Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Characterization of variation in the canine suppressor of cytokine signaling-2 (SOCS2) gene

G. Rincón[1], A.E. Young[2], D.L. Bannasch[2] and J.F. Medrano[1]

[1]Department of Animal Science, University of California, Davis, CA, USA
[2]Department of Population Health and Reproduction,
School of Veterinary Medicine, University of California, Davis, CA, USA
Corresponding author: J.F. Medrano
E-mail: Jfmedrano@ucdavis.edu

Genet. Mol. Res. 6 (1): 144-151 (2007)
Received August 25, 2006
Accepted January 5, 2007
Published March 28, 2007

ABSTRACT. Suppressor of cytokine signaling 2 (SOCS2) is a negative regulator of growth hormone signaling. The deletion of SOCS2 in mice results in a 30-50% increase in post-natal growth. In an effort to identify polymorphisms in the SOCS2 gene that may be associated with body size in dogs, we characterized the canine SOCS2 gene and analyzed its genetic diversity among small and large dog breeds. The study was carried out on a total of 520 dogs from 66 different breeds. Dogs were classified as large or small based on height and weight as determined by their respective American Kennel Club breed standards. The SH2 and SOCS domains of the canine SOCS2 gene were sequenced in 32 dogs from different breeds. Only one non-synonymous sequence variant (DQ415457:g.326G>T) was detected which corresponds to an amino acid change (Asp127Tyr). All samples were genotyped by PCR/RFLP and the allele frequencies were determined for each dog breed. The T allele was distributed primarily among European large dog breeds with a gene frequency ranging from 0.72 to 0.04. The nature of the nucleotide change and the effect on the protein together with the finding

 ©FUNPEC-RP www.funpecrp.com.br

of a QTL related to body size in the same CFA15 region by other researchers suggest canine SOCS2 as a potential candidate gene for body size in dogs. Future studies will be needed to clarify the role of the 326G>T polymorphism and its interaction with genes like growth hormone and insulin-like growth factor 1.

Key words: SOCS2, Growth, SNP, Polymorphism, Dog breeds

INTRODUCTION

Cytokines consist of a large family of secreted proteins that regulate a diverse array of physiologic and pathologic responses, including body growth, hematopoiesis, immune response, inflammation, and development of the nervous system. Suppressors of cytokine signaling (SOCS) are a group of intracellular proteins that have the ability to regulate the magnitude and duration of cytokine signaling. SOCS2 is one of the members of this family that inhibits signal transduction induced by several cytokines including interleukin 6, leukemia inhibitory factor, insulin-like growth factor 1 (IGF-1), prolactin, and growth hormone (GH) (Greenhalgh and Hilton, 2001; Krebs and Hilton, 2001; Tan and Rabkin, 2005).

SOCS proteins share conserved structural and functional domains. They typically have an amino-terminal region, a Src-homology 2 (SH2) domain and a carboxy-terminal motif denominated SOCS box (Hilton et al., 1998). The SH2 domain is the prototype for protein-protein interaction modules that mediate the formation of multiprotein complexes during signaling. The SOCS box targets signaling proteins to the proteasome for degradation by recruitment of the ubiquitin transferase system (Kile et al., 2001; Pawson et al., 2001; Pawson, 2004). Both domains are evolutionarily conserved in many eukaryotes (Machida and Mayer, 2005).

A spontaneous deletion in mouse chromosome 10 disrupts expression of SOCS2 and results in the *high growth* mouse phenotype which is characterized by a 30-50% increase in postnatal body growth without altering overall body composition (Corva and Medrano, 2000; Horvat and Medrano, 2001; Wong et al., 2002). Independent gene targeting studies have shown that SOCS2 is an indispensable negative regulator of GH actions (Greenhalgh and Alexander, 2004) and that the SOCS2 knockout shares similar phenotypes with the *high growth* mouse including a large size ((Metcalf et al., 2000).

The role of SOCS2 in the regulation of murine body size suggests that this gene may be an appropriate candidate for association with body size in other animals. As evidenced by *high growth* and the SOCS2 knockout mice, the study of extreme variations in body size observed within species provides opportunities to understand the genetic basis of size and morphology. The considerable phenotypic variability in the canine population exemplified by extremes among breeds, such as the Chihuahua and the Irish wolfhound has resulted in a vast array of shapes and sizes within a single species. Moreover, as a result of strict breeding programs, purebred dogs have developed into closed breeding populations with a high degree of genetic differentiation exhibiting a degree of polymorphism not encountered in any other mammal (Galibert et al., 2004; Parker et al., 2004). As such, purebred dogs are a unique biological model to study morphology, behavior and inherited diseases (Sutter and Ostrander, 2004).

