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Research paper

Novel genes and variants associated with IgA nephropathy by co-segregating with the disease phenotypes in 10 IgAN families

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ABSTRACT

Background: Previously, a large proportion of the genetic components predisposing individuals to IgA nephropathy (IgAN) have been unidentified. Familial IgAN is enriched with genetic variations predisposing individuals to the disease. Whole exome sequencing is an effective way to explore disease-causing genes and gene variants.
Methods: We performed exome sequencing on the probands from each of ten IgAN families, and on one of the unaffected member from 7 of the families. Sanger sequencing, bioinformatics and co-segregation analysis were performed for all available family members to detect deleterious genetic variation. The relatedness of the families was tested by haplotype analyses.
Results: Six deleterious variants in 4 genes were observed to be associated with IgA nephropathy by co-segregating with the disease phenotypes in study families. *MYCT1* p.Asp22Glufs*34 was associated with IgAN by co-segregating with its phenotypes in families 2, 7, and 9; *DEFA4* p.Ala8Pro, p.Ala8Val, c.172 + 1G>T co-segregated in families 1, 2, and 3; *ZNF543* p.Pro226Ala co-segregated in families 3, 5, and 6 and *CARD8* p.Val98Lysfs*26 co-segregated in families 7 and 8. Among these genes, *MYCT1*, *CARD8* and *ZNF543* are novel. Our haplotype analyses showed that families in which the same variation(s) were co-segregating with IgAN were unrelated, except for *DEFA4*. Of the families carrying *DEFA4*, families 2 and 3 were possibly related, but not family 1, indicating that common genes/variations in these families were not due to the same founder. Inter-familial sharing of different co-segregating genes was also observed, demonstrating the polygenic nature of this disease.
Conclusions: We discovered 6 deleterious variants in 4 genes associated with familial IgAN. These genes are good candidate genes that appear to be causally related to IgAN and warrant further study.

1. Introduction

IgA nephropathy (IgAN) is the most prevalent primary glomerulonephritis worldwide. Approximately 20% to 30% of individuals with IgAN develop end-stage renal disease (ESRD) within 10 to 20 years following initial diagnosis (Bisceglia et al., 2006; Le et al., 2012; Wyatt and Julian, 2013). Despite varying clinical presentations, IgAN is diagnosed by immunohistochemical analysis on renal biopsies and is characterized by predominant mesangial IgA deposition. The etiology of IgAN remains unclear. Genetic contribution to the etiology is implicated by the presence of large extended IgAN families and familial aggregation of IgAN. Evidence for a genetic component is further revealed by genome-wide association studies and candidate association studies (Klein et al., 2005; Feehally et al., 2010; Kiryluk et al., 2010, 2014; Gharavi et al., 2011; Yu et al., 2012; Xie et al., 2013). Intriguingly, the prevalence of IgAN varies greatly among different ethnicities, being higher in Asians (especially among Chinese and Japanese) but lower in Africans (Hall et al., 2004; Kiryluk et al., 2010, 2012).

IgAN families are enriched in genetic components predisposing individuals to IgAN. Next-generation sequencing has been proven to be an effective way to interrogate the whole exome or the whole genome to

Abbreviations: *CARD8*, *caspase recruitment domain-containing protein 8*; *DEFA4*, *Defensin, Alpha 4*; IgAN, IgA nephropathy; *MYCT1*, *Myc target 1*; SNP, single nucleotide polymorphisms; WHO, World Health Organization; *ZNF543*, *Zinc Finger Protein 543*.

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identify genes and gene variants that underlie both monogenic and complex diseases. We performed exome sequencing on individuals from 10 families with IgAN (9 families were unrelated) and identified 6 variations in 4 genes that co-segregated with the IgAN disease phenotype. These variants are good candidates for the genes causing IgAN.

2. Subjects and methods

2.1. Study subjects

Ten families with at least two members that had IgAN as demonstrated by a biopsy, were recruited from the Queen Mary Hospital, University of Hong Kong (Fig. 1). A total of 20 IgAN subjects were included in this cohort. Detailed clinical and laboratory investigations were performed on each of the family members available for the study. IgAN was diagnosed following the WHO criteria on renal biopsy, with the exclusion of systemic lupus erythematosis, Henoch–Schonlein purpura and hepatic diseases as previously described (Li et al., 2004). All families were of Chinese Han ethnicity. All participants gave their written informed consent. The study was approved by the Ethics Committee of the University for Human Study and was conducted in accordance with the principles of the 1975 Declaration of Helsinki.

2.2. Methods

2.2.1. Exome sequencing and candidate selection in the discovery group

DNA was extracted from peripheral blood from each of the available family members by using the QIAamp DNA Mini Kit (Qiagen, Germany). Ten patients, one from each of the 10 families, and 7 unaffected members from families 1, 2, 5, 6, 8, 9 and 10 were selected as the discovery group for exome sequencing.

