Tomino Y (ed): IgA Nephropathy Today.
Contrib Nephrol. Basel, Karger, 2007, vol 157, pp 80–89

Searching for IgA Nephropathy Candidate Genes: Genetic Studies Combined with High Throughput Innovative Investigations

F.P. Schena[a], G. Cerullo[a], D.D. Torres[a], G. Zaza[a], S. Cox[a], L. Bisceglia[b], F. Scolari[c], G. Frascà[d], G.M. Ghiggeri[e], A. Amoroso[f], on behalf of the European IgA Nephropathy Consortium

[a]Renal, Dialysis and Transplant Unit, Department of Emergency and Organ Transplant, University of Bari, Bari, [b]Medical Genetic Service, IRCCS – Casa Sollievo della Sofferenza, San Giovanni Rotondo, [c]Renal Unit, Spedali Civili, Brescia, [d]Renal Unit, Ospedale Riuniti, Ancona, [e]Laboratory of Pathophysiology of Uremia, G. Gaslini Children's Hospital, Genoa, and [f]Genetic Unit, University of Turin, Turin, Italy

Abstract

Idiopathic IgA Nephropathy (IgAN) is the most common biopsy-proven glomerulonephritis worldwide. All races with the exception of Blacks and Indians are involved. Families with two or more relatives affected by IgAN may be observed in 15–20% of pedigrees of IgAN patients. Genome wide linkage study has been considered the most promising approach to identify IgAN susceptibility genes. Therefore, some European investigators constituted the European IgAN Consortium which was initially funded by the European Union. Data from linkage analysis studies, family association studies and case-control association studies are reported. To date, the Consortium has identified two loci (located on chromosomes 4q26–31 and 17q12–22), in addition to the previous study which described the first IgAN locus on chromosome 6q22–23. The functional mapping of genes involved in the disease proceeds from the identification of susceptibility loci identified by linkage analysis (step 1) to the isolation of candidate genes within gene disease-susceptibility loci, after obtaining information by microarray analysis carried out on peripheral leukocytes and renal tissue samples (step 2). Then, the process will proceed from the design of RNA interference-agents against selected genes (step 3) to the application of systematically tested effect of RNA agents on functional cellular assay (step 4). The above combined high-throughput technologies will give information on the pathogenic mechanisms of IgAN. In addition, these data may indicate potential targets for screening, prevention and early diagnosis of the disease and more appropriate and effective treatment.

Idiopathic IgA nephropathy (IgAN) is the most common biopsy-proven glomerulonephritis, which is defined by the predominant deposition of the sub-class IgA1 in the mesangial area of the glomeruli occurring in individuals with recurrent episodes of macroscopic hematuria in concomitance of upper respiratory tract infections or other mucosal infections. However, the disease may be diagnosed in the presence of persistent microscopic hematuria with or without proteinuria. Clinical and laboratory findings should exclude other renal diseases characterized by glomerular IgA deposits such as Schönlein-Henoch purpura, lupus nephritis and chronic hepatitis.

Renal biopsy reports from different parts of the world and from renal registries show that IgAN is the most common primary glomerulonephritis among all races from Europe, Asia and Australia, with the exception of the Blacks and Indians [1–9]. A recent paper from North America demonstrated that IgAN is a common primary glomerulonephritis in USA with particular reference to the midwestern and southern states [10]. Therefore, IgAN may be considered the most common cause of end-stage kidney disease in young adult American Caucasians.

Families with two or more relatives affected by IgAN, first described in 1973 by de Werra et al. [11], were then reported by other investigators in later years [12–15]. Thus, today it is possible to distinguish familial IgAN from the sporadic form which is more frequent. Nevertheless, familial IgAN may be diagnosed when physicians accurately analyze the history of all relatives of at least 3 generations who receive the routine urinalysis to identify persistent urinary abnormalities [15]. Previous reports demonstrated that outcomes such as rapid progression of renal damage and end-stage kidney disease are more frequent in patients with familial IgAN [16]. A more recent paper, which analyzed a higher number of familial and sporadic forms of IgAN, demonstrated that there is no difference in outcome severity of the disease [17]. The contradiction between the papers could be attributed to the low number of families analyzed in the first paper, even though it documented a higher relative risk of disease in first degree relatives.

