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SHORT REPORT

Support of the histaminergic hypothesis in Tourette Syndrome: association of the histamine decarboxylase gene in a large sample of families

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ABSTRACT

Background Gilles de la Tourette Syndrome is a neurodevelopmental disorder that is caused by the interaction of environment with a complex genetic background. The genetic etiology of the disorder remains, so far, elusive, although multiple promising leads have been recently reported. The recent implication of the histamine decarboxylase (*HDC*) gene, the key enzyme in histamine production, raises the intriguing hypothesis of a possible role of histaminergic dysfunction leading to TS onset.

Methods Following up on the finding of a nonsense mutation in a single family with TS, we investigated variation across the *HDC* gene for association with TS. As a result of a collaborative international effort, we studied a large sample of 520 nuclear families originating from seven European populations (Greek, Hungarian, Italian, Polish, German, Albanian, Spanish) as well as a sample collected in Canada.

Results and Conclusions Interrogating 12 tagging SNPs (tSNP) across the *HDC* region, we find strong over-transmission of alleles at two SNPs (rs854150 and rs1894236) in the complete sample, as well as a statistically significant associated haplotypes. Analysis of individual populations also reveals signals of association in the Canadian, German and Italian samples. Our results provide strong support for the histaminergic hypothesis in TS etiology and point to a possible role of histamine pathways in neuronal development.

INTRODUCTION

Gilles de la Tourette Syndrome (TS) is a multifactorial neurodevelopmental disorder resulting from the complex interaction of environment with genetic backround. TS is characterised by the appearance of multiple motor and vocal tics, and high comorbidity rates with other neuropsychiatric disorders of childhood such as obsessive compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD) and autism, suggesting the possibility of a common aetiological background. Due to lack of education of health professionals and the general public, TS remains poorly understood and severely underdiagnosed in many countries despite the fact that recent investigations have reported a prevalence of 0.4–1% in populations of European origin.[1] Results from large-scale studies and collaborative efforts have only very recently started to become available (eg, refs. 2–4) and the genetic basis of the disorder remains, so far, elusive, although multiple chromosomal regions have been implicated, providing several promising leads that warrant further investigation.[5]

Tics are thought to result from dysfunctions in cortical and subcortical regions that are involved in habit formation, including the basal ganglia and related cortical and thalamic structures.[6] TS has traditionally been viewed as a disorder of dopaminergic neurotransmission; however, other transmitters and neuromodulators have also been implicated.[6] In fact, the recent implication of the L-histidine decarboxylase (*HDC*) gene in TS aetiology has raised the intriguing hypothesis of the involvement of histaminergic neural pathways in the onset of the disorder.[7] *HDC* is highly conserved throughout different species and catalyses the oxidative decarboxylation of histidine to histamine.[8] Ercan–Sencicek *et al*[7] studied a family with one father and eight of his children affected with TS, and found a linkage signal on chromosome 15. Sequencing of all genes within the linked interval led to the discovery of a single rare coding mutation, a premature termination codon (p. W317*, c.951G>A) in exon 9 of the *HDC* gene. The mutation could not be found in any of the 3000 control individuals of Western European origin who were screened. At the same time, resequencing of the coding region of *HDC* in 720 patients with TS and 360 controls revealed no additional nonsense variants, demonstrating the fact that the nonsense mutation identified in the index family is extremely rare.

Since the original study that implicated *HDC* in TS aetiology, Lei *et al*[9] screened the *HDC* gene for exonic mutations in 100 Chinese Han patients with TS, and could only find three variants, not predicted to result in amino acid changes: a C>T intronic transition (IVS1 +52C>T), which did not affect the splicing site, a synonymous C>A transition (c.426C>A) in exon 4 and a synonymous G>A transition (c.1743G>A) in exon 12.[9] The first genomewide association study for TS only produced a weak association signal close to the *HDC*

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gene (p value of 0.02 with rs7166052).[3] However, a recent genomewide scan for de novo or transmitted rare CNVs in TS found enrichment of genes within histamine receptor signalling pathways.[10] Furthermore, a genomewide study of 95 French Canadian trios with familial history of TS[11] showed association to a chromosome 15 microsatellite marker (D15S1016) that lies within the same interval found to be linked with TS in the original study that implicated the *HDC* gene in TS aetiology.[7]

Following up on the novel hypothesis of the involvement of *HDC* and histaminergic neural pathways in TS aetiology, we investigated the possible association of variation across the *HDC* gene in a large sample of families originating from Canada as well as multiple countries in Europe. We find significant association to the *HDC* gene in our sample, providing strong support for the role of histamine in the genesis and mediation of tics, and pointing to new lines of research in this area.