In an effort to identify polymorphisms in the SOCS2 gene that are related to body size in dogs, we characterized the canine SOCS2 gene and analyzed its genetic diversity among small and large dog breeds.

MATERIAL AND METHODS

DNA samples

The present study was carried out on a total of 520 dogs from 66 different breeds. Samples were obtained from patients of the University of California at Davis Veterinary Medical Teaching Hospital. Genomic DNA was isolated from EDTA-preserved venous whole blood using the QiaAmp DNA Blood Mini Kit (Qiagen Inc.). Breed information and body weight were obtained from medical records. For the purposes of this study, dogs were classified as large or small based on American Kennel Club (AKC) breed standards (www.akc.org). Breed standards that call for dogs to be greater than 20 inches in height or greater than 50 lbs in weight were considered large, and standards that indicated a height less than 15 inches or weight less than 20 lbs were classified as small.

In addition, six DNA samples from wolves were included to ascertain possible ancestral variation in the gene. Wolf samples were obtained from the Center for the Reproduction of Endangered Species at the San Diego Zoo.

Sequence analysis

Nucleotide BLAST programs at Ensembl (http://www.ensembl.org/Multi/blastview) were used for sequence homology searches in public databases. The complete sequence of the gene was assembled using the scaffold from the dog genome project (http://www.ensembl.org/Canis_familiaris/index.html) and the available traces using Seqman (DNAStar Inc., Madison, WI, USA). Multiple sequence alignment was performed with the ClustalW program (Thompson et al., 1994) in order to confirm the gene and protein structure of the dog SOCS2 gene.

A pair of primers was designed from the consensus sequence to amplify a 604-bp fragment that included the SH2 and SOCS domains in the canine SOCS2 gene: S2DF: 5'TGTTGCCAAGTATTTGCCCTA3', S2DR: 5'TTTACATAGCTGCATTCGGAGA3'.

PCR protocol

PCR reactions were performed in a total volume of 25 µL, containing 100 ng of template DNA, 10 pmol of each primer, 200 µM dNTP, 3.0 mM $MgCl_2$, 1X PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl), and 0.5 U *Taq* polymerase (Invitrogen). Samples were overlaid with one drop of mineral oil and incubated for 2 min at 94°C, followed by 30 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s with a final 5-min extension at 72°C on an MJ DNA Engine Thermal Cycler.

Sequencing and genotyping

To screen for polymorphisms in the canine SOCS2 gene, 32 dog samples were se-

quenced in both directions with the ABI Prism Big Dye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems). Sequencing products were purified over Centrisep columns (Fisher) and analyzed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). The sequences were visualized and aligned using Seqman (DNAStar Inc.).

All the samples were genotyped by PCR/RFLP using the restriction enzyme Taq I (Promega). The digested fragments were visualized under UV light on ethidium bromide-stained 2.5% agarose gels. Allele frequencies were determined for each dog breed using the GENEPOP software v3.1c (Rousset and Raymond, 1995).

Gene and protein structure analysis

The comparative analysis of the canine SOCS2 gene was performed using the Genome Vista Alignment program (http://genome.lbl.gov/vista/index.shtml) (Couronne et al., 2003). "*In silico*" analysis of the protein structure was performed using the PHD software (http://cubic.bioc.columbia.edu/predictprotein/) (Rost, 1996).

RESULTS AND DISCUSSION

The canine SOCS2 gene contains 6102 bp corresponding to exons 1 to 3 and a 597-bp cDNA. The genome Vista analysis revealed that the genomic sequence homologies of canine with human, mouse, rat, and cow are 95.5, 91.3, 89.1, and 95%, respectively. The percentage of similarity was high not only within the exons but also in the non-coding regions, confirming the high level of conservation of this gene among different species.

Our effort to identify possible natural variation that may be associated with body size in dogs revealed only one non-synonymous sequence variant (DQ415457:g.326G>T) which corresponds to an amino acid change (Asp127Tyr) in the protein.