European IgAN Consortium Biobank

Genome-wide linkage study involving IgAN families with large pedigrees was considered the most promising approach to identify IgAN susceptibility genes. The first genome-wide scan study was performed in Japanese IgAN families and demonstrated the existence of some chromosomal locus candidates containing genes responsible for the disease. However, those data remained only in an abstract presented at the 1996 ASN Congress [18]. Then, a well organized

genome-wide scan study, performed in 30 large extended IgAN multiplex families (24 from Italy and 6 from the USA), identified the locus called IGAN1 located on chromosome 6q22–23 in linkage with IgAN [19]. It yielded a significant peak LOD score of 5.6, with 60% of families linked, assuming an autosomal dominant mode of inheritance with incomplete penetrance. This mode of inheritance of familial IgAN is more consistent with the involvement of a single gene with a large effect located in IGAN1. Nevertheless, multifactorial determination, with the interplay of many genes, each conferring a small effect, cannot be excluded. The knowledge gained from the studies of Mendelian diseases has shown that genetic dissection of a complex trait is more powerful when combined linkage-based, association-based and sequence-based approaches are performed. Moreover, considering that no single study design consistently produces more significant results, multivariate analysis carried out by Artmüller et al. [20] showed that the only factors independently associated with increased study success are (a) an increase in the number of individuals studied and (b) study of a sample drawn from only one ethnic group. Gene discovery in complex human disease is complicated by substantial etiological heterogeneity; in addition, the possibility of genes of small effect and the concomitant requirement for large samples make a DNA Bank absolutely necessary.

In consideration of the above statements we believed it opportune to organize a IgAN-oriented Biobank [21]. Therefore, some European investigators, involved in the study of IgAN, constituted the European IgAN Consortium which was initially funded by the European Union. The collaborative study group includes expert nephrologists from Italy, Germany and Greece, and geneticists from Italy and Germany. Additional funds were obtained from other institutions. The organization of a multi-center biobank for the collection of biological samples and clinical data from IgAN patients and relatives following a common protocol was considered the start-up for the identification of the disease susceptibility genes. DNA samples of IgAN patients and relatives belonging to 74 multiple extended pedigrees were collected. Moreover, 166 trios (affected sons or daughters and their healthy parents), 1,085 patients with biopsy-proven IgAN and 1,125 healthy subjects were included in the Biobank. An electronic database was created to include data on the enrolled individuals, laboratory findings and other information from the collected biological fluids (blood, urine and saliva). A website (www.igan.net) was constructed to allow scientific information to be shared between partners and to divulge obtained data [22]. The European IgAN Consortium competency increased in time involving genetic epidemiologists, statisticians and bioinformaticians. We hope that all involved specialists, working together, can avoid problems in study design, data management, analysis and interpretation that make gene discovery and replication of findings so difficult.

Linkage Analysis Studies

Genome-wide linkage strategy identifies regions of the human genome that are likely to contain gene(s) conferring susceptibility to the disease. It is based on extended pedigrees and is effective in localizing gene(s) that are highly penetrant and affect mainly Mendelian diseases. This method alone is often inefficient in discovering genes responsible for complex diseases, such as IgAN, diabetes and hypertension. In combination with linkage analysis, we are also applying family-based candidate gene association studies. The European IgAN Consortium has performed the first genome-wide scan involving 22 Italian multiplex IgAN families [23]. A total of 186 individuals (59 affected and 127 unaffected) were genotyped and included in a two-stage linkage analysis. The regions 4q26–31 and 17q12–22 exhibited the strongest evidence of linkage by non-parametric analysis (best p values of 0.0025 and 0.0045, respectively). These localizations were also supported by multipoint parametric analysis where a peak LOD score of 1.83 ($\alpha = 0.50$) and of 2.56 ($\alpha = 0.65$), respectively, were obtained using the affected-only dominant model, and by allowing for the presence of genetic heterogeneity. These regions are becoming the second (IGAN2) and third (IGAN3) genetic locus candidates to contain causative and/or susceptibility genes for familial IgAN. Other regions did not reach the threshold of a suggestive or significant LOD score; however, the enrolment of additional IgAN families means that these chromosomal regions may be explored in the near future. Our results provide further evidence for genetic heterogeneity among IgAN families. Evidence of linkage to multiple chromosomal regions is consistent with both an oligo/polygenic and a multiple susceptibility gene model for familial IgAN with small/moderate effects in determining the pathological phenotype. The analysis of the known genes located in these two novel loci (positional information procedure), carried out consulting the National Center for Biotechnology Information, identified some potential candidate genes such as the transient receptor potential channel 3 (TRPC3) gene, the interleukin-2 (IL-2) gene, and the IL-21 gene located in 4q26–31, which could be largely involved in the unbalanced Th1/Th2 immune response reported in IgAN patients. In addition, we will also consider the histone deacetylase 5 (HD5) gene and the granulin (GRN) gene located on the 17q12–22 region, which could be involved in the immune-response deregulation. Family-based association studies, evaluating the distribution of these candidate gene polymorphisms, are in progress.