MATERIAL AND METHODS

Genetic association study samples

Five independently collected samples of TS nuclear families were analysed (total of 520 families). Details of age of onset per sample, sex distribution as well as comorbid OCD and ADHD are shown in online supplementary table S1. The Tourette Syndrome Genetics—Southern and Eastern Europe Initiative sample included European-descent families of Polish (36 trios), Italian (50 trios), Hungarian (84 trios), Greek (10 trios) and Albanian origin (6 trios). Assessment was performed by on-site clinicians using the tools provided by the TS Association International Consortium for Genetics.[2] TS was ascertained according to DSM-IV-TR criteria for Italy, Hungary, Albania and Greece, and DSM-IV for Poland. Our study also included two independent samples of German descent. The first was the German Collaborative TS Research Group sample (96 trios), collected using DSM-III-R criteria.[12 13] Our second sample of German ancestry was collected in Munich (69 trios), using DSM-IV-TR criteria. Our fourth European sample was of Spanish ancestry (19 families), collected using DSM-IV-TR criteria. Finally, we also analysed a large sample of TS trios collected in Canada (150 families), using DSM-III-R criteria. Differences between DSM-III-R, DSM-IV and DSM-IV-TR are minimal; the upper age limit of onset is 18 in DSM-IV (and DSM-IV-TR) and 21 in DSM-III-R, and the 'marked distress' criterion, possibly pointing to more severe cases, only appears in DSM-IV (applied only to the Polish families).[14] For all samples, collection was approved by local Ethics Boards and informed consent was taken from all participating individuals or their parents.

Selection of single nucleotide polymorphisms, genotyping and statistical analysis

Using the HapMap CEPH European population as reference, we selected tagging single nucleotide polymorphisms (tSNPs) in order to capture variation for a 40 kb region including the *HDC* gene. A total of 12 tSNPs were selected (see online supplementary table S2) capturing variation at an additional nine SNPs with a mean r^2 of 0.968. All selected SNPs are intronic. Genotyping was performed using the KASP genotyping method, a competitive allele-specific assay based on Fluorescent Resonance Energy Transfer from LGC Genomics and Kbioscience (http://www.lgcgenomics.com). The average genotyping success was greater than 99%. The linkage disequilibrium (LD) structure of each gene in the different studied populations was analysed using Haploview.[15] In order to test for association of the studied SNPs with the TS phenotype, the transmission test for linkage disequilibrium (TDT) was performed, as also implemented in Haploview.[15] We report here association results on single SNPs as well as SNP haplotypes of haplotype blocks defined with the Gabriel *et al*[16] criteria (tests with one degree of freedom, as implemented by Haploview). In order to correct for multiple comparisons, 1000 permutations were performed (using Haploview) and the adjusted p values are also reported here (table 1).

RESULTS

Genotyping results for all SNPs conformed to Hardy–Weinberg equilibrium proportions and Mendelian transmission within families. Rare allele frequencies for each analysed sample are shown in online supplementary figure S1. Since the transmission disequilibrium test (TDT) is robust to population stratification, we were able to perform analysis for the complete sample that was available to us, as well as families within each individual population. Single SNP analysis for association with the TS phenotype in the complete sample (520 families) produced

