RP1 and retinitis pigmentosa: report of novel mutations and insight into mutational mechanism

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ABSTRACT

Background/aim Retinitis pigmentosa (RP) is the commonest form of retinal dystrophy and is usually inherited as a monogenic trait but with remarkable genetic heterogeneity. RP1 is one of the earliest identified disease genes in RP with mutations in this gene known to act both recessively and dominantly although the mutational mechanism remains unclear. This study is part of our ongoing effort to characterise RP in Saudi Arabia at the molecular level.

Methods Homozygosity mapping and candidate gene analysis.

Results The authors have identified four novel mutations, all recessive, in a number of families with a typical RP phenotype.

Conclusion The distribution of these novel and previously reported RP1 mutations makes it challenging to describe a unifying mutational mechanism for dominant versus recessive RP1-related RP.

INTRODUCTION

Retinitis pigmentosa (RP) (MIM 268000) represents the most common form of retinal dystrophies and is characterised by progressive degeneration of rod and, subsequently, cone photoreceptors.[1 2] Symptoms usually start with night blindness during adolescence, followed by peripheral visual loss in young adulthood, and finally central visual loss and even total blindness as the disease progresses.[1 2] The classic triad of ocular changes encompasses optic disc pallor, attenuated retinal vessels and diffuse pigmentary changes in the retina. RP is genetically heterogeneous with more than 60[1] chromosomal loci identified of which 53 have been resolved at the gene level.[3] Our improved understanding of the genetics of RP has led to new insights into disease mechanisms, which in turn have led to cautious optimism regarding retinal cell rescue.[4]

RP1 (oxygen regulated photoreceptor protein) was the fourth dominant RP gene to be identified,[5–7] after *RHO*, *RDS* and *NRL*, which encode rhodopsin, peripherin/RDS and NRL, respectively.[8–10] *RP1* is located on chromosome 8q12 and consists of four exons with an open reading frame of 6468 bp, encoding a protein of 2156 amino acids, mostly by exon 4 (788–6468 bp). Previous studies revealed that mutations in *RP1* can cause both autosomal dominant (adRP) and autosomal recessive (arRP) forms of RP. Although the mechanism behind this dual mutational effect is unclear.[11–13] In this study, we report four novel truncating mutations in *RP1* associated with arRP. Based on the location of these novel mutations, and the mutations we and others have previously reported, we speculate on the mutational mechanism that explains the dominant versus recessive nature of RP1 mutations.

SUBJECTS AND METHODS

Patients

The data we present are part of an ongoing national study to characterise the molecular basis of RP in Saudi Arabia. Patients with RP were identified using established ophthalmological criteria, electroretinography was only carried out when possible,[13] and enrolled using a written informed consent (KFHSRC IRB RAC#2070023). Blood samples were obtained from the affected patients and their relatives as dictated by the nature of the family history but included at a minimum the parents and the unaffected siblings. Careful family history was obtained from all patients who were accordingly categorised as simplex or familial cases.

DNA extraction

DNA extraction from blood samples collected in EDTA tubes was carried out using the Gentra DNA Extraction Kit (Qiagen, Germantown, Maryland, USA) in accordance with the protocol provided by the manufacturer.

SNP genotyping, homozygosity mapping and linkage analysis

Genome-wide genotypes were obtained using Affymetrix SNP 250K or Axiom Chip platform (Affymetrix, Santa Clara, California, USA) following the manufacturer's instructions. Homozygosity mapping was carried out using both the Affymetrix® Genotyping Console (Affymetrix) and autoSNPa as described previously.[14–16] In multiplex families, linkage analysis was performed using the SNP genotypes run on EasyLinkage software as previously described.[15]

Mutation analysis

Homozygous regions overlapping with known retinal dystrophy genes were further investigated by direct sequencing of the previously identified retinal dystrophy gene mapping to the regions of interest. The entire coding and flanking intronic regions of the mapped gene were PCR amplified (primers and conditions are available upon request). Direct bidirectional sequencing was performed using BigDye Terminator Cycle Sequencing v3.1 kit and the Prism 3730XL Genetic Analyzer (Applied Biosystems, Carlsbad, California, USA). Sequences were analysed using the Seqman II program of the