The canine SOCS2 protein sequence was compared with human, mouse, rat, pig, and cow in a ClustalW alignment (http://www.ebi.ac.uk/clustalw/) (Figure 1). The alignment includes the SH2 domain, extended SH2 subdomain (ESS) and the SOCS domain. The amino acid change observed in the SH2 domain occurs in the region where SOCS2 interacts with the IGF-1 receptor (see Figure 1, amino acids YVQM highlighted in black) (Dey et al., 1998). This region is also an essential conserved domain for the SOCS box ESS/SH2 interface (Bullock et al., 2006). A recent study showed that SOCS2 also interacts with the leptin receptor (Lavens et al., 2006). The hypothalamic leptin receptor signaling plays a central role in weight regulation by controlling fat storage and energy expenditure. This novel regulatory role for SOCS2 reinforces the functionality of this gene's interactions in different pathways related to growth.

In silico structural analysis performed using the PHD software predicted conformational changes in the secondary structure of the protein with Tyr in amino acid position 127. This may lead to a modification in the binding affinity of the SOCS2 SH2 domain. Additional experiments will be needed to clarify the role of the mutation and interactions with genes like GH, IGF-1 and leptin receptor.

The breed-specific gene frequencies for the 326G>T polymorphism were examined using the PCR/RFLP genotyping system shown in Figure 2. The T allele was present in 17% of the breeds tested (Table 1). Nine of eleven dogs belong to European large breeds that are reported to have a large size originating from ancient Mastiff-type ancestors (Parker et al.,

```
Bovine          MTLRCLESSGNGAEGAQSQWGTAGSAEEPSPEAARLAKALRELSHTGWYWGSMTVNEAKE
Pig             MTLRCLEPSGNGAEGTQSQWGTSGSAEEPSPEAARLAKALRELSHTGWYWGSMTVNEAKE
Dog             MTLRCLEPSGNGAEGTPSQWGPAGPAEEPTPEAARLAKALRELSHTGWYWGNMTVNEAKE
Human           MTLRCLEPSGNGGEGTRSQWGTAGSAEEPSPQAARLAKALRELGQTGWYWGSMTVNEAKE
Mouse           MTLRCLEPSGNGADRTRSQWGTAGLPEEQSPEAARLAKALRELSQTGWYWGSMTVNEAKE
Rat             MTLRCLEPSGNGADRTRSQWGTAGSPEDQSPEAARLAKALRELSQTGWYWGSMTVNEAKE
                *******.****.: : ****.:* .*: :*:***********.:******.********

Bovine          KLKEAPEGTFLIRDSSHSDYLLTISVKTSAGPTNLRIEYQDGKFRLDSIICVKSKLKQFD
Pig             KLKEAPEGTFLIRDSSHSDYLLTISVKTSAGPTNLRIEYQDGKFRLDSIICVKSKLKQFD
Dog             KLKEAPEGTFLIRDSSHSDYLLTISVKTSAGPTNLRIEYQDGKFRLDSIICVKSKLKQFD
Human           KLKEAPEGTFLIRDSSHSDYLLTISVKTSAGPTNLRIEYQDGKFRLDSIICVKSKLKQFD
Mouse           KLKEAPEGTFLIRDSSHSDYLLTISVKTSAGPTNLRIEYQDGKFRLDSIICVKSKLKQFD
Rat             KLKEAPEGTFLIRDSSHSDYLLTISVKTSAGPTNLRIEYQDGKFRLDSIICVKSKLKQFD
                ************************************************************

Bovine          SVVHLIDYYVQMCKDKRTGPEAPRNGTVHLYLTKPLYTSAPPLQHLCRLTINKCTSTVWG
Pig             SVVHLIDYYVQMCKDKRTGPEAPRNGTVHLYLTKPLYTSAPPLQHLCRLTINKCTGTIWG
Dog             SVVHLIYYVQMCKDKRTGPEAPRNGTVHLYLTKPLYTSAPPLQHLCRLTINKCTGTIWG
Human           SVVHLIDYYVQMCKDKRTGPEAPRNGTVHLYLTKPLYTSAPSLQHLCRLTINKCTGAIWG
Mouse           SVVHLIDYYVQMCKDKRTGPEAPRNGTVHLYLTKPLYTSAPTLQHFCRLAINKCTGTIWG
Rat             SVVHLIDYYVQMCKDKRTGPEAPRNGTVHLYLTKPLYTSAPTLQHFCRLSINKCTGTIRG
                ****** ***********************************.***:***:*****.:: *

Bovine          LPLPTRLKDYLEEYKFQV
Pig             LPLPTRLKDYLEEYKFQV
Dog             LPLPTRLKDYLEEYKFQV
Human           LPLPTRLKDYLEEYKFQV
Mouse           LPLPTRLKDYLEEYKFQV
Rat             LPLPTRLKDYLEEYKFQV
                ******************
```