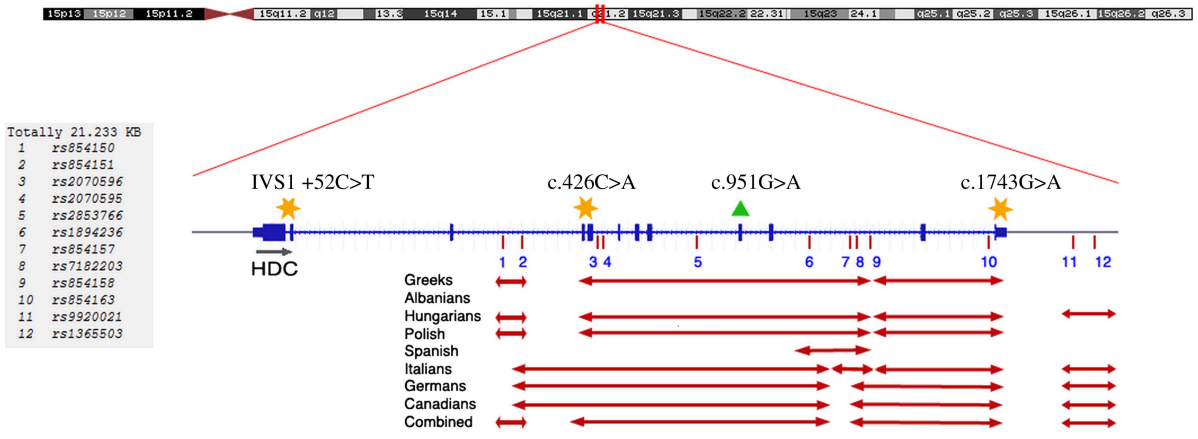

Figure 1 Linkage disequilibrium structure of studied region covering the L-histidine decarboxylase (*HDC*) gene (chromosome 15: 48 316 000–48 356 000 NCBI build 36). Unrelated individuals from a sample of 520 nuclear families were analysed using Haploview and haplotype blocks were defined using the Gabriel *et al*[16] criteria and visualised with the software Haplot. *HDC* variants found in individuals with Gilles de la Tourette Syndrome (TS) in previous studies are also shown. The triangle indicates the position of the originally described mutation that led to a premature termination codon (p. W317*, c.951G>A) in a family with TS,[7] and the stars indicate the position of variants described in Chinese Han patients with TS (not predicted to result in amino acid changes).[9] Access the article online to view this figure in colour.

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Table 1 Transmission test for linkage disequilibrium for L-histidine decarboxylase variation tSNPs and haplotypes

Rs#	Over-transmitted allele	T:U	χ^2	p Value	Permutation p value
rs854150	**G**	**284 : 220**	**8.127**	**0.0044**	**0.0290**
rs854151	G	205 : 190	0.57	0.4504	
rs2070596	T	192 : 154	4.173	0.0411	0.2640
rs2070595	G	232 : 224	0.14	0.7079	
rs2853766	A	215 : 174	4.321	0.0376	0.2370
rs1894236	**C**	**201 : 143**	**9.779**	**0.0018**	**0.0150**
rs854157	T	277 : 245	1.962	0.1613	
rs7182203	G	217 : 215	0.009	0.9233	
rs854158	G	246 : 216	1.948	0.1628	
rs854163	A	223 : 188	2.981	0.0843	
rs9920021	A	227 : 198	1.979	0.1595	
rs1365503	A	249 : 223	1.432	0.2314	
Haplotype	**Frequency**	**T : U**	**χ2**	**p Value**	**Permutation p value**
Block 1					
CA	**0.620**	**224.5 : 286.7**	**7.56**	**0.006**	**0.0140**
GG	0.268	227.2 : 195.0	2.465	0.1164	
GA	**0.107**	**123.3 : 87.1**	**6.212**	**0.0127**	**0.0440**
Block 2					
TGGC	0.387	243.0 : 273.2	1.761	0.1845	
TGAC	0.248	218.0 : 173.0	5.179	0.0229	0.0790
ATGT	**0.216**	**151.0 : 198.9**	**6.561**	**0.0104**	**0.0420**
TTGC	0.140	147.5 : 122.3	2.346	0.1256	

The studied sample consists of 520 TS nuclear families of Greek, Albanian, Italian, Hungarian, Polish, Spanish, German and Canadian origin. The permutation p value was computed after 1000 permutations of the datasets. Results for the associated haplotype blocks 1 and 2 are shown (figure 1, block 1:SNPs rs854150-rs854151; block 2:SNPs rs2070596-rs2070595-rs2853766-rs1894236).
Statistically significant signals (permutation p-value lower than 0.05) are shown in bold.
T:U, transmitted–untransmitted; tSNP, tagging SNP.

