



Nonsyndromic cleft lip with or without cleft palate and cancer: Evaluation of a possible common genetic background through the analysis of GWAS data



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ABSTRACT

Previous research suggests a genetic overlap between nonsyndromic cleft lip with or without cleft palate (NSCL/P) and cancer. The aim of the present study was to identify common genetic risk loci for NSCL/P and cancer entities that have been reported to co-occur with orofacial clefting. This was achieved through the investigation of large genome-wide association study datasets. Investigations of 12 NSCL/P single nucleotide polymorphisms (SNPs) in 32 cancer datasets, and 204 cancer SNPs in two NSCL/P datasets, were performed. The SNPs rs13041247 (20q12) and rs6457327 (6p21.33) showed suggestive evidence for an association with both NSCL/P and a specific cancer entity. These loci harbor genes of biological relevance to oncogenesis (MAFB and OCT4, respectively). This study is the first to characterize possible pleiotropic risk loci for NSCL/P and cancer in a systematic manner. The data represent a starting point for future research by identifying a genetic link between NSCL/P and cancer.

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1. Introduction

Orofacial clefting (OFC) is a common congenital malformation comprising several subtypes. The most frequent form is nonsyndromic 'cleft lip with or without cleft palate' (NSCL/P), which is characterized by clefting of the upper lip and facultative clefting of the palate [46]. NSCL/P etiology involves both environmental and genetic factors. Genome-wide association studies (GWAS) have provided insights into the genetic background of NSCL/P through the identification of several risk loci [40,42].

Research suggests a common genetic etiology for congenital malformations - including OFC - and specific cancer entities. Miller [45] analyzed the death certificates of approximately 30,000 pediatric

cancer cases in order to determine the prevalence of co-morbid congenital abnormalities. Miller reported associations between Down's syndrome and leukemia, and aniridia and Wilms' tumor. Various epidemiological study designs have since been applied to identify associations between specific cancer entities and OFC or particular clefting subtypes. One of the largest investigations to date is the Danish registry cohort study of Bille et al. [4]. The authors found a significantly higher prevalence of: breast cancer in females with cleft lip and/or cleft palate; brain cancer in females with cleft palate; and lung cancer in males with cleft lip and palate. Further studies have supported an association [13, 43,47,72], whereas others have not [6,8,56].

As both cancer and OFC have a multifactorial etiology, shared risk factors might be genetic, environmental, or a combination of both. Molecular studies - in particular recent GWAS - have identified susceptibility factors for various cancer subtypes [14]. The first GWAS of cancer in the late 2000s reported a limited number of loci for the most common

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malignancies. To date, individual studies and meta-analyses have identified approximately 50 susceptibility loci for colorectal cancer, >70 for breast cancer, and >100 for prostate cancer. Of particular interest is the finding that chromosomal region 8q24.21 contains several cancer-risk single nucleotide polymorphisms (SNPs) [25], and a major risk locus for NSCL/P [5].

The majority of published investigations into a common genetic etiology for OFC and cancer have been purely descriptive. To date, strong molecular evidence for an association between cancer and OFC has been generated for only one gene. In the respective resequencing study, a causative role for the gene *CDH1* was identified in both gastric cancer and OFC [9]. A limitation of previous studies is that they were based on the analysis of candidate genes and/or particular pedigrees. To overcome this, the present study used genome-wide SNP data from large cohorts of patients with sporadic cancers or NSCL/P. Analyses were performed to identify common genetic risk loci for NSCL/P and those cancer entities for which co-occurrence with NSCL/P has been reported. The identification of shared risk loci would provide insights into common underlying mechanisms.

2. Materials and methods

2.1. Cancer entity search strategy

In a first step, a Pubmed search was performed to locate original studies published prior to July 2012 that had investigated an association between OFC and cancer. The following search terms were used: “cleft cancer”, “cleft tumor”, “cleft lip cancer”, “cleft lip tumor”, “cleft palate cancer”, “cleft palate tumor”, “facial cleft cancer”, “facial cleft tumor”, “oral cleft cancer”, “oral cleft tumor”, “orofacial cleft cancer”, and “orofacial cleft tumor”. In the OFC literature, nomenclature is applied inconsistently, and these broad search terms were used in order to ensure that relevant studies were not overlooked due to the use of alternative morphological classification. The reference lists of these publications were then scrutinized to identify additional studies. On the basis of these search results, a list of cancer entities with a reported association with any form of OFC was compiled.