Microarray Studies

Different high-throughput gene analysis techniques can be used for obtaining transcriptome profiling of renal diseases. Microarray analysis represents the

best and the latest approach to gain information on global gene expression. IgAN is a complex disease which results from the joint influence of genetic and environmental factors. Important categories of environmental exposures such as mucosal infections have been identified, but the genetic architecture of the disease remains obscure. Genome-wide linkage analyses have identified at least three locus candidates containing IgAN susceptibility genes, although no specific gene(s) have been identified. Microarrays are now in use to fingerprint the pathological process.

A recently published study postulated that changes in gene expression patterns in circulating leukocytes of IgAN patients may correlate with renal disease activity [24]. The investigators identified 14 upregulated genes. The BTG2, NCUBE1, FLJ2948, SRPK1, LYZ, GIG2 and IL-8 genes correlated mathematically with serum creatinine levels and the PMAIP1, SRPK1, SSI-3, LYZ and PTGS2 genes correlated with higher values of creatinine clearance, thus implying that the latter group of genes may provide a protective effect, while the overexpression of other genes such as B3GNT5, AXUD1 and GIG-2 indicates a worse prognosis. This gene signature reflected kidney function and did not correlate with hematuria or proteinuria. The authors concluded that studies carried out on large populations of IgAN patients will be necessary to confirm that the leukocyte gene expression profile can be used as a marker for diagnosis and for predicting outcome. The European IgAN Consortium has recently organized a protocol for studying gene expression in peripheral blood mononuclear cells (PBMC) and their subclasses from IgAN patients with different clinical and histological patterns. Some genes overexpressed in PBMC are located in the chromosomal regions linked with IgAN. Extensive studies are in progress in a large population of IgAN included in the database of the IgAN Consortium.

Expression profiling using serial analysis of gene expression (SAGE) and microarray techniques allows global description of expressed genes present in renal tissue. This is a high throughput genomics technology which enables the simultaneous determination of a large number of genes from tissue samples. Waga et al. identified 13 upregulated genes in IgAN renal biopsy samples. The cluster analysis identified 3 clusters with 7, 12 and 1 involved gene, respectively [25]. The expression levels of these genes were then examined on expanded RNA samples from other renal biopsies, leukocyte samples and cultured primary cells. Data demonstrated the involvement of the genes GABP and STAT3 in cluster I, and gp330 (megalin), MBP45K, MEF2, Oct1 and GABX in cluster II. We recently used laser-capture microdissection applied to renal biopsy samples in combination with extraction of intact RNA and differential gene expression analysis. First, we defined the clinical groups based on differences in the manifestation of characteristic phenotypes; then, we proceeded

with gene expression analysis by gene chips. The analysis of the data is in progress.

Association Studies

The IgAN Consortium takes care of the collection of biological samples from large homogeneous cohorts of IgAN patients, their parents and their first degree relatives, and family-based association studies are preferred to analyze the role of some candidate genes. A family-based association study, including 53 patients, 45 complete trios, 4 incomplete trios and 36 discordant siblings, evaluated the impact of some Th1/Th2/Th3/TR-type lymphocyte and monocyte/macrophage cytokines on IgAN susceptibility [26]. Cytokine gene polymorphisms with a potential regulatory role on their production were investigated using the family-based association test (FBAT): IFNγ intron-1 CA-repeat at position 1349–1373; IL-13 –1055C/T; TGFβ 915G/C; IL-10 5′-proximal and distal microsatellites; TNFα –308G/A, –238G/A.

The FBAT multi-allelic analysis showed an association between IFNγ polymorphism and susceptibility to IgAN ($p = 0.03$). The bi-allelic analysis showed that the 13-CA repeat allele was preferentially transmitted to the affected individuals ($p = 0.006$; Bonferroni p value = 0.04). The direct sequencing of IFNγ amplicons showed a strict association between the 13-CA repeat allele and the A variant of the +874T/A single nucleotide polymorphism (SNP rs2430561) directly adjacent to the 5′ end of the microsatellite. The in vitro production of IFNγ evaluated in PBMC from 10 genotyped patients demonstrated a correlation between the +874A allele and a lower production of IFNγ ($p = 0.028$). This SNP affects IFNγ production lying within a binding site for the transcription factor NF-κB.

