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# The *PITX3* gene in posterior polar congenital cataract in Australia

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**Purpose:** Congenital cataract is a significant cause of blindness worldwide. Many genes are known to cause the disorder. A large multigenerational pedigree was investigated for the genetic cause of a posterior polar autosomal dominant congenital cataract.

**Methods:** A genome wide scan was conducted in a large multigenerational family with autosomal dominant cataract to identify the linked region of the genome. The *PITX3* gene was investigated through direct sequencing and detection of fluorescently labeled PCR products.

**Results:** Linkage was detected to a region of chromosome 10q23-26 which contains the candidate gene *PITX3*. A segregating 17 bp insertion mutation was identified. This mutation was not identified in 100 additional unrelated sporadic and familial congenital cataract patients. No mutations of the *PITX3* gene were identified in 9 families with posterior polar congenital cataract.

**Conclusions:** The 657ins17bp duplication of the *PITX3* gene is the cause of the cataract phenotype in the large pedigree, however, this gene appears responsible for only a small proportion of congenital cataract in Australia.

Bilateral congenital cataract is the leading cause of treatable blindness among children worldwide [1]. It is a heterogeneous group of disorders, characterized by onset of cataract at birth or during early childhood. The cataract is classified according to its location in the lens and its appearance can be either progressive or static. It can occur in isolation or as a component of numerous syndromes [2]. While vision can be restored through surgery to remove the opacified lens and implant an artificial one, complications of the procedure such as glaucoma or opacification of the posterior lens capsule can lead to lifelong visual deficits [3-5]. Thus, despite treatment, congenital cataract remains a serious health problem of considerable financial burden.

The estimated incidence of congenital cataract in Australia is 2.2 per 10,000 births [6]. While autosomal dominant is the most common inheritance pattern observed for isolated congenital cataract, recessive and X-linked forms have also been described [7]. To date, 13 genes and an additional 5 loci have been reported to cause various congenital cataract phenotypes. Due to the location of the opacity in the lens, the posterior polar phenotype has a profound affect on vision and can be severely debilitating. Two genes (*CRYAB* [8] and *PITX3* [9,10]) and two additional loci (1p36 [11] and 20p12-q12 [12]) have been described in association with this form of cataract.

## METHODS

Family members were recruited with written informed consent obtained from all participating individuals or their guardians. Ethics approval for this study was obtained from the Human Research Ethics Committees of the Royal Children's Hospital, Melbourne, the Royal Victorian Eye and Ear Hospital, Melbourne, the University of Tasmania, Hobart and Flinders University, Adelaide, and adhered to the tenets of the Declaration of Helsinki. DNA was extracted from whole blood using the QIAamp DNA blood maxi kit (Qiagen Pty Ltd., Doncaster, Australia) and from buccal swabs using the PureGene genomic DNA isolation kit (Gentra Systems Inc. Minneapolis, MN).

Following simulation studies in SLINK to determine a minimal set of individuals to detect significant linkage, a genome wide scan was conducted on 15 family members (Figure 1) using the 10 cM ABI screening panel Vr 2.5 (Applied Biosystems, Foster City, CA). Genotyping was carried out by the Australian Genome Research Facility (AGRF). Two-point linkage was assessed in the MLINK component of the LINKAGE package (version 5.1) [13] and multipoint location scores calculated in SIMWALK2 (version 2.6) [14], in both cases assuming a disease gene frequency of 0.001 and a penetrance of 0.9. Marker distances were taken from Marshfield Genetics [15] and allele frequencies estimated from the Australian population (AGRF).

A previously reported 17 bp insertion in exon 4 of the *PITX3* [9,10] gene was assessed through the detection of fluorescently labeled PCR products in an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). PCR primers used

