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Homozygosity Mapping of Lethal Congenital Contractural Syndrome Type 2 (LCCS2) to a 6 cM Interval on Chromosome 12q13

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We have recently described a novel autosomal recessive disorder, lethal congenital contractural syndrome type 2 (LCCS2) (OMIM 607598), in a large Israeli Bedouin kindred. The phenotype, which is lethal in the neonatal period, is distinguished by the presence of a markedly distended urinary bladder. Association of LCCS2 to the known loci associated with arthogryposis was excluded. In the present study, we set out to determine the genetic locus harboring the gene defective in this disease. We performed genome-wide linkage analysis, demonstrating linkage to a ~6 cM (corresponding to ~7.2 Mb) homozygosity region on chromosome 12q13 between markers D12S1604 and D12S83. Based on recombination events, the interval harboring the disease-associated locus was further narrowed to a region spanning ~6 cM (~6.4 Mb) between D12S325 and D12S1072. Linkage of LCCS2 to that locus was established, with two significant maximum peaks at markers D12S1604 ($Z_{max} = 10.56$ at $\theta = 0.01$) and D12S1700 ($Z_{max} = 9.23$ at $\theta = 0.00$).

KEY WORDS: Arthrogryposis; LCCS2; linkage analysis; gene; contractural syndrome

INTRODUCTION

Lethal congenital contractural syndrome type 2 (LCCS2) (OMIM 607598) is an autosomal recessive syndrome prevalent

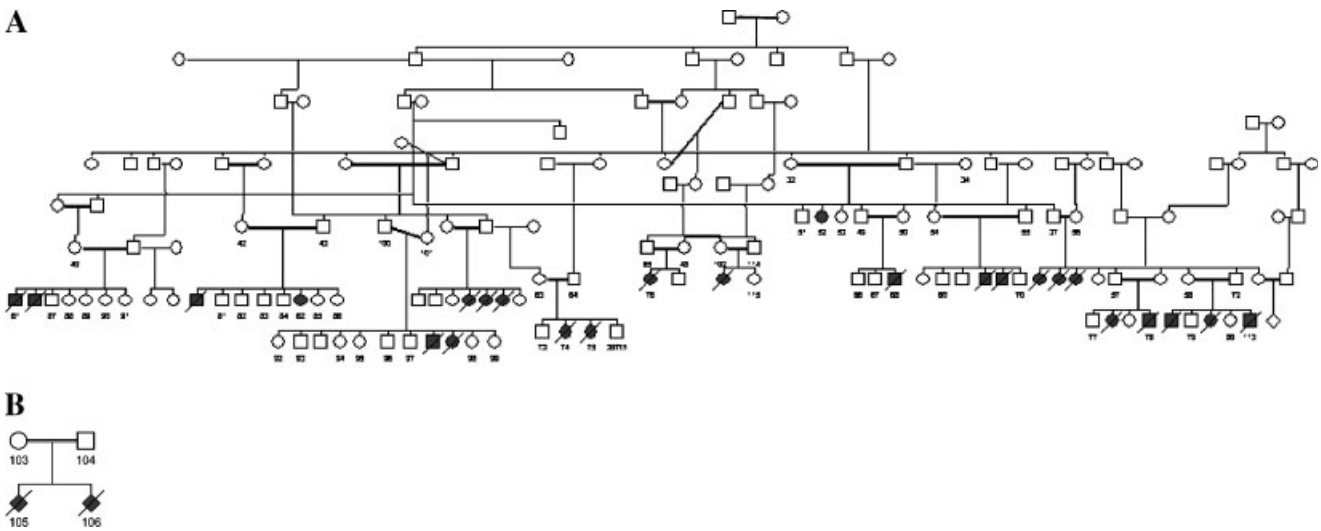

Fig. 1. Pedigrees of Israeli-Bedouin kindreds affected with LCCS2. Solid and open symbols represent affected and unaffected individuals, respectively. The numbers denote individuals whose DNA samples were analyzed. **A**: Family 1. **B**: Family 2.

Daniella Landau and Esther Manor contributed equally to this study.