The occurrence of the +874A variant is responsible for the low production of IFNγ and predisposes to a preferential Th2-mediated immune response. The predominance of this variant in individuals with IgAN may be responsible for the onset of the disease. This unbalanced Th2 cytokine production in response to upper respiratory tract infections, which may be a significant pathogenic factor in human IgAN, has recently been confirmed by another paper on Th2 predominance produced by our group. This case-control association study shows a significantly higher frequency of the IL-10–1082 G/G genotype in IgAN patients [27]. The high producer IL-10 genotype (–1082 GG) was significantly more prevalent in IgAN patients than controls (OR 2.41; CI 1.45–4.00; $p = 0.0008$).

Since this genotype is characterized by high production of IL-10, which is a Th2 cytokine, our data support once again the hypothesis that individuals

genetically determined to an overproduction of Th2 cytokines may be predisposed to IgAN.

The prevalence of Th2 cytokines may also explain the abnormality in IgA1 glycosylation occurring in IgAN patients and the concomitant formation of circulating IgA1-IgG immune complexes. Hyperfunction of Th2 cells and cytokine polarity are linked to a more nephritogenic pattern of IgA1 glycosylation in the animal model, and the decreased glycosylation of IgA1 elicited by Th2 cytokines is blunted in vitro by the addition of IFNγ [28].

The core 1 β1,3-galactosyltransferase (C1GALT1) is suspected to be involved in the abnormal glycosylation process of IgA1 in IgAN. The C1GALT1 gene complete sequence analysis was performed in 284 IgAN patients and 234 healthy controls. We found a statistically significant association of the genotype 1365G/G with susceptibility to IgAN ($\chi^2 = 17.58$, $p < 0.0001$, odds ratio 2.57 [95% CI: 1.64–4.04]). No association was found with the progression of the disease.

Our case-control association study demonstrates that the low expression of C1GALT1 seems to confer susceptibility to IgAN [29].

Conclusions

The application of genome-wide expression analysis requires some infrastructure such as: (1) the collaboration between scientists and clinicians with different skills to develop an effective methodological strategy; (2) the accumulation of sufficient technical expertise to generate high-quality, large-scale, biochemical, genetic and physiological data; (3) the development of effective mechanisms and tools to properly store, disseminate and analyze the data that will be generated from large-scale scientific projects. These appropriate infrastructures have been set up over the last five years by the European IgAN Consortium and the actual high-throughput innovative program is articled in the following projects: (1) management of the European IgAN Biobank collecting large samples of biological specimens from well-characterized IgAN patients, their relatives, and healthy subjects. This material is an invaluable source for genetic and genomic studies in IgAN; (2) the fine mapping of the chromosomal regions in linkage with familial IgAN and further linkage analyses of new multiplex IgAN families to receive additional information; (3) the global gene expression analysis performed in PBMC and renal tissue to define a set of genes, up- or downregulated in IgAN patients, of which some could be located in the linked chromosomal regions and could be responsible for the onset and/or progression of the disease; (4) the association studies organized and partially followed by the applied functional genomics of individualized

