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GLCCI1 single nucleotide polymorphisms in pediatric nephrotic syndrome

Hae Il Cheong[1,2,3], **Hee Gyung Kang**[1,2], and **Johannes Schlondorff**[4]

[1]Department of Pediatrics, Seoul National University Children's Hospital, Seoul, Korea

[2]Research Center for Rare Diseases, Seoul National University Hospital, Seoul, Korea

[3]Kidney Research Institute, Medical Research Center, Seoul National University College of Medicine, Seoul, Korea

[4]Division of Nephrology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA

Abstract

Background—Empiric steroid therapy is the first-line therapy for pediatric nephrotic syndrome, but treatment response is variable. There are few predictors of steroid-responsiveness, though evidence for genetic factors exists. Recently, single nucleotide polymorphisms (SNPs) were identified in the promoter region of *glucocorticoid-induced transcript 1* gene (*GLCCI1*) which effect steroid-responsiveness in asthmatic patients.

Independently, GLCCI1 was identified as a podocyte protein, loss of which disrupts the function of the glomerular filtration barrier. We therefore examined whether SNPs associated with the steroid-responsive expression of *GLCCI1* might predict steroid-responsiveness in nephrotic syndrome.

Case-Diagnosis/Treatment—A cohort of 211 pediatric patients with nephrotic syndrome and 102 controls were genotyped; among the cases, 117 were initial steroid responders while 94 did not respond to oral steroids. No statistically significant differences were noted among the groups, though there was a trend in comparing the small subgroups of steroid-responsive and non-responsive patients with biopsy proven minimal change disease.

Conclusions—While larger cohorts are needed to ascertain the possibility of a small effect of *GLCCI1* SNPs on steroid-responsiveness of nephrotic syndrome, the *GLCCI1* SNPs associated with steroid responsiveness in asthmatic patients are unlikely to have a clinically actionable impact in pediatric nephrotic syndrome.

INTRODUCTION

Idiopathic nephrotic syndrome is the most common kidney disease in children. In these cases, empiric treatment with high dose steroids is the current standard of care [1]. While the relatively high response rates in this population warrants the approach, the treatment does carry the risk of significant adverse effects. Identifying predictors to differentiate steroid-sensitive nephrotic syndrome (SSNS) from steroid-resistant nephrotic syndrome (SRNS) has the potential to limit exposure to steroids in patients unlikely to benefit from these medications. Age at disease onset is the only well-established clinical parameter that influences response to treatment. There are no established laboratory tests that can

Corresponding author: Johannes Schlondorff Division of Nephrology, BIDMC Research North RN304B 99 Brookline Ave Boston, MA 02215 USA jschlond@bidmc.harvard.edu.

differentiate SSNS and SRNS. Histological factors found on renal biopsy, such as the underlying primary glomerular pathology, have clear implications on the likelihood of a clinical response to steroids, but require an invasive procedure usually deferred in the pediatric patient. Over the last decade, there have been substantial efforts to identify factors that influence responsiveness to treatment, including serum cytokine levels [2] and urinary biomarkers [3], but with little success. There has been substantial progress in identifying genetic mutations which lead to hereditary forms of nephrotic syndrome [4]. Many of these Mendelian forms of disease are generally unresponsive to conventional treatment. This provides clinical utility to testing for these mutations [5], allowing affected patients to avoid unnecessary exposure to steroids. Efforts have also been made to identify common genetic polymorphisms that confer the risk of developing disease or steroid-responsiveness. Variants and haplotypes of various genes have been reported to influence disease risk, including *apolipoprotein L1* (*APOL1*) [6, 7] and *glypican-5* (*GPC5*) [8], among others. However, reported associations have been inconsistent in many cases, as exemplified by the results of a recent meta-analysis of the *ACE* I/D polymorphism [9].