Figure 1. Multiple sequence alignment of the protein sequence of SOCS2 including the SH2 domain (grey), extended SH2 subdomain (italics) and the SOCS domain (underlined). The position of the dog Asp127Tyr mutation is identified with the corresponding Y (Tyr) highlighted in white on the SH2 domain. The four amino acids, YVQM, highlighted in black represent the domain where SOCS2 interacts with the IGF-1 receptor.

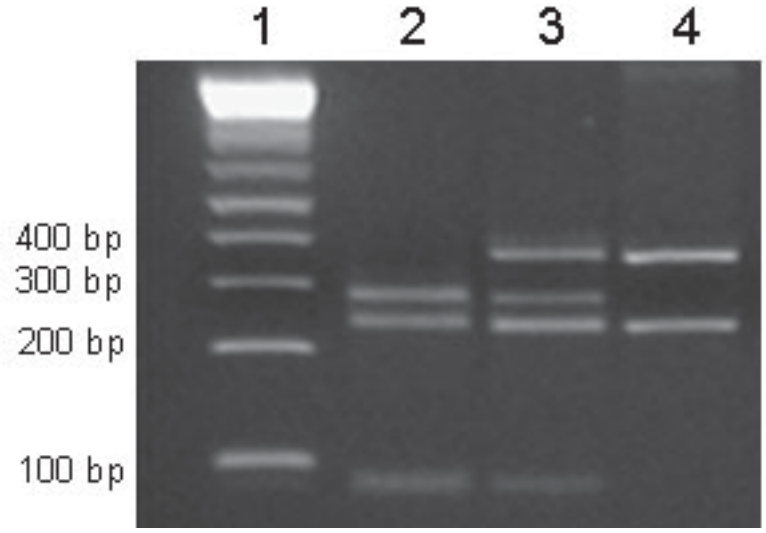

Figure 2. PCR/RFLP genotyping for the canine SOCS2 326G>T . *Lane 1*, 1 kb plus DNA ladder (Invitrogen); *lanes 2* to *4* correspond to DNA samples of genotype GG, GT and TT, respectively. A 604-bp fragment was digested with Taq I to classify allele G (87, 238 and 279 bp) and allele T (238 and 366 bp).

Table 1. Allele frequencies of the 326G>T SNP in large and small dog breeds ordered by geographic origin.