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in a large inbred Israeli-Bedouin kindred, with 23 affected individuals described to date [Landau et al., 2003]. The phenotype is similar to the Finnish type lethal congenital contractural syndrome (LCCS) (OMIM 253310). However, unlike LCCS patients, individuals affected with LCCS2 have a markedly distended urinary bladder as well as other urinary abnormalities. LCCS2 patients, in contrast with those affected with LCCS, have also additional craniofacial/ocular findings, yet do not present with hydrops, multiple pterygia and fractures, that are characteristic of LCCS. The genetic defect underlying LCCS has been mapped to chromosome 9q34 [Makela-Bengs et al., 1998].

Another autosomal recessive contractural syndrome, arthrogryposis multiplex congenita, neurogenic type (AMCN) (OMIM 208100) [Shohat et al., 1997], has been described in a large Israeli-Arab inbred kindred. Individuals affected with this nonlethal disorder have non-progressive joint contractures that involve more than one part of the body, presenting with arthrogryposis of the spinal neuropathic type. The disease locus for AMCN in this kindred has been assigned to chromosome 5q35 [Shohat et al., 1997; Tanamy et al., 2001]. The specific gene defects underlying LCCS and AMCN are yet to be elucidated. No genetic loci have been assigned so far to any of the other forms of autosomal recessive contractural syndromes.

Linkage to the 9q34 and 5q35 loci in the inbred LCCS2 Israeli Bedouin kindred described by Landau et al. [2003] was excluded. Assuming that a founder effect underlies the genetic