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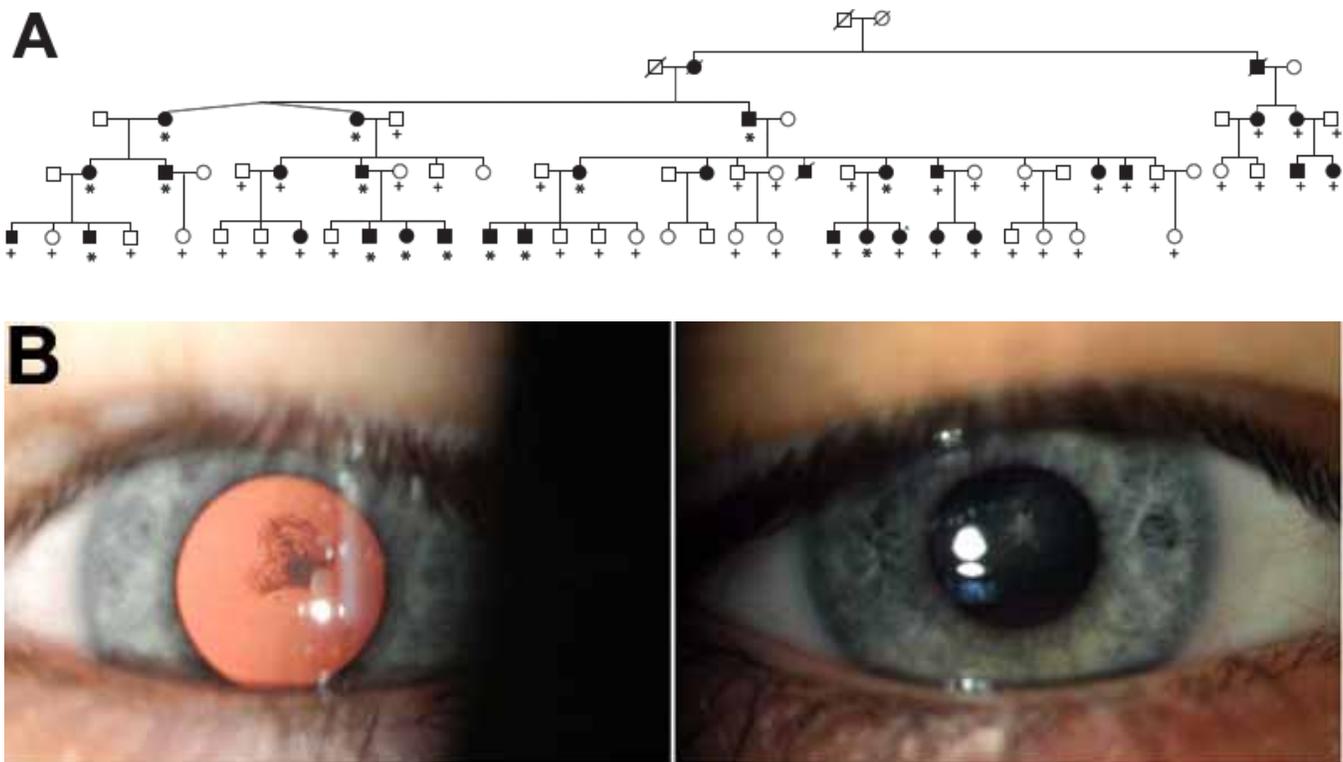


Figure 1. **A:** A multigenerational pedigree with posterior polar congenital cataract. Squares represent males and circles females. Solid symbols indicate the presence of cataract. The asterisk indicates DNA included in the genome wide scan. The plus sign indicates DNA available for mutation screen only. **B:** Clinical photographs of typical posterior plaque-like cataract with gradual progression. Anterior segment dysgenesis was not observed in this family.