This list was then reduced to cancer entities with a reported association with NSCL/P. Studies that had investigated other OFC phenotypes (i.e., cleft palate only, syndromic OFC, or anomalies such as tooth agenesis and bifid uvula) were excluded. Data from case reports and animal models were also excluded from the analyses (Supplementary Table S1).

2.2. Cancer GWAS search strategy

In a second step, a search of the “NHGRI GWAS Online Catalog” ([29], <http://www.genome.gov/gwastudies>) was performed to identify GWAS of those cancer entities for which an association with NSCL/P had been reported (cancer entities of interest listed in Supplementary Table S1). Since this GWAS catalog is not exhaustive, the search was complemented using PubMed. The following search terms, followed by the respective cancer subtype, were used: “genome-wide association studies”, “genome-wide association study”, “gwas”, “gwa”.

2.3. Identification of NSCL/P risk SNPs from the literature

Prior to July 2012, three GWAS of NSCL/P were performed in European case-control samples [5,26,41], and one GWAS was performed in European and Asian trios [2]. Subsequently, two meta-analyses of GWAS data from Mangold et al. [41] and Beaty et al. [2] were performed [40]. In total, 12 loci in these studies showed genome-wide significance. These comprised one established NSCL/P risk locus (*IRF6*), and 11 novel loci. Twelve lead SNPs at these loci were chosen as NSCL/P risk SNPs for the present study (Table 1).

Table 1
NSCL/P-associated risk SNPs identified in GWAS.

SNP-ID	Allele*	Chr. region	Position (Mb)	Reference
rs560426	G-A	1p22.1	94.32–94.35	[2]
rs861020	A-G	1q32.2	208.00–208.12	[2]
rs742071	T-G	1p36	18.85	[40]
rs7590268	G-T	2p21	43.39	[40]
rs7632427	C-T	3p11.1	89.61	[40]
rs12543318	C-A	8q21.3	88.93	[40]
rs987525	A-C	8q24.21	129.77–130.30	[5]
rs7078160	A-G	10q25	118.81–118.83	[41]
rs8001641	A-G	13q31	79.57–79.60	[40]
rs1873147	C-T	15q22	61.09	[40]
rs227731	C-A	17q22	52.12	[41]
rs13041247	C-T	20q12	38.70–38.71	[2]

Chr. = chromosomal.

* Minor allele first, risk allele for NSCL/P in bold.

2.4. Identification of cancer risk SNPs from the literature

All autosomal SNPs with genome-wide significance in at least one cancer GWAS published prior to July 2012 were listed. According to the guidelines of the International HapMap Consortium [32], an SNP should be considered genome-wide significant if it achieves a *P*-value below a threshold of 5×10^{-8} . As an exception to this rule, the present study also included SNPs with a *P*-value of $>5 \times 10^{-8}$ if they had been defined as genome-wide significant in the original study.

2.5. Exploration of genome-wide SNP datasets

Data on NSCL/P-associated genetic variants were retrieved from the meta-analyses of the two largest GWAS of NSCL/P to date [40]. Ludwig et al. [40] included 497,084 SNPs, which had been genotyped in 666 complete European trios, 795 complete Asian trios, and 399 patients and 1318 controls of Central European origin. This study included 95% of all individuals available at that time with both NSCL/P and genome-wide data. For SNPs in the cancer SNP list, analyses were performed to determine association with NSCL/P in the two meta-analyses: i) European (meta_Euro); and ii) the combined European/Asian population (meta_all) datasets.

Additionally, the corresponding authors of all cancer GWAS used for SNP selection were contacted. These researchers were asked to retrieve association information from their cancer GWAS datasets for each lead SNP from the 12 NSCL/P risk loci (Table 1).

For both analyses, cancer and NSCL/P SNPs that were not represented in the respective analyzed data were replaced by a proxy SNP ($r^2 > 0.5$ in the HapMap CEU population, Supplementary methods). For the *P*-values of cancer-associated SNPs in the NSCL/P meta-analyses, correction for multiple testing was performed using a simulation procedure. This was based on 10,000 replicated samples, and involved permutation of: (i) the case and control status of individuals; and (ii) the transmitted and non-transmitted parental alleles.