In a recently reported genome-wide association study examining response to inhaled glucocorticoids in asthmatic patients, two SNPs in complete linkage disequilibrium in the promoter region of *GLCCI1* were found to associate with a poorer response to steroid treatment [10]. The variants were associated with decreased expression levels of *GLCCI1* in lymphoblastoid B cells, both at baseline and in response to dexamethasone treatment. Independently, Tryggvason and colleagues reported that GLCCI1 is specifically expressed in podocytes and mesangial cells in the kidney, and that knockdown of GLCCI1 in zebrafish embryos leads to the development of proteinuria, abnormal glomerular capillary loops, and podocyte foot process defects [11]. Based on these results, we hypothesized that the SNPs that alter *GLCCI1* expression and steroid-responsiveness in asthmatic patients might also impact the risk of developing nephrotic syndrome or of responding to glucocorticoid therapy for nephrotic syndrome. In genotyping 211 pediatric patients with nephrotic syndrome and 102 controls, there were no statistically significant associations between the SNPs associated with a poorer response to glucocorticoid therapy in asthmatics and either the development of nephrotic syndrome, or with initial response to steroid therapy.

METHODS

Patients

Two-hundred and twenty-six pediatric patients diagnosed with nephrotic syndrome by the Department of Pediatrics, Seoul National University Children's Hospital, Seoul, Korea were enrolled in the study between 1983 and 2011. Diagnostic criteria, initial treatment and response criteria were as previously described [12]. Briefly, nephrotic syndrome was defined as proteinuria >40 mg/h/m^2 with associated hypoalbuminemia below 2.5 g/dL. Initial treatment was with oral prednisolone 60mg/m^2/day or equivalent dose of deflazacort for 4 weeks, followed by a dose of 40mg/m^2 every other day for 4 additional weeks. Initial steroid response was defined as the absence of proteinuria on dipstick test or <4mg/m^2/day for at least 3 consecutive days. Of the 226 patients, 15 were excluded from analysis due to incomplete clinical records, the presence of secondary forms of disease (including 6 patients with familial forms involving mutations in *LMX1B*, *ACTN4*, *INF2* and *MYH9* genes), or poor quality DNA for genotyping. All patients were screened to rule out mutations in *NPHS2* and *WT1* (exons 8 and 9). One hundred and two controls were enrolled at the same clinical center.

Informed consent for genetic analysis was obtained from all patients and/or their parents. The Institutional Review Board of Seoul National University Hopsital, Seoul, Korea approved the protocols used in this study.

Genotyping

Genomic DNA was obtained from a sample of peripheral blood using a QIA Amp DNA Blood Mini kit (Qiagen). The genotypes of SNPs rs37972 and rs37973 were determined using validated TaqMan SNP Genotyping assays from Applied Biosciences and TaqMan Genotyping Master Mix. Genotyping reactions were run in 384 well plates on an ABI Real Time PCR machine as per the manufacturer's recommendation, with automated allele calling.

Statistical Analysis

Analysis for an association of genotypes with different groups in an additive model was performed using the Cochran-Armitage trend test in XLSTAT (Addinsoft) and Excel (Microsoft). Odds ratios, p values and 95% confidence intervals of allele and genotype frequencies between groups were calculated using Fisher's exact test on Graph Pad Prism 5.

Power calculations assuming an additive model, an odds ratio of 2.4 between persons homozygous for the major allele and persons homozygous for the minor allele, and a minor allele frequency of 0.4, gave a power of 62% at an alpha of 0.05 for evaluation of cases and controls, and a 50% power for comparison of steroid-responsive and non-responsive cases.

RESULTS

The average patient age at the time of diagnosis was 6.0 ±3.8 years (range 6 months to 18 years); 71 % were male. Of the 211 patients, 117 (55%) were deemed initial steroid responders. 120 patients underwent renal biopsy. All steroid-resistant patients underwent biopsy unless there was a contra-indication; frequent relapses and steroid dependence were relative indications for a renal biopsy, and some of these patients underwent biopsy. The pathologic diagnosis was minimal change disease (MCD) in 42 (35%), focal and segmental glomerulosclerosis (FSGS) in 76 (63%), and C1q nephropathy in two cases.

Genotype and allele frequencies for the SNPs rs37972 and rs37973 are shown in Table 1; no significant deviation from Hardy-Weinberg equilibrium was observed. Minor allele frequencies are similar to those reported by the HapMap consortium for Han Chinese in Beijing (43% for both SNPs) and Japanese in Tokyo (38% and 41%, respectively for rs37972 and rs37973) [13].