strong results of overtransmission of alleles for SNP rs854150 and rs1894236 (table 1). These results remained statistically significant after performing 1000 permutations of the data. Using the Gabriel *et al*[16] criteria for the combined sample, four haplotype blocks were defined across the studied region. The first of the two TS-associated SNPs in our analysis (rs854150) resides within a two-SNP haplotype block, which is also significantly associated with the TS phenotype, both for the protective and the susceptibility alleles (table 1). Significant association is also found with the haplotype block that carries the second TS-associated SNP in our analysis (rs1894236). This is a five-SNP haplotype spanning 9.2 Kb (SNPs rs854151, rs2070596, rs2070595, rs2853766 and rs1894236). The rs1894236 allele that is overtransmitted to TS patients is found on four different haplotypes. On the other hand, the undertransmitted, and thus, protective allele, is only found on a single haplotype at a frequency of 0.21%, which is also significantly undertransmitted to TS patients (permutation p=0.04) (table 1).

Individual population analysis also revealed interesting results that further support a possible role for *HDC* in TS aetiology (see online supplementary tables S3 and S4). In our combined sample of German origin (our largest individual population sample with 165 trios), SNP rs854150 retained a trend of overtransmission to TS patients (uncorrected p value of 0.04), which, however, did not withstand permutation testing. Nevertheless, an additional association signal was found, including the haplotype block of the two SNPs most distal to our studied region (rs9920021 and rs1365503) with a permutation p value of 0.048. Interestingly, in our Canadian sample (150 families), these two same SNPs produced the strongest signals of association with permutation p values of 0.001 for rs1365503 and 0.021 for rs9920021. SNP rs1894236 was also significantly associated with TS in the Canadian sample. In the Italian population (50 trios), SNP rs7182203 was associated with the TS phenotype (permutation p value of 0.043), as well as a three-site haplotype including this SNP as well as rs854158 and rs854163. Individual analysis of the remaining samples did not produce any remarkable results, which could be due to small individual sample size. We should also note that analysis of the Canadian, German and Italian samples revealed an identical haplotype block structure, while a slightly different structure was observed for the remaining populations that we analysed.

DISCUSSION

Following up on the recent evidence about the involvement of the *HDC* gene and histaminergic neural pathways in the aetiology of TS, we studied variation across *HDC* for association with TS in a large sample of 520 nuclear families from seven European populations (Greece, Hungary, Poland, Italy, Albania, Germany and Spain) as well as a sample collected in Canada. We employed a tSNPs approach and identified strong overtransmission of alleles at two SNPs (rs854150 and rs1894236). Strong association was also found with a five-SNP haplotype carrying the protective rs1894236 allele, as well as two-SNP haplotypes carrying rs854150. Both SNPs that were found to be associated with TS in our study reside in intronic regions of *HDC*. However, it is worth noting that rs1894236 lies under one of the Encyclopedia of DNA Elements (ENCODE) project peaks for an enhancer-associated histone mark (UCSC genome browser NCBI36/hg18, http://genome.ucsc.edu). The ENCODE project has systematically mapped regions of transcription, transcription factor association, chromatin structure and histone modification, and histone marks are specific chemical modifications in histone sequence which may, for instance, enhance gene

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transcription.[17] Thus, this particular region may be directly involved in regulation of transcription of the *HDC* gene. Individual population analysis produced additional signals of positive association. Thus, our results strongly support the histaminergic hypothesis for TS development.

Histamine is an evolutionary conserved signalling molecule that plays a central role in gastric acid secretion, innate and acquired immunity and immunomodulation, bronchoconstriction, vasodilation and neurotransmission. The neuronal histaminergic system is involved in a number of basic physiological functions, such as circadian rhythmicity, energy metabolism, neuroendocrine homeostasis, stress, sensory and motor functions, cognition, attention, learning and memory.[18] Neuronal histamine is exclusively synthesised in the tuberomamillary nucleus of the hypothalamus (TMN), while histamine-positive fibres have been described projecting to cortex, hippocampus, basal ganglia, thalamus and virtually all other regions of the central nervous system.[18] HDC is the key enzyme for histamine production from histidine. In the brain, its mRNA is expressed exclusively in the posterior hypothalamus. The specific regulation of *HDC* gene expression in the brain is not understood. However, neuroactive peptides and steroids are among the factors that control *HDC* transcription and degradation in various tissues and contexts (eg, oxidative stress).[8 18] Recently, diurnal variation in the levels of *HDC* mRNA in the TMN has been observed in agreement with a role of neuronal histamine in regulating day–night patterns.[19]