For the analysis of NSCL/P-associated SNPs in the cancer GWAS, a correction factor of 384 was used (12 risk loci, 32 cancer GWAS datasets).

3. Results

The cancer entity search for studies that had analyzed the co-occurrence of orofacial anomalies and cancer identified 36 publications (Supplementary Table S1). Of these, 10 contained sufficient information to deduce that the described associations were with the NSCL/P phenotype. These 10 studies covered 11 different cancer entities, all of which were primary forms of cancer, i.e., they were not metastatic tumors. These cancer entities comprised: brain cancer [4,22,43]; breast cancer [4,43]; colorectal cancer [43]; leukemia [43,44,48,69,72]; liver cancer

Table 2
Cancer-associated SNPs with nominal significance in NSCL/P.

SNP	Risk allele cancer ^a	Risk allele NSCL/P ^b	Proxy needed?	Chr. region	P _{meta_Euro}	P _{meta_all}	Associated cancer entity	Reference
rs6457327	C	C	NO	6p21.33	1.92×10^{-4}	4.18×10^{-3}	Lymphoma (FL)	[54]
rs17505102	G	G	rs16864725	3q28	1.93×10^{-3}	2.82×10^{-2}	Leukemia (ALL)	[20]
rs3131379	n/s	A	NO	6p21.33	4.29×10^{-3}	5.69×10^{-3}	Lung cancer	[65]
rs3117582	C	C	NO	6p21.33	4.74×10^{-3}	5.46×10^{-3}	Lung cancer	[65]
rs4779584	n/s	C	NO	15q13	8.76×10^{-3}	7.76×10^{-2}	Colorectal cancer	[49]
rs10934853	A	A	NO	3q21.3	1.99×10^{-2}	1.13×10^{-3}	Prostate cancer	[27]
rs6712055	C	T	NO	2q35	2.29×10^{-2}	1.05×10^{-1}	Neuroblastoma	[10]
rs17728461	G	C	rs9614158	22q12.2	2.51×10^{-2}	1.90×10^{-1}	Lung cancer	[31]
rs4857841	A	A	NO	3q21.3	2.56×10^{-2}	1.22×10^{-3}	Prostate cancer	[27]
rs204999	A	A	NO	6p21.32	2.72×10^{-2}	2.25×10^{-1}	Lymphoma (cHL)	[15]
rs11170164	A	G	rs11170148	12q13.13	3.94×10^{-2}	3.94×10^{-2}	Skin cancer (BCC)	[55]
rs2055109	C	T	NO	3p11.2	4.24×10^{-2}	4.07×10^{-2}	Prostate cancer	[1]
rs1321311	A	A	NO	6p21.2	4.28×10^{-2}	6.41×10^{-2}	Colorectal cancer	[17]
rs10995190	G	A	NO	10q21.2	4.47×10^{-2}	4.76×10^{-2}	Breast cancer	[63]
rs4635969	C	C	NO	5p15.33	9.66×10^{-2}	3.35×10^{-2}	Lung cancer	[35]
rs17021918	C	T	NO	4q22.3	1.24×10^{-1}	1.74×10^{-2}	Prostate cancer	[19]
rs1862748	C	T	NO	16q22.1	1.50×10^{-1}	3.57×10^{-2}	Colorectal cancer	[30]

n/s = not specified; P_{meta_Euro} and P_{meta_all} = P-value from Likelihood ratio test in the European or European/Asian meta-analysis respectively; FL = follicular lymphoma; ALL = acute lymphoblastic leukemia; cHL = classical Hodgkin lymphoma; BCC = basal cell carcinoma.

^a Risk allele in cancer GWAS.

^b Risk allele in NSCL/P GWAS (in identical strand orientation).

[43]; lung cancer [4,43]; lymphoma [72]; neuroblastoma [47]; prostate cancer [43]; retinoblastoma [7]; and skin cancer [43]. The remaining 26 studies did not meet the present inclusion criteria.