Prespecified comparisons were performed between cases and controls and between steroid-responsive and non-responsive patients. In an additive model, no statistically significant differences were noted between these groups (p values 0.567 for both SNPs comparing cases and controls; p values of 0.414 and 0.344 for rs37972 and 37973, respectively, comparing steroid responders and non-responders; Cochran-Armitage trend test). Analysis of the data using various models, as well as by comparison of allele frequencies, did not demonstrate any statistically significant associations (Supplementary Table 1). Post-hoc analysis of subgroups with biopsy proven MCD or FSGS did suggest a relatively lower proportion of patients homozygous for the major alleles at both loci in the steroid non-responsive group compared to those who were steroid responsive. However, the differences did not reach statistical significance and the small number of individuals in these subgroups, as well as potential selection biases in pursuing biopsy, warrant caution in interpreting these results.

DISCUSSION

In this study, we determined the allele and genotype frequencies of *GLCCI1* SNPs rs37972 and rs37973 in a cohort of pediatric patients with idiopathic nephrotic syndrome and control samples. Interest in these particular SNPs was based on two recent reports: 1) these SNPs

were reported to confer a higher risk of poor response to inhaled steroids in asthmatic patients and decrease *GLCCI1* expression in B cell lines [10], and 2) *GLCCI1* was reported to be highly expressed in the renal glomerulus, and knockdown of the transcript impairs the glomerular filtration barrier in developing zebrafish [11]. We found no statistically significant association with the minor alleles, linked to poorer response to inhaled glucocorticoids in asthmatic patients, and nephrotic syndrome or initial steroid-responsiveness. A post-hoc subgroup analysis does suggest that further examination of these SNPs in patients with biopsy-confirmed MCD or FSGS is warranted.

Our understanding of the function of *GLCCI1* remains limited. It was initially identified as a transcript rapidly up-regulated in response to glucocorticoid treatment in cells derived from a thymoma [14], and is found predominantly expressed in lymphoid tissue and cell lines, lung and brain [10, 11]. In the kidney, it appears to be expressed specifically in mesangial cells and podocytes [11]. Morpholino-mediated knockdown of the gene in zebrafish embryos impairs proper formation of the glomerulus and of podocyte foot processes. However, at a cellular level, the function of GLCCI1 remains unknown, and it has no well-defined protein domains other than a short coiled-coil sequence.

