account that in Denmark patients with a classic ADPKD phenotype and a family history are mainly diagnosed using Pei’s criteria, only, unless prenatal diagnostics or predictive testing is requested (Autosomal dominant polycy, 2012), which should cause an increase in the fraction of patients with a mild or otherwise unusual phenotype among those analysed. Consistently, a non-significant (p = 0.7215 and p = 0.1452, respectively) but higher frequency of PKD2 (21%) and non-truncating PKD1 variants (36%) was identified among the Danish patients (Table 3) than in similar studies from other countries (Rossetti et al., 2007; Hwang et al., 2016; Audrezet et al., 2012; Heyer et al., 2016), in which the averages were 18% and 27%, respectively.

Identifying the cause of ADPKD is hampered by the fact that many cases are caused by missense variants that are difficult to classify beyond variant of uncertain significance (VUS). Furthermore, since most of these variants are unique to one family, it is difficult to obtain information that indicates association. As observed in Supplementary Table 2, several variants have been classified differently using VarSome and PKDB. While currently it is not known which classification method most correctly classifies ADPKD variants, aggregated data from multiple studies should improve variant classification. Furthermore, our classification system is strong with regard to reproducibility in that we used

<table>
<thead>
<tr>
<th>PKD1</th>
<th>Pathogenic</th>
<th>Likely pathogenic</th>
<th>ACMG</th>
<th>Variant of uncertain significance</th>
<th>Likely benign</th>
<th>Benign</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definitively pathogenic</td>
<td>P&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Highly likely pathogenic</td>
<td>-&lt;sup&gt;b&lt;/sup&gt;</td>
<td>PP</td>
<td>PP</td>
<td>PP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>-</td>
<td>PP</td>
<td>PP</td>
<td>PP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Likely hypomorphic</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>PP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Variant of uncertain significance</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>PP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Not available</td>
<td>PP&lt;sup&gt;c&lt;/sup&gt;</td>
<td>PP</td>
<td>PP</td>
<td>PP</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> P: Pathogenic.
<sup>b</sup> Combination not observed.
<sup>c</sup> PP: Possibly pathogenic.

3.5. Statistics

Differences in the distribution of variants in PKD2 and non-truncating variants in PKD1 (in-frame indels, alternative splicing and missense variants) in this study compared to other studies, were evaluated using Fisher’s exact test (p<0.05).

<table>
<thead>
<tr>
<th>Gene Classification</th>
<th>Known variants (N = 53)</th>
<th>Novel variants (N = 41)</th>
<th>Total distribution of variants (N = 94)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PKD1</strong> Pathogenic</td>
<td>53% (20/38)</td>
<td>47% (18/38)</td>
<td>40% (38/94)</td>
</tr>
<tr>
<td>Possible pathogenic</td>
<td>53% (19/36)</td>
<td>47% (17/36)</td>
<td>38% (36/94)</td>
</tr>
<tr>
<td><strong>PKD2</strong> Pathogenic</td>
<td>62% (8/13)</td>
<td>6% (7/94)</td>
<td></td>
</tr>
<tr>
<td>Possible pathogenic</td>
<td>86% (6/7)</td>
<td>14% (1/7)</td>
<td>7% (7/94)</td>
</tr>
</tbody>
</table>
families had a recurrent variant (Table 4), which is similar to the closely related. Furthermore, some of the families had been subjected to present study were registered in 1
we examined if they were included in The Danish Cystic Kidney Disease system. none of the variants described as recurrent in the literature, see Table 4. Distribution of pathogenic and possibly pathogenic variants in PKD1 and PKD2, by variant type.

Table 4
Recurrent variants in PKD1 and PKD2.