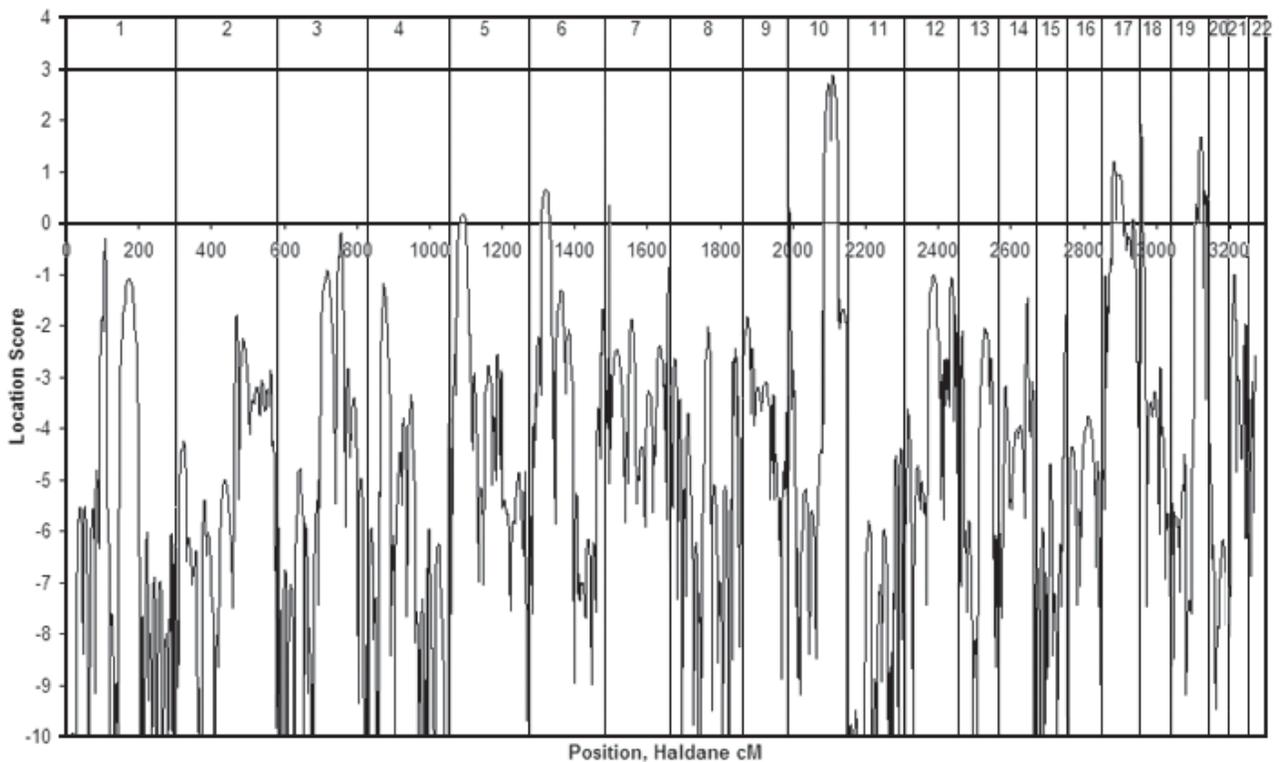


Figure 2. A genome wide scan using a 10 cM screening panel was conducted. Location scores were calculated using SIMWALK2 software. The peak on chromosome 10 is the only region approaching significance, with a location score of 2.7.

to amplify the region of interest were; Forward 5'-CCT TCA ACT CGG TCA ACG TG-3' and Reverse 5'-GTA CTG GCA CGG ACT AAG GTT G-3' with the forward primer carrying a 5' FAM label. Products were amplified with 0.5 units of HotStarTaq DNA Polymerase (Qiagen) in the presence of Q solution (Qiagen). To characterize the observed insertion, the product was sequenced bidirectionally with the BigDyeTerminator cycle sequencing mix (Applied Biosystems) and unlabeled primers, as above.

All exons of the *PITX3* gene were sequenced in index cases from nine families with posterior polar congenital cataract using the BigDyeTerminator cycle sequencing mix (Applied Biosystems). Primer sequences and PCR conditions are available on request.

### RESULTS

The large five generation family was recruited through an index case treated at the Royal Childrens Hospital, Melbourne, Australia. Subsequent tracing of the large pedigree revealed clear autosomal dominant inheritance with high penetrance. The majority of the family resided in rural New South Wales. Of 29 living available affected individuals, all except two had previously undergone bilateral cataract surgery. During the course of the molecular investigation, the remaining two affected children also had cataract surgery. In one case, the cataract morphology was able to be photographed prior to cataract extraction (Figure 1B). This confirmed previous clinical descriptions indicating posterior polar, or posterior subcapsu-

lar cataract predominantly. The severity was variable, with some older individuals remaining aphakic with nystagmus and poor visual acuity. Younger individuals had modern cataract surgery with IOL implants and excellent results with a typical age for surgery being between 4-18 years. Anterior segment dysgenesis was not documented in this family in those individuals examined in detail. However, some older individuals were not available for detailed examination.

DNA was obtained from whole blood from 25 family members (15 of which were included in the genome wide scan) and from buccal swabs from an additional 33 members. DNA was available from all but one of the affected individuals in the last three generations (Figure 1).