Our study has several limitations that should be noted. Sample size, coupled with the usually low effect size of relatively common variants, is frequently a limitation of genetic studies examining the potential role of genetic variants on disease prevalence. While we limited our analysis of *GLCCI1* to two SNPs with an established association with gene expression and a relatively robust odds ratio for glucocorticoid response in asthmatics [10], our cohort was underpowered to be able to reliably detect an effect size similar to that reported for rs37973 in asthmatics. The use of a dichotomous outcome in our study, as opposed to the continuous variable of change in forced expiratory volume in 1 second (FEV1) used by Tantisira et al [10], contributed to this. Nonetheless, our results do suggest that these SNPs are unlikely to provide clinically actionable information for initial treatment of pediatric nephrotic syndrome. Secondly, our study was limited to patients of Korean nationality. While this strategy helps to limit the possibility of underlying population stratification between groups, we cannot rule out the possibility that the SNPs examined might be associated with a significant effect on steroid-responsiveness in the background of a different ancestry. Finally, a review of dbSNP (www.ncbi.nlm.nih.gov/projects/SNP/) reveals dozens of SNPs surrounding and within *GLCCI1*, including several missense variants. It is certainly possible that one or more of these variants could influence either gene function or expression specifically in glomerular cells, and thereby alter susceptibility to proteinuric kidney disease or its response to treatment. Development of a larger cohort of patients and controls, or additional results pointing to the potential relevance of a particular variant, will be needed before evaluation of additional variants is feasible. Similarly, analysis of an adult cohort of biopsy-proved MCD or FSGS will likely be needed to follow-up the potential correlation of rs37973 and rs37972 and steroid-responsiveness in these subtypes of nephrotic syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. Gipson DS, Massengill SF, Yao L, Nagaraj S, Smoyer WE, Mahan JD, Wigfall D, Miles P, Powell L, Lin JJ, Trachtman H, Greenbaum LA. Management of childhood onset nephrotic syndrome. Pediatrics. 2009; 124:747–757. [PubMed: 19651590]
2. Araya CE, Wasserfall CH, Brusko TM, Mu W, Segal MS, Johnson RJ, Garin EH. A case of unfulfilled expectations. Cytokines in idiopathic minimal lesion nephrotic syndrome. Pediatr Nephrol. 2006; 21:603–610. [PubMed: 16525836]
3. Traum AZ. Urine proteomic profiling to identify biomarkers of steroid resistance in pediatric nephrotic syndrome. Expert Rev Proteomics. 2008; 5:715–719. [PubMed: 18937561]
4. Benoit G, Machuca E, Antignac C. Hereditary nephrotic syndrome: a systematic approach for genetic testing and a review of associated podocyte gene mutations. Pediatr Nephrol. 2010; 25:1621–1632. [PubMed: 20333530]
5. Santin S, Bullich G, Tazon-Vega B, Garcia-Maset R, Gimenez I, Silva I, Ruiz P, Ballarin J, Torra R, Ars E. Clinical utility of genetic testing in children and adults with steroid-resistant nephrotic syndrome. Clin J Am Soc Nephrol. 2011; 6:1139–1148. [PubMed: 21415313]
6. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, Bowden DW, Langefeld CD, Oleksyk TK, Uscinski Knob AL, Bernhardy AJ, Hicks PJ, Nelson GW, Vanhollebeke B, Winkler CA, Kopp JB, Pays E, Pollak MR. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. Science. 2010; 329:841–845. [PubMed: 20647424]
7. Tzur S, Rosset S, Shemer R, Yudkovsky G, Selig S, Tarekegn A, Bekele E, Bradman N, Wasser WG, Behar DM, Skorecki K. Missense mutations in the APOL1 gene are highly associated with end stage kidney disease risk previously attributed to the MYH9 gene. Hum Genet. 2010; 128:345–350. [PubMed: 20635188]
8. Okamoto K, Tokunaga K, Doi K, Fujita T, Suzuki H, Katoh T, Watanabe T, Nishida N, Mabuchi A, Takahashi A, Kubo M, Maeda S, Nakamura Y, Noiri E. Common variation in GPC5 is associated with acquired nephrotic syndrome. Nat Genet. 2011; 43:459–463. [PubMed: 21441931]
9. Zhou TB, Qin YH, Su LN, Lei FY, Huang WF, Zhao YJ. ACE I/D gene polymorphism can't predict the steroid responsiveness in Asian children with idiopathic nephrotic syndrome: a meta-analysis. PLoS One. 2011; 6:e19599. [PubMed: 21611163]
10. Tantisira KG, Lasky-Su J, Harada M, Murphy A, Litonjua AA, Himes BE, Lange C, Lazarus R, Sylvia J, Klanderman B, Duan QL, Qiu W, Hirota T, Martinez FD, Mauger D, Sorkness C, Szefler S, Lazarus SC, Lemanske RF Jr, Peters SP, Lima JJ, Nakamura Y, Tamari M, Weiss ST. Genomewide association between GLCCI1 and response to glucocorticoid therapy in asthma. N Engl J Med. 2011; 365:1173–1183. [PubMed: 21991891]
11. Nishibori Y, Katayama K, Parikka M, Oddsson A, Nukui M, Hultenby K, Wernerson A, He B, Ebarasi L, Raschperger E, Norlin J, Uhlen M, Patrakka J, Betsholtz C, Tryggvason K. Glcci1 deficiency leads to proteinuria. J Am Soc Nephrol. 2011; 22:2037–2046. [PubMed: 21949092]
12. Choi HJ, Cho HY, Ro H, Lee SH, Han KH, Lee H, Kang HG, Ha IS, Choi Y, Cheong HI. Polymorphisms of the MDR1 and MIF genes in children with nephrotic syndrome. Pediatr Nephrol. 2011; 26:1981–1988. [PubMed: 21553324]
13. Consortium IH. The International HapMap Project. Nature. 2003; 426:789–796. [PubMed: 14685227]
14. Chapman MS, Qu N, Pascoe S, Chen WX, Apostol C, Gordon D, Miesfeld RL. Isolation of differentially expressed sequence tags from steroid-responsive cells using mRNA differential display. Mol Cell Endocrinol. 1995; 108:R1–7. [PubMed: 7758820]