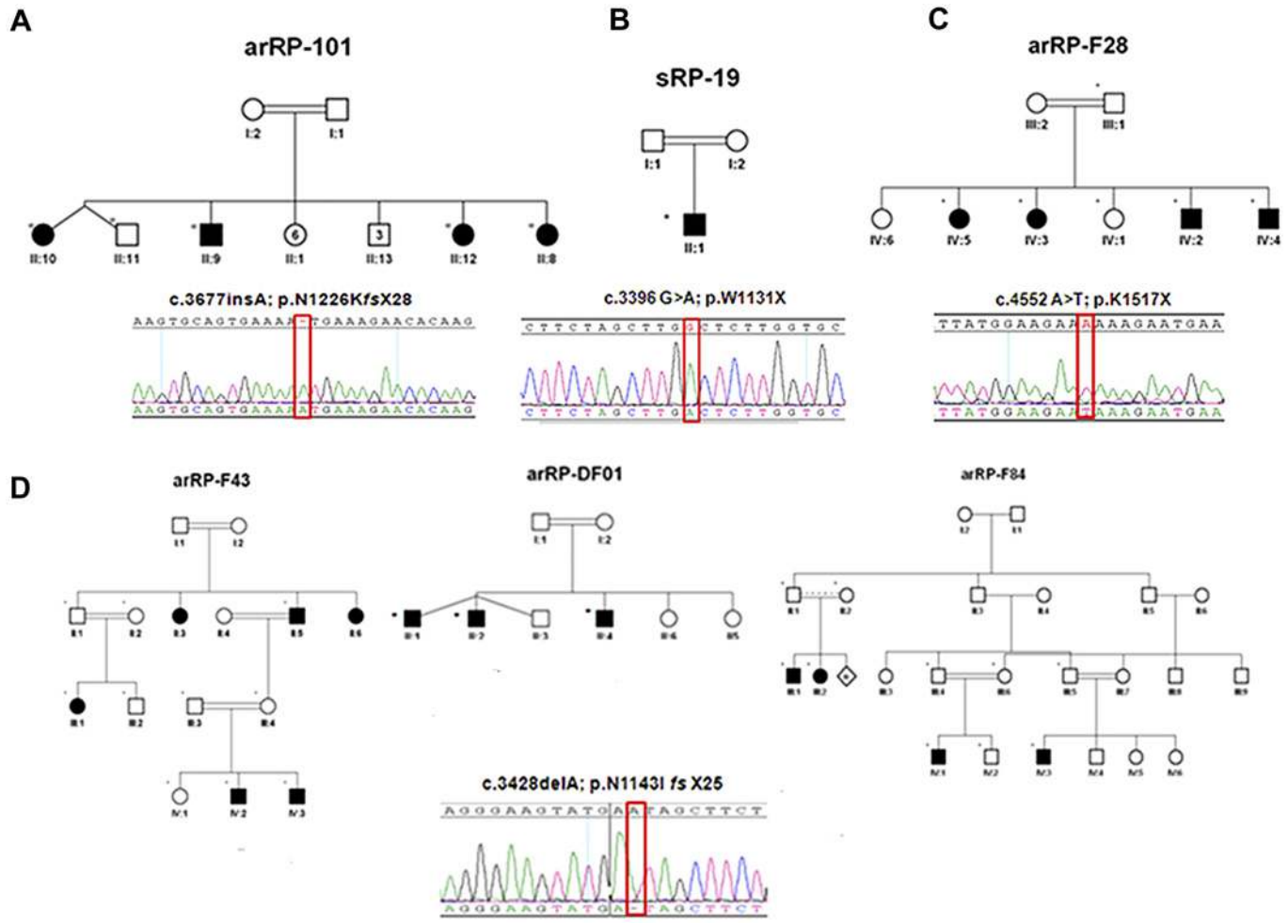

Figure 1 (A) A novel homozygous frameshift mutation in exon 4 of the *RP1* gene (c.3677_3678dupA) in family arRP-F101. Top: Family pedigree. Bottom: Sequence chromatogram of the affected proband (II:12) showing a homozygous single base insertion (c.3677_3678dupA). (B) A novel homozygous nonsense mutation in exon 4 of the *RP1* gene (c.3396 G>A) in family sRP-19. Top: Family pedigree. Bottom: Sequence chromatogram of the affected proband (II:1) showing a homozygous nonsense mutation. (C) A novel homozygous nonsense mutation in exon 4 of the *RP1* gene (c.4552 A>T) in family arRP-F28. Top: Family pedigree; star indicates DNA sample available. Bottom: Sequence chromatogram of the affected proband IV:2 showing a homozygous nonsense mutation (c.4552 A>T). (D) A novel homozygous frameshift mutation in exon 4 of the *RP1* gene (c.3428delA) in families arRP-DF01, arRP-F43 and arRP-F84. Top: Family pedigrees. Bottom: Sequence chromatogram of the affected proband (II:4) from the arRP-DF01 family showing a homozygous single base deletion (c.3428delA). Star in pedigrees indicates available DNA sample.

DNASTAR analysis package (Lasergene, Madison, Wisconsin, USA) using the reference sequence (hg19) for comparison. Familial segregation analysis of observed variants was performed whenever applicable.

RESULTS

Clinical description

We have identified 20 patients representing six Arab families (figure 1), five of which are multiplex, with autosomal recessive RP caused by RP1 mutation (see below). Consanguinity was reported in all cases. Table 1 summarises the clinical features of all index patients.

Molecular studies

Five families (arRP-DF01, arRP-F28, arRP-F43, arRP-F84 and arRP-F101) had three or more affected members and were deemed suitable for traditional linkage analysis assuming a fully penetrant autosomal recessive model. Families arRP-F43 and

Table 1 Summary of the clinical features for all index patients

Family	Patient	Age	Age of onset	Visual acuity	Refraction	Fundus findings
arRP-F28	IV:2	21	4 years	OD 20/400 OS CF 3 ft = 20/2666	−5.00−2.00× 90−4.75−1.50×80	Attenuated retinal blood vessels, diffuse pigmentery changes, macular atrophy, optic disc pallor
arRP-DF01	II:4	16	5 years	OD HM OS CF 2ft = 20/4000	−4.00−1.00× 140−4.50−1.25×160	Attenuated retinal blood vessels, diffuse pigmentery changes, macular atrophy, optic disc pallor