Large breeds	Dogs	G	T	Average weight (lb)	Small breeds	Dogs	G	T	Average weight (lb)
African Breeds					American Breeds				
Rhodesian Ridgeback	8	1	0	89.40	Chihuahua	7	1	0	11.06
Saluki	1	1	0	55.55	Asian Breeds				
North American Breeds					Pekinese	2	1	0	13.86
Australian Shepherd	2	1	0	62.92	Pug	17	1	0	21.30
Chesapeake Bay Retriever	2	1	0	86.75	Shih Tzu	18	1	0	14.45
Flat-coated Retriever	2	1	0	76.72	Australian Breeds				
Labrador Retriever	20	1	0	81.46	Australian Terrier	1	1	0	15.80
Newfoundland	8	**0.94**	**0.06**	**142.57**	Silky Terrier	2	1	0	12.70
Asian Breeds					European Breeds				
Afghan Hound	2	1	0	55.44	Beagle	4	1	0	31
Akita	13	1	0	101.19	Bichon Frise	7	1	0	17.78
Alaskan Malamute	6	1	0	84.30	Cairn Terrier	4	1	0	22.1
Borzoi	2	1	0	66.35	Cavalier King Charles Spaniel	3	1	0	22.8
European Breeds					Cocker Spaniel	4	1	0	34.2
Airedale	4	1	0	68.97	Dachshund	26	**0.94**	**0.06**	**19.06**
Anatolin Shepherd	2	1	0	112.64	Italian Greyhound	1	1	0	14.33
Bearded Collie	1	1	0	59.84	Jack Russell Terrier	7	1	0	19.3
Belgian Tervuren	1	1	0	68.34	Maltese	11	1	0	8.7
Bernese Mountain Dog	13	**0.96**	**0.04**	**97.68**	Manchester Terrier	1	1	0	13.22
Border Collie	3	1	0	45.43	Miniature Dachshund	1	1	0	13.70
Bouvier de Flandres	7	**0.86**	**0.14**	**85.01**	Miniature Pinscher	3	1	0	12.46
Boxer	19	1	0	63.40	Miniature Poodle	9	1	0	17.16
Briard	2	1	0	66.01	Miniature Schnauzer	13	1	0	20.50
Bullmastiff	3	1	0	126.50	Norfolk Terrier	1	1	0	12.78
Collie	2	1	0	76.12	Pembroke Welsh Corgi	1	1	0	36.5
Doberman	10	**0.95**	**0.05**	**88.99**	Pomeranian	11	1	0	11.40
German Shepherd	30	1	0	86.21	Schipperke	3	1	0	18.70
Golden Retriever	56	**0.79**	**0.21**	**79.46**	Scottish Terrier	8	1	0	25.70
Great Dane	7	**0.28**	**0.72**	**124.63**	Shetland Sheepdog	25	1	0	27.30
Great Pyrenees	4	1	0	106.26	Smooth Fox Terrier	3	1	0	19.20
Greyhound	2	1	0	72.60	Toy Poodle	7	1	0	10.65
Irish Setter	2	1	0	86.90	West Highland White Terrier	4	1	0	22.85
Irish Wolfhound	3	**0.83**	**0.17**	**124.90**	Yorkshire Terrier	17	1	0	6.82
Mastiff	14	**0.79**	**0.22**	**170.86**	Total	221			
Old English Sheepdog	1	1	0	90.83					
Rottweiler	38	**0.32**	**0.68**	**104.10**					
Scottish Deerhound	2	1	0	64.02					
St. Bernard	7	**0.93**	**0.07**	**127.16**					
Weimaraner	7	1	0	78.87					
Total	299								

Allele frequencies of breeds that carry the T allele are shown in bold. Dachshund, the only small breed carrying the T allele, is highlighted in grey.

2004). In the small breed group only three standard Dachshunds showed the T allele, but those with the T allele were the largest dogs in that group weighing more than 20 pounds. According to the AKC, during the development of the Dachshund breed, two different sizes emerged based on the type of game being pursued: Dachshunds weighing 30-35 pounds were used on badgers and wild boar, while smaller 16- to 22-pound Dachshunds proved effective against foxes and hare. It is likely that the larger Dachshunds inherited the T allele along with other characteristics from European breeds including German, French, and English hounds and terriers.

The T allele was predominant in Rottweilers and Great Danes with a frequency of 0.68 and 0.72, respectively. In the rest of the large breeds, T allele frequencies ranged from 0.04 in the Bernese Mountain Dog to 0.22 in the Mastiff. All of the wolves were homozygous for the G allele. Detailed gene frequencies and average weights per breed are presented in Table 1.

It is noteworthy that a study by Carrier et al. (2005) analyzed the genetic basis for skeletal variation in dogs and reported a quantitative trait locus regulating size variation associated with microsatellite marker FH2017 on autosome 15 (CFA15: 37911229-37911504). They suggested canine IGF-1 (CFA15: 44226303-44226481) as the most likely candidate gene. The SOCS2 gene described in this paper is located on CFA15: 36795986-36796444 approximately 1 cM from marker FH2017. The proximity of the SOCS2 gene related to marker FH2017 and its role in the regulation of GH signaling make the canine SOCS2 a putative candidate gene for body size in dogs.

The present analysis characterized a new sequence variation (DQ415457:g.326G>T) producing an Asp127Tyr change in the highly conserved SH2 domain in the canine SOCS2 protein. The T allele was found primarily among European large dog breeds. The nature of the nucleotide change and the predicted effect on the protein together with the finding of a QTL related to body size in the same CFA15 region by other researchers suggest that the SOCS2 gene is a potential candidate gene for body size in dogs that should be examined in future association studies.