Histamine binds to and acts through four known G protein–coupled receptors (H1 through H4) and a polyamine binding site on glutamatergic *N*-Methyl-D-Aspartate (NMDA) receptors.[18] High densities of H2R and H3R are found in the basal ganglia,[18 20] especially on the principal neurons of the striatum, the GABAergic medium spiny neurons (MSN).[18 21] Furthermore, H3R mRNAs in the cortex and in the substantia nigra pars compacta indicate the presence of H3 heteroreceptors on the major inputs to the striatum.[20] Thus, histaminergic innervations of anatomical structures that have been related to TS has been confirmed. Furthermore, H3R regulates a variety of neurotransmitters, including dopamine and serotonin. On the other hand, *Hdc*−/− deficient mice have several traits relevant to features of TS and have shown decreased brain histamine and increased sensitivity to stereotypic behaviours upon administration of dopamine agonists.[22] Such stimulant-induced movements, including rearing, sniffing and biting have previously been proposed as a model of human tics.[23]

So far, the histaminergic system has not received as much attention as other monoaminergic systems of the brain. Classically established as a 'peripherally' important mediator of inflammation, the importance of histamine in neurotransmission and its role in neuropsychiatric disorders are only recently starting to be appreciated. For instance, it has been found severely affected in age-related neurodegenerative diseases such as Parkinson disease (PD) and Alzheimer disease (AD), while histamine receptors are becoming a prime target for pharmaceutical research and development and a number of agents have entered into clinical assessment as cognition enhancers for ADHD, schizophrenia, AD and PD.[24] Adding to the multiple facets of neural histamine, our findings further support the implication of histaminergic dysfunction in TS aetiology, providing new insight. An interesting next step could be the functional characterisation of RNA transcripts in samples that carry the associated *HDC* genetic variants in order to help elucidate their role and clarify the possible involvement of histamine pathways in neurodevelopmental disorders.

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Contributors IK performed experiments and wrote the paper. SD contributed samples, participated in the design of experiments and analysis and in manuscript preparation. PS contributed samples, participated in the design of experiments and analysis and in manuscript preparation. ZT contributed samples, participated in the design of experiments and analysis and in manuscript preparation. RR contributed samples, participated in the design of experiments and analysis and in manuscript preparation. TW contributed samples, participated in the design of experiments and analysis and in manuscript preparation. MM contributed samples, participated in the design of experiments and analysis and in manuscript preparation. JH contributed samples, participated in the design of experiments and analysis and in manuscript preparation. MMN contributed samples, participated in the design of experiments and analysis and in manuscript preparation. GL contributed samples, participated in the design of experiments and analysis and in manuscript preparation. LF contributed samples, participated in the design of experiments and analysis and in manuscript preparation. PN contributed samples, participated in the design of experiments and analysis and in manuscript preparation. US contributed samples, participated in the design of experiments and analysis and in manuscript preparation. ZA performed experiments and analysis. Vassilios Stathias performed experiments and analysis. CA contributed samples, participated in the design of experiments and analysis and in manuscript preparation. VT contributed samples, participated in the design of experiments and analysis and in manuscript preparation. AK contributed samples, participated in the design of experiments and analysis and in manuscript preparation. CB contributed samples, participated in the design of experiments and analysis and in manuscript preparation. PZ participated in the design of experiments and analysis and in manuscript preparation. PM contributed samples, participated in the design of experiments and analysis and in manuscript preparation. NM contributed samples, participated in the design of experiments and analysis and in manuscript preparation. CB contributed samples, participated in the design of experiments and analysis and in manuscript preparation. PP conceived the study, designed experiments and analysis and prepared the manuscript.