Convincing GWAS results were found for nine of the 11 cancer entities. For retinoblastoma, no GWAS was found and this cancer entity was therefore excluded. The single GWAS of liver cancer had identified susceptibility variants for hepatitis B and C virus-induced hepatocellular carcinoma only [11,34,36,53,70]. Given the virus-related origin of liver cancer in these GWAS, this cancer entity was excluded from further analysis. For the remaining nine cancer entities, 233 SNPs were reported to show genome-wide significance (Supplementary Tables S2.1–S2.9). Most of these SNPs had been identified in GWAS of prostate cancer

(n = 57). Only eight SNPs were derived from three GWAS of brain cancer (glioma). Sixty-six of the 233 SNPs required replacement by a proxy SNP for further analysis, and nine variants were excluded due to the lack of a proxy SNP (for details see Supplementary Tables S2.1–S2.9). In total, 204 cancer-associated SNPs were analyzed in the NSCL/P meta-analysis data.

Nominal significance ($P < 0.05$) was achieved for a total of 17 cancer SNPs: 14 cancer SNPs in the meta_Euro subsample; and 12 cancer SNPs in the meta_All subsample (Table 2). In both instances, this is more than would have been expected by chance (expected number per subsample: 10.2). For 15 of the 17 nominally significant cancer SNPs, the “cancer risk allele” was reported in the literature. For eight of

Table 3
NSCL/P associated SNPs with nominal significance in cancer GWAS.

SNP-ID (risk allele in NSCL/P)	Proxy required?	Cancer entity	Risk	Ref	P-value ^a	OR	OR type	Number of cases/controls	Sample ethnicity
rs13041247 (T)	*† NO	skin cancer (SCC)	C	T	4.73×10^{-6}	1.23	allelic	973/>60,000	EU
rs13041247 (T)	*† NO	skin cancer (BCC)	C	T	1.04×10^{-3}	1.10	allelic	2807/>60,000	EU
rs13041247 (T)	*† NO	skin cancer (CM)	T	C	3.54×10^{-3}	1.16	allelic	725/>60,000	EU
rs13041247 (T)	*† NO	lymphoma (CLL)	T	C	3.31×10^{-2}	1.27	genotypic	407/296	EU ^b
rs13041247 (T)	*† NO	lymphoma (CLL)	T	C	4.14×10^{-2}	1.25	allelic	148/>60,000	EU
rs13041247 (T)	* NO	brain cancer (glioma)	T	C	4.18×10^{-2}	1.14	n/s	846/1310	EU ^c
rs1873147 (C)	* NO	brain cancer (glioma)	A	G	4.50×10^{-2}	1.17	n/s	846/1310	EU ^c
rs227731 (C)	*† NO	prostate cancer	T	G	1.40×10^{-3}	1.10	allelic	2682/>60,000	EU
rs227731 (C)	*† NO	skin cancer (BCC)	T	G	2.35×10^{-3}	1.09	allelic	2807/>60,000	EU
rs227731 (C)	*† NO	skin cancer (CM)	T	G	1.83×10^{-2}	1.14	allelic	725/>60,000	EU
rs227731 (C)	* NO	skin cancer (BCC)	T	G	3.29×10^{-2}	1.08	allelic	2045/6013	EU
rs560426 (G)	* NO	prostate cancer	T	C	5.83×10^{-4}	1.19	n/s	1583/4944	AS
rs560426 (G)	*† NO	colorectal cancer	C	T	1.85×10^{-3}	1.06	allelic	12,620/15,110	EU
rs560426 (G)	* NO	lymphoma (DLBCL)	C	T	4.23×10^{-2}	1.23	allelic	256/747	EU
rs7078160 (A)	*† NO	skin cancer (BCC)	G	A	1.62×10^{-3}	1.14	allelic	2807/>60,000	EU
rs7078160 (A)	* NO	brain cancer (glioma)	A	G	3.12×10^{-2}	1.16	n/s	1247/2236	EU ^d
rs7078160 (A)	*† NO	skin cancer (CM)	G	A	3.15×10^{-2}	1.16	allelic	725/>60,000	EU
rs7632427 (T)	* NO	skin cancer (SCC)	C	T	1.46×10^{-2}	1.12	allelic	973/>60,000	EU
rs7632427 (T)	*† NO	brain cancer (glioma)	T	C	4.84×10^{-2}	1.10	additive	2331/3077	AS
rs861020 (A)	*† rs1962735	leukemia (ALL)	G	A	3.13×10^{-2}	1.28	n/s	1696/3535	EU
rs861020 (A)	* NO	lymphoma (FL)	G	A	4.97×10^{-2}	1.33	allelic	213/750	EU
rs987525 (A)	* NO	brain cancer (glioma)	A	C	1.15×10^{-2}	1.20	n/s	846/1310	EU ^c
rs987525 (A)	* NO	brain cancer (glioma)	A	C	3.65×10^{-2}	1.14	n/s	1423/1190	EU ^e