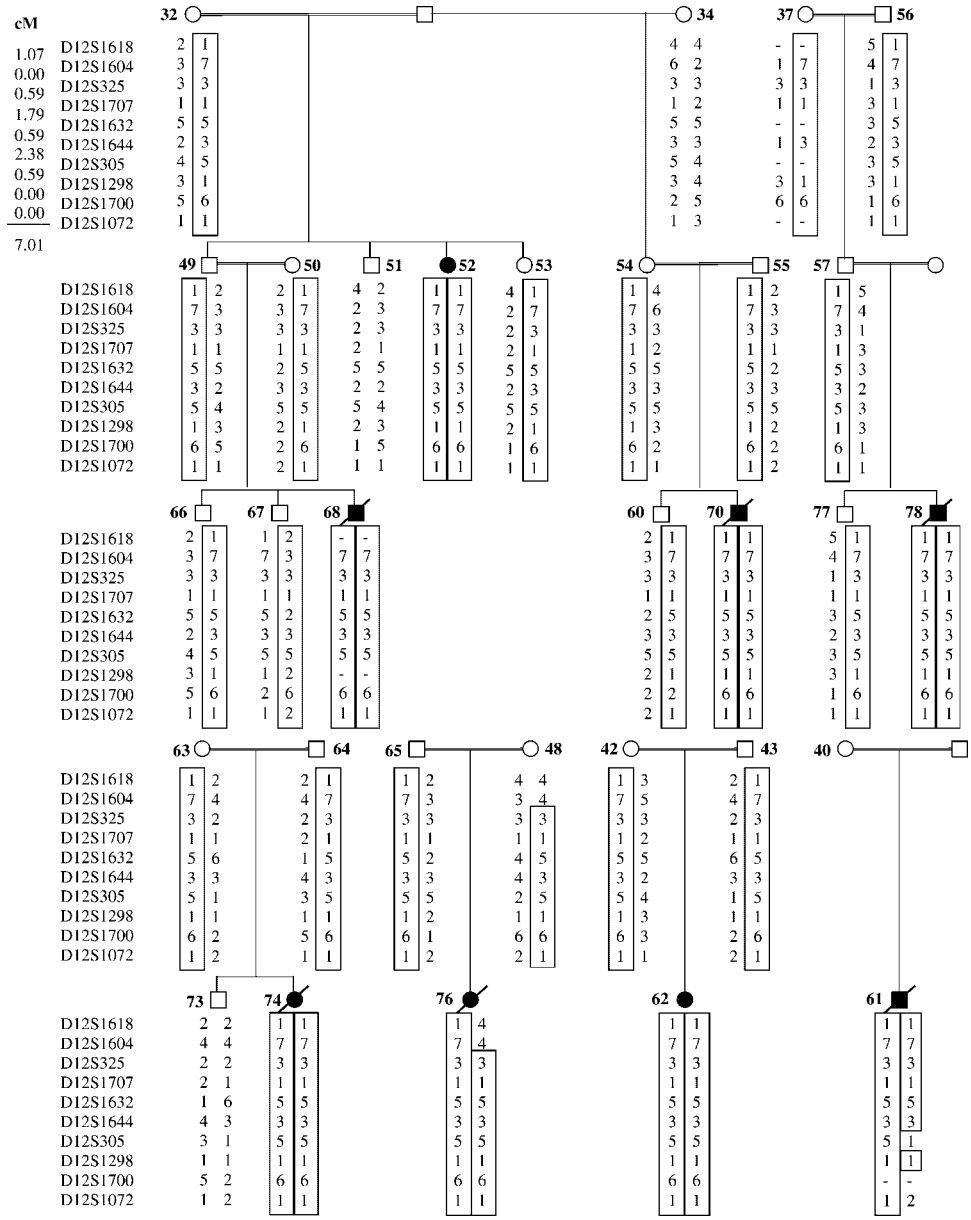

Fig. 2. Partial pedigree of the large Israeli-Bedouin kindred. The haplotype showing the homozygosity region is boxed. Physical distances between the markers are shown.

defect causing LCCS2 in this extended family, we performed genome-wide homozygosity mapping to identify the disease locus for this severe disorder.

MATERIALS AND METHODS

Patients

The pedigree of the extended family studied is shown in Figure 1A. A smaller pedigree with an identical phenotype can be seen in Figure 1B. Detailed clinical characterization of the affected individuals is given in Landau et al. [2003]. In short, the phenotype is similar to the Finnish type lethal congenital contractural syndrome, but is distinguished by the presence of a markedly distended urinary bladder. Ten affected individuals with a clinical diagnosis of LCCS2, and 44 unaffected individuals of the same consanguineous Bedouin tribe, were subjected to genetic analysis after submitting informed consent. In addition, DNA samples of members of another remotely related family, in which two offsprings of a consanguineous marriage were affected with the same disorder (Fig. 1B), were analyzed.

Linkage and Haplotype Analysis

Genomic DNA was extracted from whole blood using standard procedures. Genome-wide linkage analysis was undertaken on DNA samples, using the ABI PRISM Linkage Mapping Set MD10 (Applied Biosystems, Weiterstadt, Germany). Four hundred fluorescent-labeled microsatellite markers, spaced at approximately 10 cM intervals, were amplified from genomic DNA by PCR, according to the manufacturers' instructions. Products were separated by electrophoresis on an ABI PRISM 377 DNA Sequencer (Applied Biosystems), and analyzed using Gene-Scan software.

Fine-mapping was carried out using polymorphic markers (listed in the "Results" section) as follows: PCR products were separated on a 6% polyacrylamide gel, and visualized by silver-staining [Harel et al., 2003]. Haplotypes were manually constructed and analyzed.

Statistical analysis was done using an autosomal recessive disease model assuming complete penetrance in both sexes. Because allele frequencies of markers are unknown in this particular study, we calculated the LOD score by assuming equal frequencies.

Two-point linkage analysis on parts of the kindred was computed by means of the MLINK option of the FASTLINK package [Cottingham et al., 1993; Schaffer et al., 1994]. Extended two-point linkage analysis on the entire kindred was then performed using another, recently published software [Fishelson and Geiger, 2002], called SUPERLINK.

RESULTS

Genome-Wide linkage Analysis

As the disease locus for LCCS2 could not be assigned to any of the genetic loci known to be associated with autosomal recessive arthrogryposis, we went on to study seven affected individuals by genome-wide linkage analysis. Non-informative regions were excluded using additional adjacent markers. We identified only a single locus in which most affected individuals demonstrated increased homozygosity; the locus, spanning ~9 cm (corresponding to 8.3 Mb) on chromosome 12q13, resides between marker D12S368 (homozygosity in seven of seven affected individuals) and D12S83 (homozygosity in six of seven affected individuals). While all obligatory carriers tested were shown to be heterozygous at D12S83, marker D12S368 was non informative in this family (results not shown).