ACKNOWLEDGMENTS

Research supported by the National Research Initiative grant number 2005-35205-15453 from the USDA Cooperative State Research, Education and Extension Service.

REFERENCES

Bullock AN, Debreczeni JE, Edwards AM, Sundstrom M, et al. (2006). Crystal structure of the SOCS2-elongin C-elongin B complex defines a prototypical SOCS box ubiquitin ligase. *Proc. Natl. Acad. Sci. USA* 103: 7637-7642.

Carrier DR, Chase K and Lark KG (2005). Genetics of canid skeletal variation: size and shape of the pelvis. *Genome Res.* 15: 1825-1830.

Corva PM and Medrano JF (2000). Diet effects on weight gain and body composition in high growth (hg/hg) mice. *Physiol. Genomics* 3: 17-23.

Couronne O, Poliakov A, Bray N, Ishkhanov T, et al. (2003). Strategies and tools for whole-genome alignments. *Genome Res.* 13: 73-80.

Dey BR, Spence SL, Nissley P and Furlanetto RW (1998). Interaction of human suppressor of cytokine signaling (SOCS)-2 with the insulin-like growth factor-I receptor. *J. Biol. Chem.* 273: 24095-24101.

Galibert F, Andre C and Hitte C (2004). Dog as a mammalian genetic model. *Med. Sci.* 20: 761-766.

Greenhalgh CJ and Hilton DJ (2001). Negative regulation of cytokine signaling. *J. Leukocyte Biol.* 70: 348-

356.
Greenhalgh CJ and Alexander WS (2004). Suppressors of cytokine signalling and regulation of growth hormone action. *Growth Horm. IGF. Res.* 14: 200-206.
Hilton DJ, Richardson RT, Alexander WS, Viney EM, et al. (1998). Twenty proteins containing a C-terminal SOCS box form five structural classes. *Proc. Natl. Acad. Sci. USA* 95: 114-119.
Horvat S and Medrano JF (2001). Lack of Socs2 expression causes the high-growth phenotype in mice. *Genomics* 72: 209-212.
Kile BT, Nicola NA and Alexander WS (2001). Negative regulators of cytokine signaling. *Int. J. Hematol.* 73: 292-298.
Krebs DL and Hilton DJ (2001). SOCS proteins: negative regulators of cytokine signaling. *Stem Cells* 19: 378-387.
Lavens D, Montoye T, Piessevaux J, Zabeau L, et al. (2006). A complex interaction pattern of CIS and SOCS2 with the leptin receptor. *J. Cell Sci.* 119: 2214-2224.
Machida K and Mayer BJ (2005). The SH2 domain: versatile signaling module and pharmaceutical target. *Biochim. Biophys. Acta* 1747: 1-25.
Metcalf D, Greenhalgh CJ, Viney E, Willson TA, et al. (2000). Gigantism in mice lacking suppressor of cytokine signalling-2. *Nature* 405: 1069-1073.
Parker HG, Kim LV, Sutter NB, Carlson S, et al. (2004). Genetic structure of the purebred domestic dog. *Science* 304: 1160-1164.
Pawson T (2004). Specificity in signal transduction: from phosphotyrosine-SH2 domain interactions to complex cellular systems. *Cell* 116: 191-203.
Pawson T, Gish GD and Nash P (2001). SH2 domains, interaction modules and cellular wiring. *Trends Cell Biol.* 11: 504-511.
Rost B (1996). PHD: predicting one-dimensional protein structure by profile-based neural networks. *Methods Enzymol.* 266: 525-539.
Rousset F and Raymond M (1995). Testing heterozygote excess and deficiency. *Genetics* 140: 1413-1419.
Sutter NB and Ostrander EA (2004). Dog star rising: the canine genetic system. *Nat. Rev. Genet.* 5: 900-910.
Tan JC and Rabkin R (2005). Suppressors of cytokine signaling in health and disease. *Pediatr. Nephrol.* 20: 567-575.
Thompson JD, Higgins DG and Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic. Acids Res.* 22: 4673-4680.
Wong ML, Islas-Trejo A and Medrano JF (2002). Structural characterization of the mouse high growth deletion and discovery of a novel fusion transcript between suppressor of cytokine signaling-2 (Socs-2) and viral encoded semaphorin receptor (Plexin C1). *Gene* 299: 153-163.