<table>
<thead>
<tr>
<th>Gene</th>
<th>DNA change</th>
<th>Protein change</th>
<th>Number of families</th>
<th>Number of meioses</th>
<th>PKDB Classification</th>
<th>GnomAD frequency (%)</th>
<th>At CpG?</th>
<th>HGMD references</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKD1</td>
<td>c.6643C&gt;T</td>
<td>p. Arg2215Trp</td>
<td>2 + 0 – 2</td>
<td>0</td>
<td>LP</td>
<td>0.001893</td>
<td>Y</td>
<td>Corne-Lee (2013)</td>
</tr>
<tr>
<td>PKD1</td>
<td>c.7115C&gt;G</td>
<td>p. Ser2372Cys</td>
<td>2 + 0 – 2</td>
<td>1</td>
<td>LP</td>
<td>0</td>
<td>N</td>
<td>Audrézet (2012)</td>
</tr>
<tr>
<td>PKD1</td>
<td>c.8293C&gt;T</td>
<td>p. Arg2765Cys</td>
<td>2 + 1 – 3</td>
<td>0</td>
<td>LH</td>
<td>0.8565</td>
<td>Y</td>
<td>Rossetti et al.</td>
</tr>
<tr>
<td>PKD1</td>
<td>c.11249G&gt;A</td>
<td>p. Arg3750Glu</td>
<td>2 + 0 – 2</td>
<td>NA</td>
<td>HLP</td>
<td>0</td>
<td>N</td>
<td>Hoebeke (2010)</td>
</tr>
<tr>
<td>PKD2</td>
<td>c.1521G&gt;A</td>
<td>p.Trp 507&gt;/?</td>
<td>2 + 0 – 2</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>Y</td>
<td>Robinson (2012)</td>
</tr>
<tr>
<td>PKD2</td>
<td>c.2392_2664</td>
<td>p. 2119-2 A &gt; G</td>
<td>2 + 4 – 6</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>N</td>
<td>NA</td>
</tr>
<tr>
<td>PKD2</td>
<td>c.2614C&gt;T</td>
<td>p.Arg 872&gt;/?</td>
<td>3 + 2 – 5</td>
<td>1</td>
<td>DP</td>
<td>0.0008818</td>
<td>Y</td>
<td>Neumann (2013)</td>
</tr>
</tbody>
</table>

PKD1 (NM_001009944.2) and PKD2 (NM_000297.3). One family harbours both variants. Number of meioses in which the variant co-segregated with the phenotype for all families harbouring the variant. LP: Likely pathogenic; LH: Likely hypomorphic; HLP: Highly likely pathogenic; NA: Not available; DP: Definitely pathogenic. NA: Not available. Variant located at CpG site; Y: Yes; N: No. Variant located at CpG site; Y: Yes; N: No.

standard software (VarSome), rather than inventing our own scoring system.

In the present study, identical variants were detected in several families. Due to the unique civil registration number assigned to all people living in Denmark, we could ensure that these families were not closely related. Furthermore, some of the families had been subjected to genetic work-up including drawing of pedigrees. In total, 26% of the families had a recurrent variant (Table 4), which is similar to the 20–30% reported in other studies in Caucasians (Rossetti et al., 2007; Audrézet et al., 2012).

To explore the recurrence of these variants in the Danish population, we examined if they were included in The Danish Cystic Kidney Disease Registry. Four of ten variants observed in two or more families in the present study were registered in 1–4 families in the registry. We found none of the variants described as recurrent in the literature, see Table 4.

Recurrent variants may be founder variants or identical variants that have arisen independently. The latter are often indels generated by non-homologous recombination or single nucleotide substitutions in CpG dinucleotides (i.e., transitions of C to T or G to A). Indels are frequently generated when recombination occurs between two similar but non-identical sites (Chen et al., 2010). One variant in PKD2 (c.(2392_2664)_(2861,?)del) was likely generated by non-homologous recombination, and four variants were located at CpG sites: c.6643C>T and c.8293C>T in PKD1, and c.1521C>T and c.2614C>T in PKD2. These variants were observed in three, two, three, two and five unrelated families, respectively, (Table 4). These five variants may have arisen independently in each family, although it cannot be excluded that some of them might be founder variants, especially the PKD2 variant observed in five unrelated families.