The interval of homozygosity was defined by testing DNA samples of 10 affected and 44 non affected members of the extended family, using additional markers in and adjacent to the interval between D12S368 and D12S83 (D12S2196, D12S1635, D12S1618, D12S1604, D12S325, D12S1724, D12S1707, D12S1632, D12S1644, D12S305, D12S355, D12S1298, D12S1700, D12S1056, D12S1072, and D12S1291). As seen in Figure 2, Figure 3 and in Table I, a 5.94 cM interval of homozygosity common to all affected individuals in family 1 lies between markers D12S1604 and D121072, with a single affected individual being heterozygous at marker D12S305 in the midst of that interval. Two additional LCCS patients, offspring of consanguineous parents in a remotely related Bedouin family, demonstrated homozygosity of the same haplotype. As shown in Table I, two affected individuals in that second family presented with a crossing over event at D12S325, narrowing down the upper limit of the homozygosity interval to D12S325. Thus, the region of homozygosity common to affected individuals in both families was narrowed down to 5.94 cM, corresponding to 6.4 Mb (Fig. 3).

Linkage was demonstrated first by two-point analysis using the MLINK option of the FASTLINK package, with two maximum peaks of LOD score [Z_{max}] = 3.41 (recombination fraction [θ] = 0.00) near marker D12S1604 and LOD score [Z_{max}] = 3.58 ([θ] = 0.00) near marker D12S1700 (Table IIA). Using SUPERLINK, we computed two-point analysis of the entire complex inbred kindred in unity, establishing linkage with two significant maximum peaks of LOD score [Z_{max}] =

TABLE I. Compilation Table Showing Genotypes of All Affected Individuals

	52	61	62	68	70	74	76	78	105	106	113	118
D12S1618	1 1	1 1	1 1	1 1	1 1	1 1	1 4	1 1	- -	4 4	- -	1 1
D12S1604	7 7	7 7	7 7	7 7	7 7	7 7	7 4	7 7	5 6	5 6	7 7	7 7
D12S325	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	2 4	2 4	3 3	3 3
D12S1707	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1
D12S1632	5 5	5 5	5 5	5 5	5 5	5 5	5 5	5 5	5 5	5 5	5 5	5 5
D12S1644	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3
D12S305	5 5	1 5	5 5	5 5	5 5	5 5	5 5	5 5	5 5	5 5	5 5	5 5
D12S355	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3
D12S1298	1 1	1 1	1 1	- -	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1
D12S1700	6 6	- -	6 6	6 6	6 6	6 6	6 6	6 6	6 6	6 6	6 6	6 6
D12S1056	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2
D12S1072	1 1	2 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1
D12S83	2 2	3 2	2 2	- -	2 2	- -	2 2	- -	- -	- -	- -	- -
D12S1291	1 1	6 -	1 1	1 1	- -	6 6	1 1	1 1	6 6	6 6	- -	1 6

Patients 52, 61, 62, 68, 70, 74, 76, 78, 113, and 118 are members of family 1 (Fig. 1A). Individuals 105 and 106 (Fig. 1B) are members of another remotely related family affected with the same disorder. The shaded areas indicate the region of homozygosity defined by the various markers.

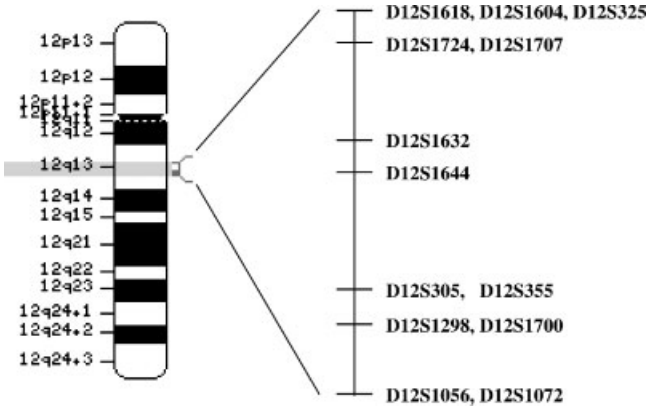

Fig. 3. Chromosomal map of the chromosome 12q13 region showing the LCCS2 locus.