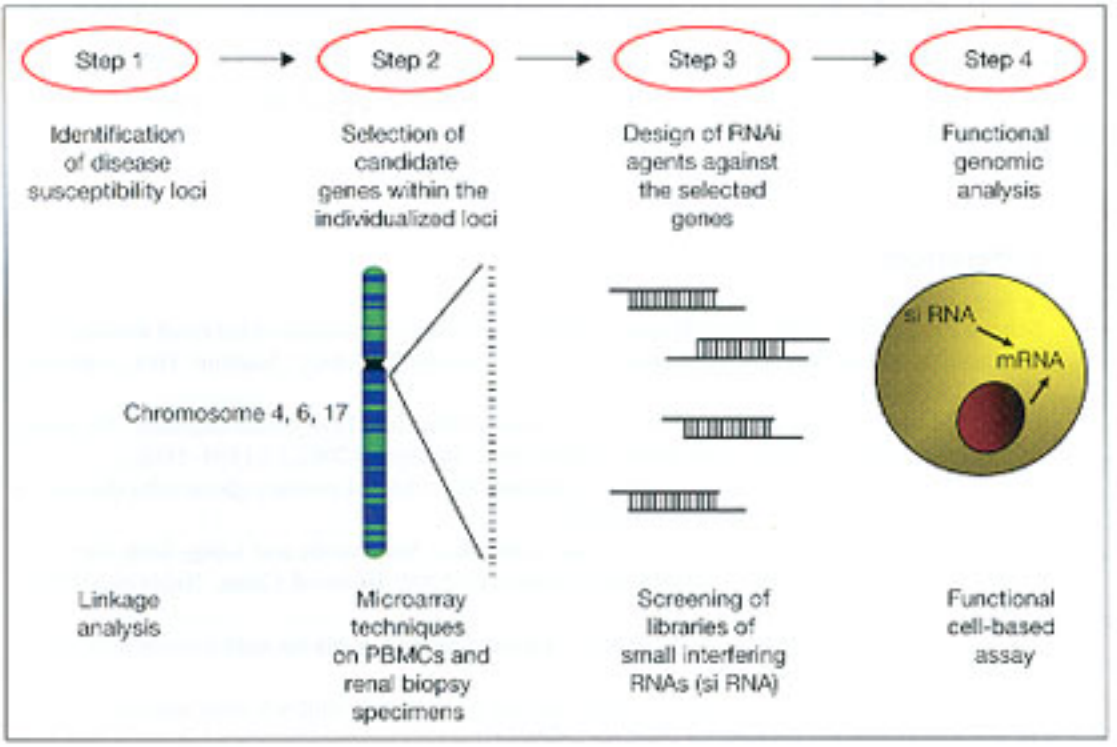

Fig. 1. The high-throughput innovative program of the European IgAN Consortium.

genes using all the available and most innovative techniques will give a fingerprint of genes involved in the disease.

Therefore, the functional mapping of genes involved in IgAN proceeds from the identification of susceptibility loci by linkage analysis (step 1) to the selection of candidate genes within the disease-susceptibility loci after obtaining accurate information by microarray analysis carried out on PBMC and renal tissue samples (step 2). Then, the process will proceed to the design of RNA interference-agents against selected genes (step 3) and finally to the application of systematically tested effect of RNA agents on functional cellular assay (step 4) (fig. 1).

The above combined high-throughput technologies aim to produce knowledge on the mechanisms linking causal determinants of disease and disease progression. In addition, they may indicate potential targets for screening, prevention and early diagnosis of the disease and more appropriate and effective treatment.

At the end of this article it is a great pleasure to invite scientists to the international scientific harmonization between IgAN Biobanks, because the pooling of data offers several benefits, of which one is the generation of a minimum of 5,000 cases, and ideally 10,000. This number is required to provide 80% power to detect sized interaction effect [30]. In addition, it will be possible

to carry out powerful analyses based on homogeneous sub-groups within the disease, e.g. based on age, gender or ethnic origin. Large genetic cohort studies have an important role in furthering our understanding of the complex disease which is IgAN.

References

1 Schena FP: Survey of the Italian Registry of Renal Biopsies. Frequency of the renal diseases for 7 consecutive years. The Italian Group of Renal Immunopathology. Nephrol Dial Transplant 1997;12:418–426.

2 Rivera F, Lopez-Gomez JM, Perez-Garcia R, Spanish Register of Glomerulonephritis: Frequency of renal pathology in Spain 1994–1999. Nephrol Dial Transplant 2002;17:1594–1602.

3 Simon P, Ramee MP, Boulahrouz R, et al: Epidemiologic data of primary glomerular diseases in western France. Kidney Int 2004;66:905–908.

4 Research Group on Progressive Chronic Renal Disease: Nationwide and Long-Term Survey of Primary Glomerulonephritis in Japan as Observed in 1,850 Biopsied Cases. Nephron 1999;82: 205–213.

5 Sinniah R, Javier AR, Ku G: The pathology of mesangial IgA nephritis with clinical correlation. Histopathology 1981;5:469–490.

6 Li LS, Liu ZH: Epidemiologic data of renal diseases from a single unit in China: analysis based on 13,519 renal biopsies. Kidney Int 2004;66:920–923.

7 Briganti EM, Dowling J, Finlay M, et al: The incidence of biopsy-proven glomerulonepluritis in Australia. Nephrol Dial Transplant 2001;16:1364–1367.

8 Jennette JC, Wall SD, Wilkman AS: Low incidence of IgA nephropathy in blacks. Kidney Int 1985;28:944–950.

9 Seedat YK, Nathoo BC, Parag KB, et al: IgA nephropathy in Blacks and Indians of Natal. Nephron 1988;50:137–141.

10 Nair R, Walker PD: Is IgA nephropathy the commonest primary glomerulopathy among young adults in the USA? Kidney Int 2006;69:1455–1458.