Five variants that were neither indels nor located at CpG sites remained: two nontruncating variants located in PKD1 were each observed in two families, and three located in PKD2 were observed in six, eight and nine families, respectively. The mutation rate in PKD1 and PKD2 is thought to be similar to the mutation rate in other large genes associated with autosomal dominant disorders (Rossetti et al., 2001). Although no definite conclusions have been reached regarding how to
predict mutational hotspots, mutations are more frequent in CpG sites and where similar sites increase the risk of non-homologous recombination. Hence, it appears unlikely that the same variants not related to non-homologous recombination or located within CpG sites have arisen independently in up to nine families. Thus, at least some of these variants most likely originated in a common ancestor, i.e. being founder variants.

Similarly, the majority of variants found in more than one family in an Italian cohort (Carrera et al., 2016) and the TGESP cohort (Hwang et al., 2016) were PKD2 or non-truncating PKD1 variants. Like our study, these studies also did not recruit patients solely diagnosed by ultrasonic criteria. Truncating PKD1 variants are associated with a more severe phenotype than variants in PKD2 and non-truncating PKD1 variants (Hwang et al., 2016). Patients with truncating PKD1 variants may therefore have reduced survival and reproductive fitness (Rossetti et al., 2001), which may cause recurrent truncating PKD1 variants to be underrepresented. Therefore, variants associated with a mild phenotype are more likely to be transmitted through multiple generations, resulting in a high number of seemingly unrelated families harbouring the same variant. In the CRISP (Rossetti et al., 2007) and Genkyst cohorts (Audrezet et al., 2012), the majority of recurrent variants were truncating PKD1 variants. However, in these cohorts, patients are diagnosed solely by Pei’s criteria, decreasing the probability that patients with a mild phenotype are included. This implies that both the frequency of recurrent variants associated with a mild phenotype and the number of families harbouring such variants may be higher than previously reported. Supporting this, the prevalence of ADPKD reported in many studies is lower than the theoretical prevalence reported by Dalgaard (1957) and others (Solazzo et al., 2018).

Conclusion: A pathogenic or possibly pathogenic variant was identified in 87% of the Danish families expected to suffer from ADPKD; 79% and 21% of the variants were identified in PKD1 and PKD2, respectively. 94/112 variants were observed once, and 41 of these were novel. Ten variants in PKD2 or non-truncating variants in PKD1 were observed in multiple families. These types of variants often cause a mild phenotype compared to truncating PKD1 variants. Among the recurrent variants, at least five were most likely founder variants. Founder variants causing a mild phenotype may be more common than previously reported, and the prevalence of ADPKD may be underestimated.

Author statement

Marlene L. Nielsen: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition; Dorte L. Lildballe: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition; Maria Rasmussen: Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition; Anders Bojesen: Conceptualization, Resources, Writing – review & editing; Henrik Birn: Writing – review & editing, Funding acquisition. Lone Sunde: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

Funding

This work was supported by Vanforefonden, Cand. Jur. Torkil Steenbecks Grant, Independent order of Odd Fellows nr. 73 Svend Fielding, Karen Elise Jensen, Danish Kidney Association, Danish Society of Nephrology, and Central Denmark Region.

Declaration of competing interest

Dr. Nielsen reports grants from: Vanforefonden, Cand. Jur. Torkil Steenbecks Grant, Independent order of Odd Fellows nr. 73 Svend Fielding, Karen Elise Jensen, Danish Kidney Association, Danish Society of Nephrology, and Central Denmark Region during the conduct of the study. None of the funders played a role in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

The results presented in this paper have not been published previously in whole or part, except in abstract format.

Acknowledgements

The authors take this opportunity to extend their gratitude to the Department of Clinical Genetics, Aarhus University Hospital, for their collaboration in providing the data for this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2021.104183.

References