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Supplementary table 1. Phenotypic characteristics of analyzed samples. (NA: Not available)

Sample origin	Number of families	Comorbid OCD (%)	Comorbid ADHD (%)	Autism Spectrum Disorder (%)	Male (%)	Female (%)
Greek	10	16.67	42.86	0	85.71	14.29
Albanian	6	NA	NA	NA	NA	NA
Hungarian	84	9.64	34.94	7.23	81.93	18.07
Polish	36	6.06	33.33	3.13	75.76	24.24
Spanish	19	66.67	41.18	0	80.00	20.00
Italian	50	79.07	69.05	0	86.05	13.95
German (Munich)	69	28.36	4.48	0	78.57	21.43
German (GCTS)	96	33	NA	NA	80	20
Canadian	150	28.28	57.24	NA	83.45	16.55

Supplementary table 2. Selection of tagging SNPs (tSNPs) at *HDC* using the HapMap CEPH European population as reference (http://hapmap.ncbi.nlm.nih.gov/). The r^2 threshold for tSNP selection was set to 0.8. Variation across *HDC* (chr15:48316000-48356000) was captured by the selected tSNPs with a mean r^2 of 0.968.

Selected tSNP	**captured SNPs**
rs1365503	rs1365503,rs2187576,rs2114447,rs10220755
rs854157	rs854160,rs860526,rs854157
rs854163	rs854159,rs854163
rs854158	rs854158,rs2238292
rs1894236	rs1894236,rs8029889
rs1677254	rs2853766,rs1677254
rs854151	rs854151
rs854150	rs854150
rs9920021	rs9920021
rs2070595	rs2070595
rs2070596	rs2070596
rs7182203	rs7182203

Supplementary Table 3. Transmission test for linkage disequilibrium in studied populations (total of 520 families) for single markers tested at the *HDC* gene. Analysis for the Greek and Albanian samples is not shown separately, because individual sample sizes are too small. (test implemented and P values determined by Haploview; T:U=Transmitted:Untransmitted)

Population	Rs#	Over-transmitted allele	T:U*	Chi square	P value	Permutation P value
Hungarian	rs854150	G	47:36	1.458	0.2273	
(84 families)	rs854151	G	33:30	0.143	0.7055	
	rs2070596	T	29:17	3.13	0.0768	
	rs2070595	G	35:27	1.032	0.3096	
	rs2853766	A	36:30	0.545	0.4602	
	rs1894236	C	29:15	4.455	0.0348	
	rs854157	T	44:42	0.047	0.8292	
	rs7182203	G	34:27	0.803	0.3701	
	rs854158	G	40:32	0.889	0.3458	
	rs854163	A	38:30	0.941	0.332	
	rs9920021	C	40:29	1.754	0.1854	
	rs1365503	G	36:34	0.057	0.8111	
Polish	rs854150	G	20:19	0.026	0.8728	
(36 families)	rs854151	A	16:15	0.032	0.8575	
	rs2070596	A	13:5	3.556	0.0593	
	rs2070595	T	18:9	3.0	0.0833	
	rs2853766	-	17:17	0	1.0	
	rs1894236	T	12:8	0.8	0.3711	
	rs854157	T	26:16	2.381	0.1228	
	rs7182203	A	17:10	1.815	0.1779	
	rs854158	-	20:20	0	1.0	
	rs854163	A	17:15	0.125	0.7237	
	rs9920021	A	15:13	0.143	0.7055	
	rs1365503	A	12:14	1.778	0.1824	
Spanish	rs854150	G	5:4	0.111	0.7389	
(19 families)	rs854151	G	7:2	2.778	0.0956	
	rs2070596	T	12:3	5.4	0.0201	
	rs2070595	G	13:6	2.579	0.1083	
	rs2853766	A	7:2	2.778	0.0956	
	rs1894236	C	13:3	6.25	0.0124	
	rs854157	C	11:5	2.25	0.1336	
	rs7182203	G	16:5	5.762	0.0164	
	rs854158	G	6:4	0.4	0.5271	
	rs854163	A	7:2	2.778	0.0956	
	rs9920021	-	8:8	0	1.0	
	rs1365503	G	10:9	0.053	0.8185	