10.56 ($[\theta] = 0.01$) near marker D12S1604 and LOD score $[Z_{max}] = 9.23$ ($[\theta] = 0.00$) near marker D12S1700 (Table IIB).

DISCUSSION

Consanguinity is prevalent in the Bedouin population of the Negev region of Israel, resulting in a very high prevalence of various autosomal recessive disorders. The unique combination of an inbred and isolated society with a high birth rate and well-ascertained clinical phenotypes is a solid basis for the identification of disease-related genes through genetic linkage analysis. The severity of some of the phenotypes urges the need for identification of disease-related gene loci to allow carrier detection and prenatal testing in affected families.

Homozygosity mapping is a powerful technique that is used to map genes underlying recessive diseases, and is very effective in studies of large consanguineous pedigrees [Sheffield et al., 1995]. The technique is based on the assumption that the disease is the result of a founder effect, i.e., homozygous inheritance of a recessive mutation from a common ancestor. In such a case, it is expected that patients affected with autosomal recessive diseases resulting from consanguineous marriages should have increased homozygosity in markers adjacent to the disease gene.

We have reported here the assignment of the LCCS2 locus to a restricted region on chromosome 12q13. We analyzed 400 polymorphic markers and identified a single chromosomal segment that was shared by all of the affected individuals in a large extended family. In addition, two affected individuals, offspring of a consanguineous marriage in a second, remotely related family, were found to be homozygous for the same haplotype.

Based on crossing over events in affected individuals of both families, we narrowed down the LCCS2 locus interval to a physical distance region of ~6.4 Mb on chromosome 12q13 between markers D12S325 and D12S1072.

TABLE II. Results of Two-Point LOD Score Analysis for 15 Markers on Chromosome 12q13

Distance (cM)	Marker	Recombination fraction 0.00	0.01	0.05	0.10	0.20	0.30	0.40	θ	Z_{max}
A										
	D12S2196	−1.11	0.29	0.75	0.75	0.48	0.20	0.04	0.05	0.75
4.27	D12S1618	−3.92	0.70	1.08	1.00	0.62	0.30	0.11	0.05	1.08
1.07	D12S1604	3.41	3.32	2.95	2.50	1.60	0.81	0.25	0.00	3.41
0.00	D12S325	1.67	1.62	1.44	1.21	0.78	0.42	0.16	0.00	1.67
0.59	D12S1724	0.79	0.77	0.66	0.53	0.30	0.11	0.02	0.00	0.79
0.00	D12S1707	0.60	0.58	0.52	0.43	0.27	0.13	0.03	0.00	0.60
1.79	D12S1632	1.80	1.76	1.58	1.36	0.93	0.54	0.22	0.00	1.80
0.59	D12S1644	2.58	2.52	2.28	1.98	1.35	0.76	0.29	0.00	2.58
2.38	D12S305	1.89	1.86	1.71	1.48	0.99	0.54	0.21	0.00	1.89
0.00	D12S355	3.33	3.24	2.88	2.42	1.53	0.75	0.22	0.00	3.33
0.59	D12S1298	2.91	2.82	2.46	2.02	1.18	0.48	0.09	0.00	2.91
0.00	D12S1700	3.58	3.49	3.12	2.64	1.72	0.91	0.34	0.00	3.58
0.00	D12S1056	2.75	2.67	2.39	2.03	1.33	0.69	0.22	0.00	2.75
0.00	D12S1072	1.05	1.02	0.89	0.74	0.45	0.21	0.06	0.00	1.05
2.97	D12S1291	0.49	0.59	1.08	1.10	0.80	0.41	0.13	0.10	1.10
B										
	D12S2196	−0.93	1.89	2.76	2.63	1.72	0.75	0.15	0.05	2.76
4.27	D12S1618	−5.29	3.58	4.71	4.49	3.26	1.91	0.81	0.05	4.71
1.07	D12S1604	$-\infty$	10.56	10.03	8.85	6.22	3.61	1.43	0.01	10.56
0.00	D12S325	4.86	4.74	4.27	3.66	2.48	1.40	0.55	0.0	4.86
0.59	D12S1724	3.75	3.63	3.21	2.70	1.75	0.96	0.37	0.0	3.75
0.00	D12S1707	3.58	3.49	3.14	2.69	1.80	1.00	0.38	0.0	3.58
1.79	D12S1632	6.55	6.39	5.79	5.01	3.47	2.06	0.88	0.0	6.55
0.59	D12S1644	7.56	7.39	6.71	5.82	3.99	2.27	0.86	0.0	7.56
2.38	D12S305	2.98	5.16	5.23	4.66	3.13	1.66	0.60	0.05	5.23
0.00	D12S355	7.45	7.27	6.56	5.67	3.82	2.10	0.78	0.0	7.45
0.59	D12S1298	8.20	8.19	7.42	6.41	4.36	2.45	0.93	0.0	8.20
0.00	D12S1700	9.23	9.01	8.14	7.03	4.81	2.71	1.04	0.0	9.23
0.00	D12S1056	6.73	6.57	5.95	5.13	3.46	1.93	0.72	0.0	6.73
0.00	D12S1072	0.02	2.13	2.39	2.17	1.50	0.87	0.37	0.05	2.39
2.97	D12S1291	$-\infty$	1.59	3.47	3.62	2.75	1.56	0.57	0.1	3.47