Table 1

Comparison of *GLPCCI1* SNPs rs37972 and rs37973 genotype and allele frequencies in patient and control groups

	rs37972						rs37973					
	Genotype			Allele		H-W Eq	Genotype			Allele		H-W Eq
	CC	CT	TT	C	T	p value	AA	AG	GG	A	G	p value
Controls (n=102)	34 (33.3%)	51 (50.0%)	17 (16.7%)	119 (58.3%)	85 (41.7%)	0.77	30 (29.4%)	53 (52.0%)	19 (18.6%)	113 (55.4%)	91 (44.6%)	0.60
Patient group (n=211)	66 (31.3%)	104 (49.3%)	41 (19.4%)	236 (55.9%)	186 (44.1%)	1.00	60 (28.4%)	102 (48.3%)	49 (23.2%)	222 (52.6%)	200 (47.4%)	0.66
Steroid responders (n=117)	40 (34.2%)	55 (47.0%)	22 (18.8%)	135 (57.7%)	99 (42.3%)	0.69	36 (30.8%)	56 (47.9%)	25 (21.4%)	128 (54.7%)	106 (45.3%)	0.71
No biopsy (n=85)	27 (31.8%)	40 (47.1%)	18 (21.2%)	94 (55.3%)	76 (44.7%)	0.66	23 (27.1%)	42 (49.4%)	20 (23.5%)	88 (51.8%)	82 (48.2%)	0.92
MCD (n=21)	9 (42.9%)	9 (42.9%)	3 (14.3%)	27 (64.3%)	15 (35.7%)	0.76	9 (42.9%)	8 (38.1%)	4 (19.0%)	26 (61.9%)	16 (38.1%)	0.38
FSGS (n=11)	4 (36.4%)	6 (54.5%)	1 (9.1%)	14 (63.6%)	7 (31.8%)	0.55	4 (36.4%)	6 (54.5%)	1 (9.1%)	14 (63.6%)	7 (31.8%)	0.55
Steroid nonresponders (n=94)	26 (27.7%)	49 (52.1%)	19 (20.2%)	101 (53.7%)	87 (46.3%)	0.64	24 (25.5%)	46 (48.9%)	24 (25.5%)	94 (50.0%)	94 (50.0%)	0.84
No biopsy (n=6)	1 (16.7%)	4 (66.7%)	1 (16.7%)	6 (50.0%)	6 (50.0%)	0.41	1 (16.7%)	4 (66.7%)	1 (16.7%)	6 (50.0%)	6 (50.0%)	0.41
MCD (n=21)	4 (19.0%)	13 (61.9%)	4 (19.0%)	21 (50.0%)	21 (50.0%)	0.28	3 (14.3%)	14 (66.7%)	4 (19.0%)	20 (47.6%)	22 (52.4%)	0.12
FSGS (n=65)	20 (30.8%)	32 (49.2%)	13 (20.0%)	72 (55.4%)	58 (44.6)	0.98	19 (29.2%)	28 (43.1%)	18 (27.7%)	66 (50.8%)	64 (49.2%)	0.26
C1q nephropathy (n=2)	1 (50.0%)	0 (0%)	1 (50.0%)	2 (50.0%)	2 (50.0%)	0.16	1 (50.0%)	0 (0%)	1 (50.0%)	2 (50.0%)	2 (50.0%)	0.16
MCD (n=42)	13 (31.0%)	22 (52.4%)	7 (16.7%)	48 (57.1%)	36 (42.9%)	0.65	12 (28.6%)	22 (52.4%)	8 (19.0%)	46 (54.8%)	38 (45.2%)	0.71
FSGS (n=76)	24 (31.6%)	38 (50.0%)	14 (18.4%)	86 (56.6%)	66 (43.4%)	0.88	23 (30.3%)	34 (44.7%)	19 (25.0%)	80 (52.6%)	72 (47.4%)	0.37
No biopsy (n=91)	28 (30.8%)	44 (48.4%)	19 (20.9%)	100 (54.9%)	82 (45.1%)	0.82	24 (26.4%)	46 (50.5%)	21 (23.1%)	94 (52.6%)	88 (48.4%)	0.91

MCD, minimal change disease; FSGS, focal segmental glomerulosclerosis; H-W Eq, Hardy-Weinberg equilibrium.