Population	Rs#	Over-transmitted allele	T:U*	Chi square	P value	Permutation P value
Italian	rs854150	G	29:22	0.961	0.327	
(50 families)	rs854151	A	20:18	0.105	0.7456	
	rs2070596	A	21:14	1.4	0.2367	
	rs2070595	T	26:11	6.081	0.0137	
	rs2853766	A	18:17	0.029	0.8658	
	rs1894236	T	21:14	1.4	0.2367	
	rs854157	T	32:16	5.333	0.0209	
	rs7182203	**A**	**26:10**	**7.111**	**0.0077**	**0.0480**
	rs854158	-	20:20	0	1.0	
	rs854163	G	20:17	0.243	0.6219	
	rs9920021	A	22:21	0.023	0.8788	
	rs1365503	G	23:20	0.209	0.6473	
German	rs854150	G	91:66	3.981	0.046	
(165 families)	rs854151	G	61:60	0.008	0.9276	
	rs2070596	T	61:56	0.214	0.6439	
	rs2070595	T	74:67	0.348	0.5555	
	rs2853766	A	63:54	0.692	0.4054	
	rs1894236	C	63:52	1.052	0.305	
	rs854157	T	82:71	0.791	0.3738	
	rs7182203	A	73:63	0.735	0.3912	
	rs854158	G	72:69	0.064	0.8005	
	rs854163	A	66:58	0.516	0.4725	
	rs9920021	A	74:64	0.725	0.3946	
	rs1365503	G	79:60	2.597	0.1071	
Canadian	rs854150	G	87:66	2.882	0.0896	
(150 families)	rs854151	G	66:55	1.0	0.3173	
	rs2070596	T	65:40	5.952	0.0147	0.1100
	rs2070595	G	91:68	3.327	0.0681	
	rs2853766	A	70:50	3.333	0.0679	
	rs1894236	**C**	**68:37**	**9.152**	**0.0025**	**0.0240**
	rs854157	-	83:83	0.0	1.0	
	rs7182203	G	79:64	1.573	0.2097	
	rs854158	G	82:66	1.73	0.1884	
	rs854163	A	73:58	1.718	0.19	
	rs9920021	**A**	**75:42**	**9.308**	**0.0023**	**0.0210**
	rs1365503	**A**	**100:55**	**13.065**	**0.0003**	**0.0010**

Supplementary Table 4. Transmission test for linkage disequilibrium in studied populations (total of 520 trios) for SNP haplotypes encompassing the identified haplotype blocks in each population across the *HDC* gene. Haplotype blocks are shown in figure 1 of the main text. Analysis for the Greek and Albanian samples is not shown separately, because individual sample sizes are too small. (test implemented and P values determined by Haploview; T:U=Transmitted:Untransmitted; Permutation P value was calculated after 1,000 permutations).

Population	Haplotype	Frequency	T:U*	Chi square	P value	Permutation P value
Hungarian	Block 1					
(84 families)	CA	0.585	35.3 : 45.6	1.317	0.2512	
	GG	0.294	38.5 : 33.0	0.419	0.5173	
	GA	0.108	18.5 : 12.4	1.186	0.2761	
	CG	0.014	2.0 : 3.2	0.283	0.5946	
	Block 2					
	TGGCCG	0.395	43.0 : 41.0	0.048	0.8262	
	TGACTG	0.269	36.0 : 28.0	1.006	0.316	
	ATGTTA	0.185	17.0 : 28.0	2.684	0.1013	
	TTGCTA	0.103	17.0 : 13.0	0.53	0.4665	
	TTGCTG	0.026	5.0 : 4.0	0.111	0.7389	
	TTGCCG	0.010	0.0 : 2.0	2.013	0.156	
	Block 3					
	AG	0.646	32.0 : 40.0	0.888	0.346	
	GA	0.295	38.0 : 31.0	0.71	0.3994	
	GG	0.054	8.0 : 7.0	0.067	0.7963	
	Block 4					
	AA	0.402	36.4 : 37.5	0.017	0.8949	
	AG	0.306	32.5 : 42.9	1.428	0.232	
	CG	0.292	41.0 : 29.5	1.88	0.1703	
Polish	Block 1					
(36 families)	CA	0.551	20.0 : 20.0	0	1.0	
	GG	0.319	16.0 : 18.5	0.187	0.6655	
	GA	0.130	8.0 : 5.5	0.48	0.4886	
	Block 2					
	TGGCCG	0.392	14.0 : 24.0	2.632	0.1048	
	TGACTG	0.297	17.0 : 16.0	0.03	0.8618	
	ATGTTA	0.139	12.0 : 7.0	1.316	0.2513	
	TTGCTA	0.107	8.0 : 6.0	0.286	0.593	
	TTGCCG	0.024	2.0 : 1.7	0.017	0.8957	
	ATGCTA	0.013	2.0 : 0.0	2.0	0.1573	
	Block 3					
	AG	0.645	20.1 : 20.0	0	0.9929	
	GA	0.316	17.5 : 16.5	0.029	0.8638	
	GG	0.038	2.5 : 3.6	0.184	0.6678	