Risk = Risk allele in cancer GWAS; Ref = Reference allele in cancer GWAS; OR = Odds Ratio; * SNP genotyped; † SNP imputed; ‡ no data available for SNP; SCC = squamous cell carcinoma; BCC = basal cell carcinoma; CM = cutaneous melanoma; CLL = chronic lymphocytic leukemia; DLBCL = Diffuse large B-cell lymphoma; FL = follicular lymphoma; EU = European ethnicity; AS = Asian ethnicity; n/s = not specified.

^a Association P-value from cancer GWAS.

^b 99% are known or assumed to be White and Not Hispanic.

^c German subgroup.

^d US subgroup.

^e French subgroup.

Table 4

Cancer risk SNPs at 8q24.21 and distance from NSCL/P risk SNP rs987525.

Cancer entity	SNP-ID	Risk allele ^a	OR ^b	Position	Distance from rs987525 (kb)	Reference
Breast cancer	rs13281615	C	1.08	128,424,800	– 1591	[18]
Colorectal cancer	rs6983267	G	1.21	128,476,625	– 1539	[62]
Colorectal cancer	rs10505477	n/s	1.12	128,482,487	– 1533	[62]
Colorectal cancer	rs7014346	A	1.19	128,493,974	– 1521	[60]
Glioma	rs4295627	G	1.36	130,754,639	739	[52]
Lymphoma (cHL)	rs2608053	G	1.20	129,261,453	– 754	[21]
Lymphoma (cHL)	rs2019960	G	1.33	129,145,014	– 870	[21]
Lymphoma (CLL)	rs2456449	G	1.26	128,262,163	– 1753	[16]
Lymphoma (CLL)	rs2466024	A	1.20	128,257,201	– 1758	[16]
Prostate cancer	rs1447295	A	1.60	128,554,220	– 1461	[28]
Prostate cancer	rs16901979	A	1.79	128,194,098	– 1821	[28]
Prostate cancer	rs6983267	G	1.27	128,482,487	– 1533	[68]
Prostate cancer	rs7837688	T	1.47	128,608,542	– 1407	[68]
Prostate cancer	rs4242382	A	n/s	128,586,755	– 1429	[61]
Prostate cancer	rs16902094	G	1.21	128,389,528	– 1626	[27]
Prostate cancer	rs445114	T	1.14	128,410,090	– 1605	[27]
Prostate cancer	rs16902104	T	1.21	128,392,363	– 1623	[27]

n/s = not specified; cHL = classical Hodgkin's lymphoma; CLL = chronic lymphocytic leukemia.

^a Risk allele in cancer GWAS.^b Allelic odds ratio in cancer GWAS.

these SNPs, the “cancer risk allele” was identical to the “NSCL/P risk allele” (Table 2). For the cancer-associated SNP rs6457327 on chromosome 6p21.33, a borderline association was reported in the European NSCL/P dataset. However, this became non-significant following correction for multiple testing ($P_{\text{adj}} = 0.0528$). In the original report, the SNP rs6457327 was associated with follicular lymphoma [54]. In the original cancer GWAS, risk was conferred by the C allele at this SNP, which is identical to the risk allele in the NSCL/P meta-analyses datasets.

Analyses were then performed to identify associations with the lead SNPs of the 12 NSCL/P loci in 32 different cancer sample datasets. Eight SNPs achieved nominal significance in at least one sample dataset. The association of rs13041247 at chromosome 20q12 with squamous cell cancer of the skin ($P_{\text{adj}} = 4.73 \times 10^{-6} \times 384 = 0.0018$, data extracted from the Icelandic Cancer Registry) remained significant after conservative Bonferroni correction for 384 tests. However, the risk allele for this SNP reported in squamous cell cancer of the skin differs from that found in the NSCL/P patients (Table 3).

Two *CDH1* SNPs (rs9929218 and rs1862748) were included in the present study as they had shown genome-wide significant results in a GWAS of colorectal cancer [30]. The SNP rs1862748 showed nominal significance ($P = 3.57 \times 10^{-2}$) in the present NSCL/P dataset (Table 2).