Exome sequences were captured by SureSelect Human All Exon v.2 Kit, Agilent Technologies (http://www.genomics.agilent.com/); this kit targets 44 Mb of the human genome, covering 98.2% of the Consensus Coding Sequences Database (CCDS, http://www.ncbi.nlm.nih.gov/CCDS/CcdsBrowse.cgi). The enriched library was sequenced on an Illumina HighSeq2000. Sequencing reads were mapped to GRCh37/hg19 (http://genome.ucsc.edu/cgi-bin/hgGateway). The results were annotated using the Consensus Coding Sequences Database at the NCBI (released in September 7, 2011). Single nucleotide variations (SNVs) were identified using Samtools (http://samtools.sourceforge.net). Small insertions and deletions (indels) were identified using Dindel (http://www.sanger.ac.uk/resources/software/dindel/). Variants were also annotated using the Consensus Coding Sequences Database at the NCBI (released in September 7, 2011).

To maximize the chance, we used the following criteria for selecting candidates. We first excluded sex-linked variants. Secondly, we selected single nucleotide variations (SNVs) and the small insertions and deletions (indels) that cause non-synonymous, frameshift, inframe changes and variants that occurred at splice sites. Thirdly, we selected variants that co-occurred in cases in at least two different families and only appeared in case group or control group. Fourthly, we identified deleterious variants by their predicted effects on gene functions by using the programs of PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/www/SIFT_chr_coords_submit.html). Finally, we selected candidate genes based on known functions and those reported as candidate genes in previous genome-wide association studies. Once candidate genes were selected, we performed Sanger sequencing, co-segregation analyses and haplotype constructions (Fig. 2).

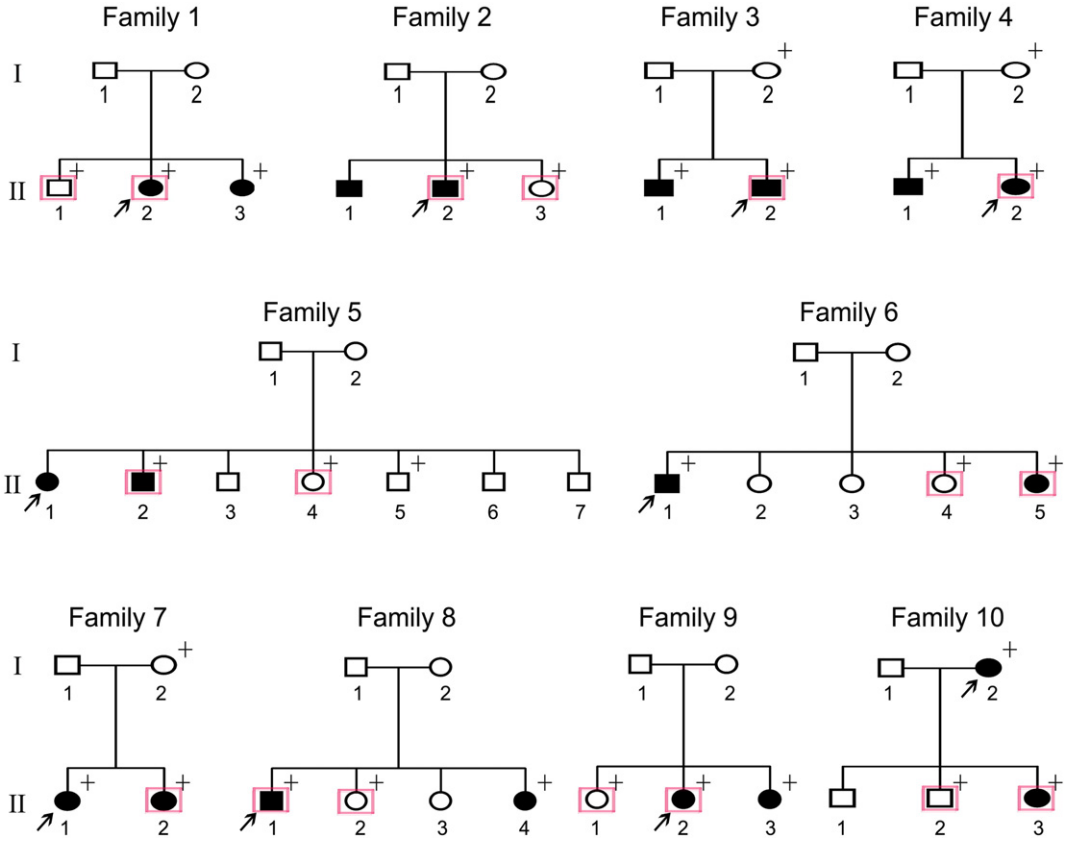

Fig. 1. Pedigrees for 10 IgAN families. Filled squares or circles indicate IgAN patients. + depicts individuals who were available for the genetic analysis. Pink frames designate the family members in whom the whole exome sequencing was performed. Arrows point to the probands for each family.