11 de Werra P, Morel-Maroger L, Leroux-Robert C, et al: Glomerulonephritis with diffuse IgA deposits in the mesangium. Study of 96 adult cases. Schweiz Med Wochenschr 1973;103: 761–768.

12 Julian BA, Quiggins PA, Thompson JS, et al: Familial IgA nephropathy. Evidence of an inherited mechanism of disease. N Engl J Med 1985;312:202–208.

13 Levy M, Lesavre P: Genetic factors in IgA nephropathy (Berger's disease). Adv Nephrol Necker Hosp 1992;21:23–51.

14 Scolari F, Amoroso A, Savoldi S, et al: Familial occurrence of primary glomerulonephritis: evidence for a role of genetic factors. Nephrol Dial Transplant 1992;7:587–596.

15 Schena FP: Immunogenetic aspects of primary IgA nephropathy. Kidney Int 1995;48:1998–2013.

16 Schena FP, Cerullo G, Rossini M, et al: Increased risk of end-stage renal disease in familial IgA nephropathy. J Am Soc Nephrol 2002;13:453–460.

17 Izzi C, Ravani P, Torres D, et al: IgA nephropathy: the presence of familial disease does not confer an increased risk for progression. Am J Kidney Dis 2006;47:761–769.

18 Fukushima T, Nomura S, Kawai S, et al: Whole genome scanning for IgA nephropathy (IgAN) (abstract). J Am Soc Nephrol 1996;7:1333.

19 Gharavi AG, Yan Y, Scolari F, et al: IgA nephropathy, the most common cause of glomerulonephritis, is linked to 6q22–23. Nature Genet 2000;26:354–357.

20 Altmüller J, Palmer LJ, Fischer G, et al: Genomewide scans of complex human diseases: true linkage is hard to find. Am J Hum Genet 2001;69:936–950.

21 Schena FP, Cerullo G, Torres DD, et al, on behalf of the European IgA nephropathy Consortium: The IgA nephropathy Biobank. An important starting point for the genetic dissection of a complex trait. BMC Nephrology 2005;6:14.

22 European IgAN Consortium website (http://www.igan.net).
23 Bisceglia L, Cerullo G, Forabosco P, et al, on behalf of the European IgA Nephropathy Consortium: Genetic heterogeneity in Italian families with IgA nephropathy: suggestive linkage for two novel IgA nephropathy loci. Am J Hum Genet 2006;79:1130–1134.
24 Preston GA, Waga I, Alcorta DA, et al: Gene expression profiles of circulating leukocytes correlate with renal disease activity in IgA nephropathy. Kidney Int 2004;65:420–430.
25 Preston GA, Waga I, Alcorta DA: Gene expression profiles of circulating leukocytes correlate with renal disease activity in IgA nephropathy. Kidney Int 2004;65:420–430.
26 Schena FP, Cerullo G, Torres DD, et al, on behalf of the European IgA Nephropathy Consortium: Role of interferon-gamma gene polymorphisms in susceptibility to IgA nepluropathy: a family-based association study. Eur J Hum Genet 2006;14:488–496.
27 Capasso M, Boschetto L, Di Noce F, et al, on behalf of the European IgAN Consortium: Influence of the Inteleukin-10 and Tumor necrosis factor-α polymorphisms on primary IgA nephropathy susceptibility (submitted).
28 Ebihara I, Hirayama K, Yamamoto S, et al: Th2 predominance at the single-cell level in patients with IgA nephropathy. Nephrol Dial Transplant 2001;16:1783–1789.
29 Pirulli D, Ulivi S, Zadro C, et al, on behalf of the European IgA Nephropathy Consortium: A polymorphism in the gene coding for the Core 1 beta 1,3 galactosyltransferase T1 contributes to the genetic susceptibility to IgA Nephropathy (submitted).
30 Davey Smith G, Ebrahim S, Lewis S, et al: Genetic epidemiology and public health: hope, hype, and future prospects. Lancet 2005;366:1484–1498.

F. Paolo Schena
Renal Dialysis and Transplantation Unit, Department of Emergency and Organ Transplants
University of Bari, Piazza G. Cesare 11
IT–70124 Bari (Italy)
Tel. +39 080 547 8869, Fax +39 080 557 5710, E-Mail fp.schena@nephro.uniba.it