The distances given are those between every two adjacent markers.
A: Analysis using the MLINK option of the FASTLINK package.
B: Analysis using SUPERLINK.

Using SUPERLINK, we computed the entire kindred of 115 individuals in unity, taking into consideration all the complexities of extreme inbreeding. This enabled us to refine the LOD score calculations reaching two peaks of LOD score $[Z_{max}] = 10.56$ ($[\theta] = 0.01$) near marker D12S1604, and LOD score $[Z_{max}] = 9.23$ ($[\theta] = 0.00$) near marker D12S1700. Although the two peaks are only ~6 cM apart, one of the affected individuals presented with heterozygosity at D12S305 in the midst of that region. As the DNA samples of the parents of this individual were not available to us for analysis, we do not know whether this heterozygosity was inherited from earlier on in that segment of the family, or whether it represents a novel event occurring in the meiosis producing this patient. We also do not know whether this heterozygosity at D12S305 represents a case of a (somewhat unlikely) novel mutation, or a recombination event. The possibility of D12S305 being an unstable microsatellite marker cannot be ruled out, although it is unlikely in view of this marker being stable throughout the rest of the pedigree. The heterozygosity at D12S305 also raises the question of which of the two parts of the defined ~6 cM region harbors the disease related gene.

About 170 genes reside within the defined 6.4 Mb interval on chromosome 12q13, some of which are known to be expressed in neural tissues. The results of this linkage study allow for carrier detection and prenatal testing for this devastating disease in a large Bedouin tribe. Further analysis and careful selection of candidate genes within the defined interval will hopefully allow for the identification of the specific gene defect, and will thus enable better understanding of the disease mechanism that leads to lethal congenital contractural syndrome type 2, and perhaps to other forms of congenital contractural syndromes.

ELECTRONIC-DATABASE INFORMATION

URLs for data presented herein are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation http://research.marshfieldclinic.org/genetics/ (for marker position), National Center for Biotechnology Information (NCBI) http://www.ncbi.nlm.nih.gov/mapview/ (for marker and gene position, as well as sequence and protein function information), Online Mendelian Inheritance in Man (OMIM) http://www.ncbi.nlm.nih.gov/Omim/ (for LCCS1, LCCS2, AMCN and *KIF5A*), UCSC Genome Bioinformatics http://genome.ucsc.edu/ (for marker and gene position and sequence information), The Laboratory of Computational Biology, Technion http://dogbert.cs.technion.ac.il/superlink/ (for statistical analysis), Whitehead Institute http://www.genome.wi.mit. edu/cgi-bin/primer/primer3.cgi. (for primers designed).

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