The most compelling evidence for linkage was obtained on chromosome 10q23-26 (Figure 2). The maximum location score for multipoint analysis in SIMWALK2 was 2.7 at marker D10S597 (Table 1). Three markers (D10S185, D10S192, and D10S597) gave a 2-point LOD score in MLINK of >1.0 (Table 2). Investigation of recombinant individuals indicated the linked interval was between markers D10S1686 and D10S1693.

This region contained the *PITX3* gene, which had recently been reported to be associated with isolated posterior polar congenital cataract in several pedigrees of various ethnicities [9,10]. Five of these pedigrees had an identical 17 bp insertion of a repeated element at nucleotide 657 (from the start of translation) causing a nonsense mutation. This apparently re-occurring mutation was assessed in our family by fragment size analysis of PCR products generated from primers flanking the insertion site. All affected individuals were found to be heterozygous for an insertion. Sequencing of the PCR product revealed a 17 bp duplication insertion, identical to that reported by Berry et al. [9] (Figure 3). All additional family members were screened for the duplication which was fully penetrant in this family, identified in all 29 affected individuals and none of the 29 unaffected individuals screened. The two-point LOD score calculated in MLINK using the mutation itself as a rare biallelic marker was 13.1, clearly demonstrating linkage to this gene.

In order to determine the relative contribution of this mutation to congenital cataract in Australia we screened an additional 100 patients, consisting of 42 index cases from congenital cataract families and 58 consecutive congenital cataract cases. None of these cases carried this mutation.

TABLE 1.

Marker	Physical position (Mb)	Location score
D10S1686	8.5	-3.84
D10S185	9.4	2.57
D10S192	10.2	1.51
D10S1268	10.5	2.35
D10S597	11.0	2.73
D10S1693	11.9	-2.03

Multipoint locations scores calculated in SIMWALK2 across 10q23-27, showing the flanking markers D1S1686 and D1S1693 where linkage is excluded.

TABLE 2.

Marker	LOD scores at $\theta$								
	0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40
D10S1686	-4.32	-0.41	0.16	0.4	0.49	0.49	0.44	0.36	0.25
D10S185	1.4	1.35	1.22	1.08	0.93	0.79	0.64	0.48	0.33
D10S192	1.02	1.49	1.48	1.37	1.2	1.01	0.8	0.59	0.38
D10S1268	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0
D10S597	1.19	1.08	0.97	0.85	0.73	0.62	0.49	0.37	0.25
D10S1693	-4.91	-0.03	0.33	0.46	0.49	0.47	0.41	0.33	0.23

Two-point LOD scores calculated in MLINK at a range of recombination fractions ( $\theta$ ).

The phenotype in 9 of the 42 congenital cataract pedigrees was noted as posterior polar or posterior subcapsular. Probands from all nine families were completely sequenced at all four exons of the *PITX3* gene. No mutations were identified in the coding regions of this gene in these nine families.

### DISCUSSION

At the outset of this study, the link between the *PITX3* gene at 10q24 and isolated congenital cataract was not clear. Mutations had been reported to generally cause anterior segment mesenchymal dysgenesis (ASMD) with cataract [10]. The recent publication of Berry et al. [9] highlights this gene as an important contributor to congenital cataract, and in particular posterior polar phenotypes in multiple ethnicities. The 657ins17bp mutation has now been identified in six families [9,10], only three of which have ASMD in addition to the cataract. Within these families the phenotype is variable with only some affected members of the pedigrees reported by Berry et al. [9] displaying ASMD. This variability within the families and between families suggests the presence of modifying genes or environmental factors. Three of the pedigrees in the report of Berry et al. [9] are of English descent, as is the current family. Additional families (with no ASMD) are reported to be Chinese and Hispanic in origin, suggesting that this is not a founded mutation and that it arose independently multiple times. As this mutation has been previously reported to be associated with posterior polar congenital cataract in a similar ethnic group [9] to this family, unaffected controls were not screened in this study.