arRP-F43	IV:2	12	4 years	OD 20/25 OS 20/28	−0.50−0.50× 65−0.50−0.50×131	Attenuated vessels, pigmentery changes, optic pallor
arRP-F84	II:1	35	At birth	NA	NA	NA
arRP-F101	II:8	27	4 years	NA	−7.00−2.00× 15−4.50−1.25×135	Attenuated vessels, pigmentery changes, optic atrophy
sRP-19	II:1	33	18	OD 20/30 OS 20/60	NA	Attenuated vessels, mild optic nerve head pallor

arRP-FD01 gave a single linkage peak with an LOD (logarithm of odds) score of 3 on chromosome 8 which harbours *RP1*, whereas in the remaining families (arRP-F28, arRP-F84 and arRP-F101), multiple linkage peaks were obtained and subsequent analysis was only possible with the aid of homozygosity mapping (see below).

Homozygosity mapping

Consistent with the fact that 100% of these patients come from consanguineous families, multiple blocks of homozygosity were seen in each patient. Homozygosity mapping was used to either confirm the linkage regions in multiplex families or to focus the search for candidate genes in the sporadic case where linkage analysis was not applicable. These blocks were shown to overlap with known retinal dystrophy genes. The number of known autosomal recessive RP genes suggested by homozygosity mapping in the sporadic case (sRP-19) was seven.

RP1 mutation analysis

Four novel mutations were identified by direct sequencing of the *RP1* gene in five consanguineous families and one sporadic case. Mutations consisted of a homozygous nonsense mutation (NM_006269.1:c.4552A>T; p.K1518X) in family arRP-F28 (figure 1); a homozygous single base deletion (NM_006269.1: c.3428delA; p.N1143I*fs*X25) in three families arRP-F43, arRP-F84 and arRP-DF01 figure 1); a homozygous nonsense mutation (NM_006269.1:c.33396G>A; p.W1131X) in a sporadic case (sRP-19) (figure 1); and a homozygous frameshift mutation (NM_006269.1:c.3677_3678dupA; p.E1227M*fs*X29) in family arRP-F101 (figure 1). All these mutations segregated with the phenotype within the families. Interestingly, these mutations are clustered in exon 4 (figure 2) and are predicted to encode a truncated RP1 protein that lacks a half to two-thirds of its full length because they are not predicted to be subject to the nonsense-mediated decay (NMD) pathway since these mutations affect the last exon of the gene.[17]