Supplementary Table 4 continued

Population	Haplotype	Frequency	T:U*	Chi square	P value	Permutation P value
Spanish	Block 1					
(19 families)	CCG	0.447	12.0 : 5.0	2.882	0.0896	
	TTA	0.327	3.0 : 13.9	6.993	0.0082	
	CTG	0.171	6.0 : 2.0	2.0	0.1573	
	CTA	0.055	3.0 : 3.1	0.003	0.954	
Italian	Block 1					
(50 families)	ATGGC	0.375	14.1 : 28.0	4.602	0.0319	
	AATGT	0.227	21.0 : 14.0	1.4	0.2367	
	GTGAC	0.193	18.0 : 18.0	0	1.0	
	ATTGC	0.170	18.0 : 10.0	2.286	0.1306	
	GTGGC	0.034	3.0 : 4.1	0.166	0.6838	
	Block 2					
	CG	0.447	16.0 : 32.0	5.333	0.0209	0.0460
	TA	**0.343**	**27.0 : 10.6**	**7.131**	**0.0076**	**0.0350**
	TG	0.210	20.0 : 20.4	0.004	0.9524	
	Block 3					
	AG	0.738	21.8 : 22.1	0.003	0.9592	
	GA	0.200	17.8 : 20.0	0.126	0.7231	
	GG	0.055	7.2 : 5.0	0.379	0.5383	
	Block 4					
	AG	0.364	24.0 : 20.0	0.364	0.5465	
	CG	0.348	21.0 : 22.0	0.023	0.8788	
	AA	0.288	20.0 : 23.0	0.209	0.6473	
German	Block 1					
(165 families)	ATGGC	0.354	71.0 : 83.4	1.0	0.3173	
	GTGAC	0.247	67.0 : 54.0	1.4	0.2367	
	AATGT	0.229	56.0 : 66.0	0.809	0.3684	
	ATTGC	0.132	44.0 : 31.3	2.153	0.1423	
	GTGGC	0.029	6.0 : 11.3	1.598	0.2062	
	Block 2					
	AAG	0.332	77.7 : 70.8	0.33	0.5657	
	GAG	0.332	69.9 : 79.7	0.644	0.4222	
	GGA	0.263	66.5 : 59.3	0.414	0.5199	
	GGG	0.073	16.0 : 20.4	0.531	0.4661	
	Block 3					
	AA	0.369	66.0 : 84.1	2.204	0.1377	
	CG	0.329	64.5 : 74.5	0.719	0.3963	
	AG	**0.302**	**92.5 : 64.4**	**5.064**	**0.0244**	**0.0480**

Supplementary table 4 continued

Population	Haplotype	Frequency	T:U*	Chi square	P value	Permutation P value
Canadian	Block 1					
(150 families)	ATGGC	0.393	88.7 : 83.1	0.183	0.6691	
	GTGAC	0.248	71.9 : 49.9	3.976	0.0461	0.3160
	AATGT	**0.199**	**39.0 : 67.0**	**7.397**	**0.0065**	**0.0430**
	ATTGC	0.126	46.9 : 44.3	0.072	0.7886	
	GTGGC	0.013	2.0 : 5.2	1.397	0.2373	
	Block 2					
	GAG	0.394	83.9 : 84.1	0.0	0.9863	
	AAG	0.295	65.0 : 80.0	1.552	0.2128	
	GGA	0.269	72.0 : 57.8	1.558	0.2119	
	GGG	0.039	13.1 : 12.1	0.04	0.842	
	Block 3					
	AA	**0.371**	**100.0 : 55.5**	**12.713**	**0.0004**	**0.0010**
	AG	0.326	65.0 : 77.5	1.09	0.2964	
	CG	**0.303**	**45.5 : 77.5**	**8.325**	**0.0039**	**0.0370**

Supplementary Figure 1. Rare allele frequencies of studied SNPs across the *HDC* region, in each analyzed population.