As this mutation has now been identified in six unrelated pedigrees we hypothesized that it may contribute to a signifi-

cant number of congenital cataract cases, however, we found no further carriers of the variant in 100 congenital cataract index cases, indicating that it is not a common cause of congenital cataract in Australia. As two other mutations have also been reported to cause a posterior polar cataract (650delG [9] and S13N [10]), we hypothesized that other mutations may be responsible for the posterior polar phenotype observed in nine additional, smaller pedigrees. Complete sequencing of the coding regions of *PITX3* failed to identify any mutations, indicating that *PITX3* is not a common contributor to the posterior polar congenital cataract in Australia.

In conclusion, the posterior polar cataract observed in the large multi-generational pedigree is caused by a 17 bp insertion in exon 4 of the *PITX3* gene. This insertion has also been observed in five other pedigrees with a posterior polar cataract. We believe the sample of families recruited for this study to be representative of Australia in general and is an almost complete collection of all congenital cataract families in southeastern Australia [6]. Therefore, we conclude that although the 17 bp insertion has been detected in a large kindred with posterior polar congenital cataract, this mutation is not a common cause of congenital cataract in Australia, nor is the *PITX3* gene a significant contributor to congenital posterior polar cataract in this country, in contrast to previous results suggesting it could be a common cause world wide [9].

### ACKNOWLEDGEMENTS

This work was funded by NHMRC project grant 275566. James McKay is a CJ Martin research fellow (NHMRC Australia). The authors would like to thank Dr. James Elder and Lindsey Scotter for assistance with patient recruitment.

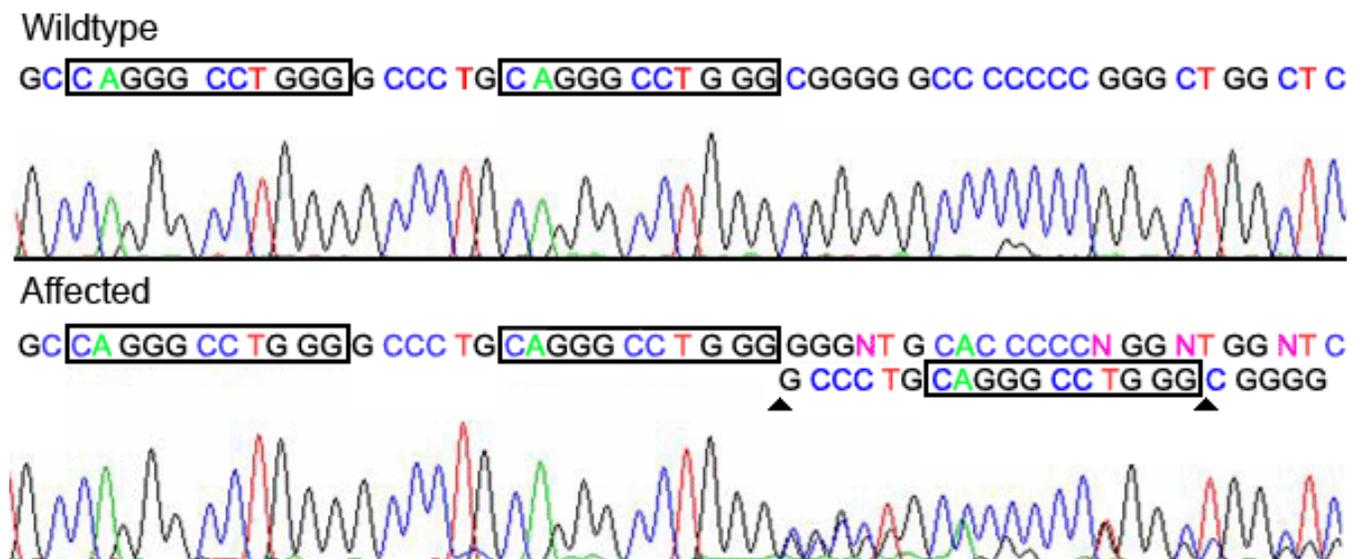


Figure 3. This figure shows the 17 bp insertion at nucleotide 657 of *PITX3* mRNA from the start of translation (GenBank NM\_005029). The repeated element is boxed. The top line of sequence in the affected individual is the raw base calls made by the sequencing instrument, highlighting the overlaid PCR products (ambiguous base calls) where the insertion begins. The bottom line shows the sequence of the mutated allele, with the duplicated 17 bp indicated by arrow heads.

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