DISCUSSION

RP1 encodes a protein of 2156 amino acids that is localised to the connecting cilia of both rod and cone photoreceptors.[18] Its expression is almost exclusive to the photoreceptor cells of the retina.[5–7] The N-terminal portion of RP1 is related to double-cortin (DCX), originally identified in the context of directing neuronal migration but has later been shown to be a member of a new family of microtubule-associated proteins.[19–22] RP1 is thus the first photoreceptor-specific microtubule-associated protein to be identified.[23] Furthermore, the location of RP1 in the connecting cilia and its homology with DCX make RP1 an attractive candidate in the transport of newly synthesised outer segment proteins from the inner segments to the site of disc membrane assembly through the connecting cilia. It is possible that RP1 interacts with microtubules through its N-terminal DCX domain, whereas the C-terminal portion of RP1 binds a protein that is destined for the outer segment. RP1 may also be involved in regulation of microtubule dynamics through its DC domain, maintenance of the structure and orientation of connecting cilia, or blockage of diffusion between the inner segments and outer segments.[18]

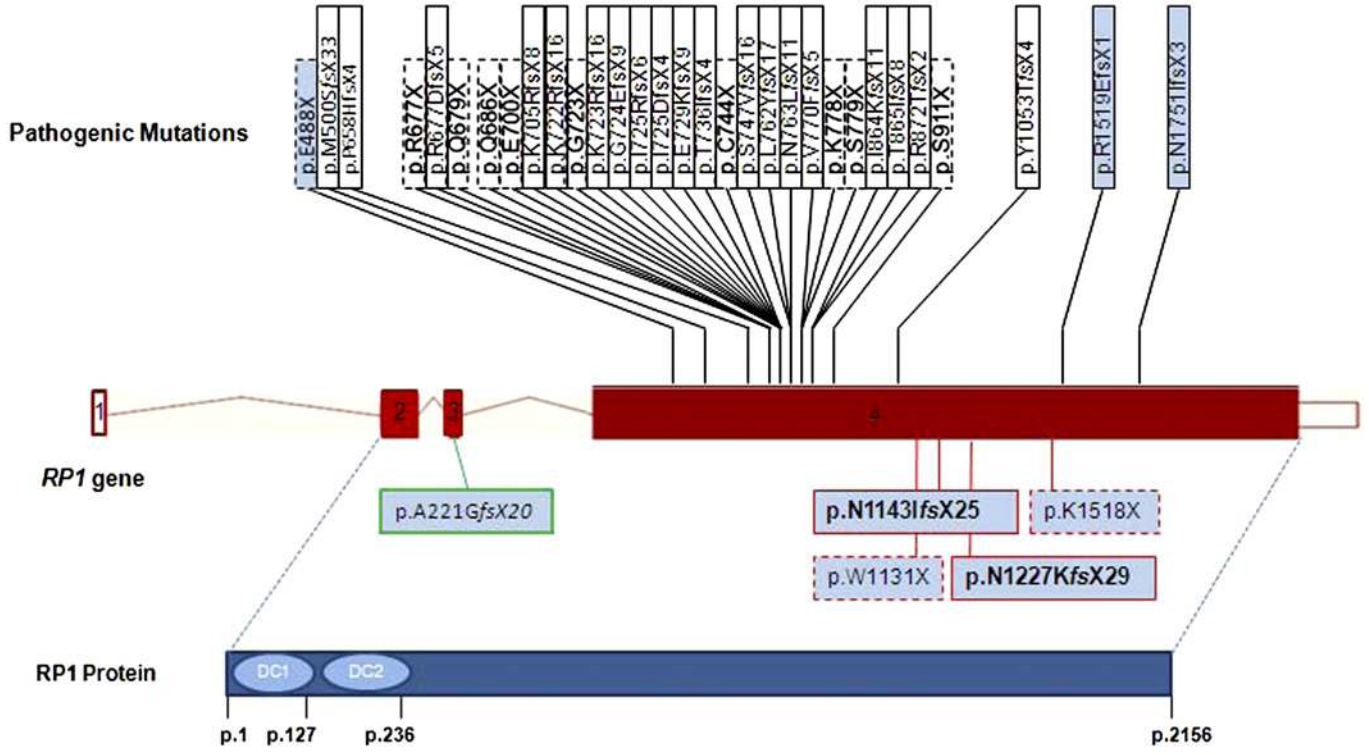

Figure 2 The *RP1* gene structure showing the locations of pathogenic truncation mutations to date. Mutations are annotated at the protein level using a reference sequence from Ensembl (ENST00000220676). The mutations above the schematic are pathogenic mutations that are responsible for adRP or arRP. Mutations causing arRP in a homozygous state are in blue shaded boxes and cluster at the C-terminal of the protein, with the exception of p. A221G*fs*X20 which is predicted to be subjected to nonsense-mediated decay. Mutations in the dashed rectangles are nonsense point mutations, and those in the longer rectangles are frameshift mutations. The mutations identified in the present study are shown below the schematic in red boxes, and the green boxes represent the homozygous arRP mutation identified previously by our group. The lower part of the diagram is the schematic RP1 protein with the two doubelcortin domain encoded by exon 2(p.29—127) and 3 (p.150—236).