The literature search for cancer risk SNPs identified 16 SNPs in the region of 8q24.21. This region also contains a key susceptibility locus for NSCL/P, with the top marker being rs987525. None of the cancer risk SNPs showed significant association in the NSCL/P datasets, and no association with rs987525 was found in any of the investigated cancer datasets (Table 4).

4. Discussion

The aim of the present study was to identify common genetic risk loci for NSCL/P and those cancer entities that have been reported to co-occur with NSCL/P in descriptive studies. Two approaches were used. First, conclusively identified cancer susceptibility variants were analyzed in a large genome-wide SNP dataset of NSCL/P patients. Second, known NSCL/P risk loci were analyzed in GWAS data for specific cancer entities. Analysis of only a subset of candidate SNPs, i.e., those identified as being genome-wide significant for one trait, reduced the number of tests and thus the requirement for correction, thereby increasing the chances of identifying common risk loci.

In principle, an overlapping genetic contribution to both traits could also be quantified using a genome wide polygenic score approach [51]. However, for this etiological overlap to be apparent in a polygenic score, a specific cancer entity and NSCL/P would have to share a large number

of genetic risk factors. Given the weak associations reported for cancer entities and NSCL/P in previous epidemiological studies, the presence of a large number of shared genetic risk factors cannot be assumed. In addition, since the polygenic score allows no conclusions to be drawn concerning a particular gene, this approach would allow no conclusions concerning a common biological pathway for NSCL/P and any specific cancer entity. We therefore considered the present methodology to be the more appropriate approach to our research question.

One association withstood correction for multiple testing. The NSCL/P-associated risk SNP rs13041247 at chromosome 20q12 showed genome-wide significant association in the dataset of the Icelandic cancer registry for squamous cell carcinoma of the skin. However, the NSCL/P risk allele (T) is not identical to the skin cancer risk allele (C). The SNP rs13041247 maps 45 kb downstream of the musculoaponeurotic fibrosarcoma oncogene homolog B (*MAFB*) gene, which encodes the v-maf transcription factor. In the GWAS of NSCL/P conducted by Beaty et al. [2], which was the first to describe association of this variant with NSCL/P, sequencing of conserved elements within the 3' region and the coding region of *MAFB* in NSCL/P cases and controls from Iowa and the Philippines revealed an overrepresentation of a rare missense variant (His131Gln). The contribution of this variant to NSCL/P awaits elucidation [2]. Animal studies have provided additional support for the hypothesis that *MAFB* is a candidate gene for NSCL/P at this locus by showing that its homolog in rodents is expressed in craniofacial structures during embryogenesis [2]. Recently, Lopez-Pajares et al. [39] demonstrated that *MAF* and *MAFB* control the expression of the transcription factor genes *GRHL3*, *ZNF750*, *PRDM1*, and *KLF4*. Together, these genes form a network that is essential for epidermal differentiation. Previous studies have demonstrated that the grainyhead-like transcription factor 3 gene *GRHL3* causes the autosomal dominant Van der Woude syndrome [50], which is the most common syndromic form of cleft lip and palate. Furthermore, Bhandari et al. [3] observed a marked reduction or absence of *GRHL3* expression in squamous cell skin carcinoma samples from mice and humans. A recent study identified dominant negative *KLF4* variants in patients with NSCL/P [38].

Of the cancer associated SNPs, the most significant *P*-value in the NSCL/P meta-analyses datasets was found for rs6457327 at 6p21.33, although this fell short of significance after correction for multiple testing. This SNP was originally reported by Skibola et al. [54] as a risk locus for follicular lymphoma, and maps 58 kb downstream of the *POU* class 5 homeobox 1 (*POU5F1*) gene, also known as *OCT4*. This gene encodes a transcription factor with an important role in embryonic development, in particular during early embryogenesis, and which is necessary for maintaining embryonic stem cell pluripotency [57]. Wang et al. [66]

showed that *OCT4* regulates and interacts with the BMP4 pathway in specifying different developmental fates in human embryonic stem cells. Notably, the BMP4 pathway is involved in mammalian palatogenesis [71], and mutations in *BMP4* have been associated with human NSCL/P [12,58,59]. Research has shown that *OCT4* is overexpressed in cancer cell lines and in diverse cancer entities [24,37,67], suggesting that aberrant transcriptional regulation of *OCT4* might be a mechanism in cancer susceptibility. Thus, a plausible hypothesis is that rs6457327 regulates *OCT4* expression, and that this regulation is a possible common process in oncogenesis and the development of NSCL/P.