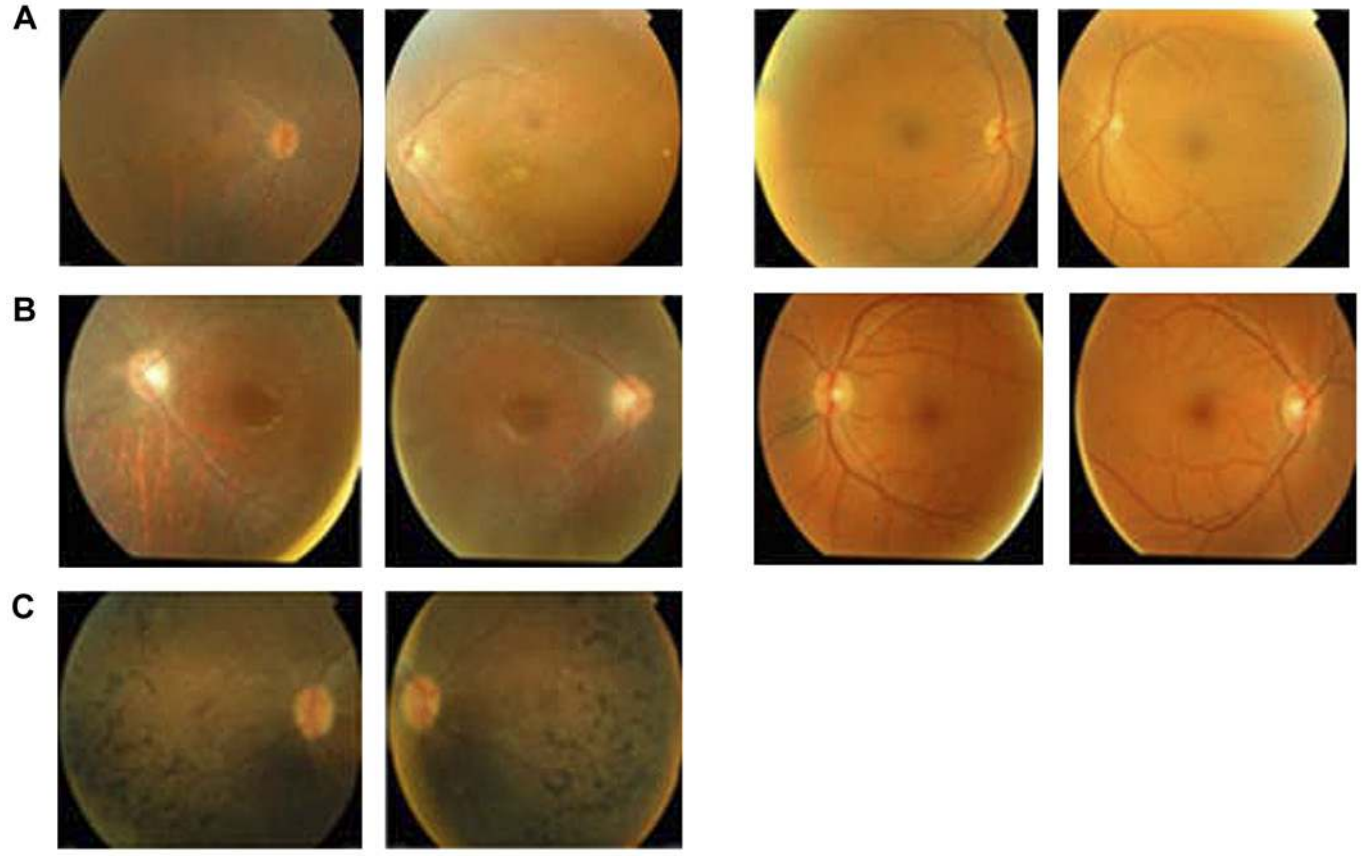

Figure 3 (A) Left: Fundus photograph of the affected proband IV:2 from family arRP-F28 showing typical retinitis pigmentosa (RP) changes. Right: Fundus photograph of the carrier III:1 showing normal findings. (B) Left: Fundus photographs of the affected proband (II:4) from family arRP-DF01 showing typical RP changes. Right: Fundus photographs of the carrier (I:1) showing normal findings. (C) Fundus photographs of the affected proband (II:1) from family sRP-19 showing typical RP changes.

Mutations in *RP1* account for approximately 5.5% of adRP and 1% of arRP.[2] To date at least 47 *RP1* mutations are reported that are causative for RP, all of which are clustered in exon 4, downstream from the DC domain (exons 2 and 3) except for one recessive mutation (p.A221G*fs*X20) in exon 3 that our group has previously described (figure 2).[15] Most *RP1* mutations are single nucleotide substitutions that produce a premature stop codon or frameshift changes from insertions/deletions, resulting in a truncated protein (see figure 2).[24] Because these mutations occur after the final intron–exon junction in *RP1*, it is likely that the mutant RP1 transcripts are not subject to NMD and truncated RP1 proteins are produced; however, this has not been experimentally proven. This is supported by the observation that mutant *RP1* mRNA was detected in homozygotes for a p.Arg677Stop mutation in exon 4.[25] The association of *RP1* mutations with arRP was first described by Khaliq *et al* 2005;[11] however, no clear model has emerged for dominant versus recessive mechanism of mutation in *RP1*. It has been suggested that RP due to mutations in *RP1* is a result of haploinsufficiency, but our previous observation that carriers of the recessive p.A221G*fs*X20 mutation in exon 3 (predicted to be subjected to NMD) were asymptomatic suggests an alternative mechanism. This is further supported by the report of compound heterozygosity in arRP, where carriers of one allele predicted to be subjected to NMD were also asymptomatic.[26] The four novel arRP protein truncating mutations we describe are downstream of the DCX domain and carrier individuals show no evidence of a retina phenotype (figure 3) suggesting each allele in a heterozygous state is not acting in a dominant negative manner, and one normal allele is sufficient for retina function. It is likely, therefore, that critical protein domains in the C-terminal domain of the RP1 protein are lost in these truncated products (truncations from aa 1131 to 1158) and RP results from homozygous loss of this important domain. Mutations causing dominant RP have only been identified in more N-terminal regions of the RP1 protein (residues up to and including aa 1053) and this suggests that dominant negative or toxic gain-of-function is the underlying mechanism leading to dominant RP as a result of mutation of the RP1 protein.

In conclusion, we show that our study population is characterised by significant allelic heterogeneity for RP1, a phenomenon we have previously documented for other genes. The alleles we report in this study and those previously reported make it possible to propose a model for how mutations in this gene cause both recessive and dominant forms of RP.

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Contributors MAR: collected and analysed data and wrote the manuscript; LAS, HA, JA, MS and MH: collected and analysed data; AJH and SH: analysed data; FSA: supervised the collection and analysis of data and helped write the manuscript.

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Competing interests None.

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