An important consideration in interpreting the present results is that the publication search adhered to very strict inclusion criteria, which might have introduced two sources of bias. First, we concentrated on investigating cancer risk association with NSCL/P and no other OFC subtype in order to reduce any existing genetic heterogeneity. However, as OFC nomenclature is applied inconsistently, we cannot exclude the possibility that our investigation included cancer entities that were associated with forms of OFC other than “pure” NSCL/P. Second, we may have rejected genuinely associated cancer entities due to a non-precise description of a possible association with NSCL/P in the respective publication. Frebourg et al. [23] and Kluijdt et al. [33] described a possible co-segregation of OFC and *CDH1*-related gastric cancer, and recent resequencing and association studies strongly support a contribution to NSCL/P of predominantly rare and moderately penetrant *CDH1* variants [9]. As this co-occurrence was not precisely related to NSCL/P in the initial studies, and no other study of this cancer entity had been published by July 2012, gastric cancer was not included in the present cancer entity list. Nonetheless, two *CDH1* SNPs (rs9929218 and rs1862748) were included, as they had shown genome-wide significant results in a GWAS of colorectal cancer [30], and rs1862748 showed nominal significance ($P = 3.57 \times 10^{-2}$) in the present NSCL/P dataset (Table 2).

Another important consideration is that most of the individuals investigated in the descriptive studies had a relatively low mean- and median age. Bille et al. [4] were the only authors to investigate the co-occurrence of OFC and cancer in adults (maximum age: 62 years). Consequently, the present analyses may have failed to consider cancers that are more frequent in later life.

In addition, we cannot exclude the possibility that NSCL/P is associated with cancer-subtypes other than those considered in the present study. Many of the GWAS concentrated on specific cancer subtypes. For example, studies of leukemia and lymphoma were conducted using case cohorts of acute lymphoblastic leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia, follicular lymphoma, and Hodgkin's lymphoma (Supplementary Tables S2.4 and S2.6), which do not represent all forms of leukemia and lymphoma. This also applies to the skin cancer studies, since most of the GWAS of skin cancer were performed for the most frequent skin cancer subtypes, such as cutaneous melanoma, basal cell carcinoma, and squamous cell carcinoma.

An interesting result of the present study is the finding for the 8q24.21 region. This contains a key susceptibility locus for NSCL/P, and although it was initially identified in a small GWAS, the genetic effect was very pronounced [5]. The finding has since been confirmed in numerous GWAS and targeted replication studies [42]. The top marker, rs987525, maps to an intergenic region, which may contain remote cis-acting enhancers that control expression of the well known proto-oncogene *Myc* in the developing murine facial prominences [64]. Sixteen of the present cancer-risk SNPs are located in the 8q24.21 region (Table 4). However, none of these SNPs showed a statistically significant association in the NSCL/P data-sets. Furthermore, none of these cancer risk variants is in linkage disequilibrium with rs987525, which suggests that this locus might contain distinct regulatory regions that are responsible for different developmental processes. This hypothesis is supported by data from Uslu et al. [64], who showed that a distinct region adjacent to rs987525 contained a specific facial enhancer element. Deletion of this medial-nasal enhancer resulted in a pronounced reduction

in *myc* expression in the facial tissues of homozygous murine embryos but not in other embryonic tissues. Therefore it is possible that the 8q24 region contains enhancers that control the expression of *Myc* in either facial development or cancer but not in both.

In summary, the present study is the first to characterize possible pleiotropic risk loci for NSCL/P and cancer using large genome-wide datasets. Suggestive evidence for a common genetic background was found for NSCL/P and follicular lymphoma at 6p21.33, and for NSCL/P and squamous cell carcinoma of the skin at 20q12. Whether, and to what extent, the development of these phenotypes is influenced by an altered function of the putative candidate genes *OCT4* and *MAFB* at these loci remains unclear. No marker in the present study showed pronounced effects on both phenotypes. Although inconclusive at the single marker level, the present data represent a starting point for further research into the common genetic etiology of OFC and cancer.

Conflicts of interest

None

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.gdata.2016.08.017>.

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